Effects of 6-Thioguanine on RNA Biosynthesis in Regenerating Rat Liver

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SUMMARY

6-Thioguanine, at a dose of 40 mg/kg body weight, was administered to rats at 12 hr after partial hepatectomy; 6 hr later, liver polysomes and cell sap were isolated and utilized to measure the effects of this antimetabolite on protein synthesis in vitro. When radioactive leucine was used to label peptides synthesized in vitro, no difference was observed between polyacrylamide gradient gel scans of systems derived from control regenerating liver and those from 6-thioguanine-treated regenerating liver. However, when radioactive tyrosine was used as the tracer to monitor synthesized peptides, a depression in the 30,000-molecular weight region of scans of products synthesized in systems derived from 6-thioguanine-treated regenerating liver was observed. Recombination experiments showed this effect to be due to the polysome component of the system. When equal amounts of polyadenylic acid-containing RNA from 6-thioguanine-treated or control regenerating liver were added to a wheat germ in vitro protein-synthesizing system, polyacrylamide gel scans of the products synthesized in the presence of radioactive tyrosine showed that more peptides were synthesized from polyadenylic acid-containing RNA from 6-thioguanine-treated rats than from control polyadenylic acid-containing RNA. That this phenomenon might be the result of incorporation of the analog into RNA was shown by the finding that all types of RNA contained 6-thioguanine, with the greatest concentration occurring in polyadenylic acid-containing RNA.

INTRODUCTION

The previous paper of this series (4) has shown that 6-TG has a major inhibitory effect on the partial hepatectomy-induced synthesis of enzymes required to support DNA replication in regenerating rat liver without altering the rate of total protein synthesis in vivo. The findings of these experiments were accompanied by a depression of the synthesis of poly(A)-containing RNA in 6-TG-treated regenerating liver, whereas the synthesis of other kinds of RNA was apparently unaffected by the purine antimetabolite. These effects were initiated in the G1 phase of the cell cycle and appeared to constitute a new metabolic lesion(s) created by 6-TG; thus, it was of importance to attempt to localize more precisely the site of action of the purine antimetabolite on these metabolic events. Earlier work by Wheeler et al. (20) has shown that 6-TG acts in the G2 phase of the cell cycle of H.Ep.2 cells to delay the G1- to S-phase transition; the biochemical alteration responsible for this action is unknown but would appear to be unrelated to incorporation of the analog into DNA.

GTP is required at several stages of the translational process; therefore, protein synthesis would appear to be a particularly vulnerable site for the action of a guanine analog. Roy et al. (14), however, demonstrated that when 6-TG triphosphate was substituted for GTP in an in vitro amino acid-incorporating system, not only did the thiopurine nucleotide not inhibit the reaction, but in contrast, it was found to partially substitute for the natural substrate. Gray and Rachmeler (6) reported that 6-TG was incorporated into Escherichia coli tRNA and that this incorporation apparently affected the amino acid acceptor activities of some of the tRNA’s.

6-TG could also be envisioned to interfere with the synthesis of certain proteins either by being incorporated into the specific mRNA molecules for these proteins or by affecting the synthesis of the specific mRNA’s themselves. The purine antimetabolite has been shown to be incorporated into both RNA and DNA (8, 9, 15, 17), but it is the incorporation into DNA to which cytotoxicity has been attributed (see, e.g., Ref. 10).

The experiments described in this paper show that in vitro translation of certain groups of peptides is altered in cell-free systems derived from 6-TG-treated regenerating liver and that the basis of this effect resides in the polysomes. Furthermore, 6-TG is preferentially incorporated into poly(A)+ RNA, and this 6-TG-containing RNA is more efficient in directing in vitro translation than is poly(A)+ RNA without 6-TG. These effects are observed at a time when DNA synthesis and 6-TG incorporation into these macro-molecules in regenerating liver are minimal.

MATERIALS AND METHODS

Wheat germ, pyruvate kinase, phosphoenolpyruvate, RNase A, dithiothreitol, creatine phosphate, creatine phosphokinase, N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid; poly(A) RNA, polyadenylic acid-containing RNA; SDS, sodium dodecyl sulfate; poly(A)+ RNA, polyadenylic acid-lacking RNA; PCA, perchloric acid.
acid, and carbonic anhydrase were obtained from Sigma Chemical Co., St. Louis, Mo.; acrylamide and N,N'-methylenebisacrylamide were purchased from Eastman Kodak Co., Rochester, N. Y. Cellex-D anion-exchange resin was obtained from Bio-Rad Laboratories, Richmond, Calif.; [4,5-3H (N)]leucine and elemental 35S were obtained from New England Nuclear, Boston, Mass.; [2,6-3H (N)]tyrosine was purchased from ICN Chemical Radioisotope Division, Irvine, Calif. Redisolv HP was obtained from Beckman Instruments, Inc., Fullerton, Calif. 6-TG was the generous gift of Dr. George H. Hitchings of Burroughs-Wellcome Research Laboratories, Research Triangle Park, N. C.

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.), weighing between 170 and 200 g, were housed routinely over corn cob bedding, and alternating periods of 12 hr dark and 12 hr light were maintained. Before surgery, Purina rat chow and water were available ad libitum. Partial hepatectomies were performed under light ether anesthesia between 8:30 and 10:30 a.m. according to the method of Higgins and Anderson (7). 6-TG was dissolved in 0.9% NaCl solution with the aid of dilute NaOH as described previously (4) and was injected i.p. at 12 hr after partial hepatectomy, at a dose of 40 mg/kg body weight; control animals received an equal volume of 0.9% NaCl solution.

The rat liver in vitro protein-synthesizing system utilized was a composite of those described by Ragnotti et al. (12) and Atkins et al. (2). Partially hepatectomized and normal animals were sacrificed by decapitation, and their livers were excised, minced, and homogenized in 2 volumes of 0.25 m sucrose-50 mM Tris-HCl, pH 7.8-25 mM KCl-6 mM MgSO4. This homogenate was centrifuged at 12,000 x g for 10 min, and the supernatant solution was recentrifuged at 145,000 x g for 2 hr. The resulting pellet, resuspended in incubation buffer; 175 µg pyruvate kinase/ml, 4 ml of 10 mM phosphoenolpyruvate, and 35 µCi [3,5-3H (N)]tyrosine, 20 µg creatine phosphokinase, and 50 µg RNase and EDTA to a final concentration of 10 mM were added. After incubation for 15 min at 37°, the peptides were precipitated with the aid of dilute acetic acid mix lacking only the radioactive amino acid

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of Roberts and Paterson (13). The peak turbid fractions were pooled, rapidly frozen in 0.5-ml batches, and stored at −70°. Wheat germ in vitro protein synthesis assays were performed in a total volume of 0.5 ml, containing 0.2 ml wheat germ S-30 fraction, 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.6 (adjusted with KOH), 2 mM dithiothreitol, 1 mM ATP, 20 µM GTP, 8 mM creatine phosphate, 3 mM magnesium acetate, 100 mM KCl, 30 µM cold amino acid mix without tyrosine, 50 µCi [3,5-3H (N)]tyrosine, 20 µg creatine phosphokinase, and 50 µg poly(A)+ RNA isolated as described previously (4). The reaction was started by the addition of the S-30 fraction and was allowed to proceed for 30 min at 25°, after which time 10 µg RNase and EDTA to a final concentration of 10 mM were added. After incubation for 15 min at 37°, the peptides were precipitated by the addition of 2 volumes of 80% acetone at 0°. The precipitate was collected by centrifugation and washed 3 times with 80% acetone, after which it was suspended in 0.0625 M Tris-HCl, pH 6.8-2% SDS-10% glycerol-0.1 M dithiothreitol-0.01% bromphenol blue for polyacrylamide gel electrophoresis on gradient polyacrylamide gels.

Linear (4 to 30%) gradient acrylamide gels were prepared by using an apparatus described by Caton and Goldstein (5). Chamber 1 contained 51.45 g acrylamide and 1.05 g N,N'-methylenebisacrylamide in 150 ml of 0.375 M Tris-HCl, pH 8.8-0.1% (w/w) SDS, and Chamber 2 contained 5.72 g acrylamide and 0.28 g N,N'-methylenebisacrylamide in 150 ml of the above Tris buffer. Immediately before starting the gradient former, 0.15 ml N,N,N',N'-tetramethylethylenediamine and 0.075 g of ammonium persulfate were added to each chamber and mixed well. The gels were allowed to polymerize overnight at room temperature, after which time they were cut free from the excess gel encasing the glass tubing. Gels prepared in this fashion could be stored under buffer in the cold for several weeks.

Before electrophoresis of peptides, gels were subjected to preelectrophoresis for 45 min at 2 ma/gel. Samples were applied in a volume of 50 µl and subjected to electrophoresis in a Buchler Polyanalyt water-jacketed electrophoresis cell at 2 ma/gel, until the bromphenol blue marker reached the bottom of the tube (approximately 4.5 to 5 hr). Electrophoresis buffer was 0.025 M Tris-0.193 M glycine, pH 8.3, containing 0.1% SDS. Gels were chopped into 2-mm segments directly from the electrophoresis tubes with a Gilson Aliquogel fractionator, and radioactivity in the gel sections was determined in Beckman Redisolv HP using a Packard Tri-Carb liquid scintillation spectrometer.

Protein markers were run each time a series of gels was subjected to electrophoresis. The marker proteins used were cytochrome c, RNase A, and carbonic anhydrase, possessing molecular weights of 1.17 x 10⁶, 1.37 x 10⁴, and 3.40 x 10⁴, respectively. Marker gels were stained with 0.1% Coomassie blue in 7.5% acetic acid-50% methanol and destained in 7.5% acetic acid-50% methanol. Following destaining, the marker gels were scanned at 600 nm in a Beckman DU spectrophotometer equipped with a Gilford Model 2140 linear transport accessory utilizing a scanning aperture of 2.4 x 0.05 mm and a scanning rate of 1 cm/min.

6-[35S]TG was synthesized according to the method of
Morávek and Nejedly (11). 6-TG was dried at 110° in a vacuum over P2O5 for several hr. To a suspension of 500 mg (2.99 moles) of 6-TG in 200 ml of dry pyridine, which had been refluxing for 3 hr, were added 10 mCi (1 to 20 mCi/mg) of elemental 35S in benzene solution. The vial containing the 35S was rinsed 5 to 7 times with pyridine, and these washes were added to the refluxing solution. The suspension was refluxed (protected by a calcium chloride tube) for 3.5 hr. The pyridine was removed in a vacuum. The residue was resuspended in toluene, and this was removed in a vacuum to eliminate all pyridine from the product. The dry residue was recrystallized from 480 ml of water after decolorization with charcoal and concentration of the filtrate to 150 ml. The crystals were collected after 48 hr of refrigeration and dried with air and acetone. The yield was 115 mg (23%) of 6-[^35S]TG (3 mCi/mmol).

For measurement of the incorporation of 6-TG into RNA, rats were given 6[^35S]TG at 40 mg/kg body weight 12 hr after partial hepatectomy. Six hr later the rats were sacrificed by decapitation, and their livers were excised and homogenized in 3 volumes of 0.32 M sucrose-3 mM MgCl2. Nuclear poly(A) RNA and poly(A)− RNA were isolated as described previously (4). Cytoplasmic RNA was isolated from the 700 x g supernatant solution after centrifugation to remove the mitochondria and precipitation with 95% ethanol-2% potassium acetate at -20° overnight. The precipitate was dissolved in 10 mM sodium acetate, pH 5.1.0.14 M NaCl-0.01% sodium dextran sulfate-0.3% SDS, and the RNA was extracted and separated into its poly(A)+ and poly(A)− components as described (4). The absorbance at 260 and 280 nm was determined for each fraction. RNA fractions were then hydrolyzed at 37° for 1 hr in 0.3 N KOH. After neutralization with PCA, the hydrolysates were applied to Cellex-D anion-exchange columns (30 x 5 mm), equilibrated with 0.02 M Tris-HCl, pH 8.0. Following elution with 11 ml of 0.02 M Tris-HCl, pH 8.0, to remove 6-TG present as the free base, nucleotides were eluted with 2 ml of 1 N HCl, and radioactivity therein was determined using Beckman Redisolv HP in a Packard Tri-Carb liquid scintillation spectrometer.

The presence of 6-TG nucleotide in DNA was determined after an i.p. injection of 6[^35S]TG at 40 mg/kg body weight. Purified nuclei were isolated from 18- and 24-hr regenerating liver as described previously (4). The 50,000 x g pellet was resuspended in 1 ml of 0.32 M sucrose-3 mM MgCl2 and precipitated with 4.5 volumes of 0.5 N PCA. Following centrifugation at 270 x g for 10 min, the pellet was dissolved in 4 ml of 0.3 N KOH and heated at 37° for 1 hr to hydrolyze the DNA. Following the addition of 2.5 ml of 1.2 N PCA and centrifugation at 270 x g for 10 min, the pellet, which consisted of DNA and protein, was dispersed in 4 ml of 0.5 N PCA and heated to 70° for 20 min. The DNA hydrolysate was chilled and centrifuged at 270 x g for 10 min. Aliquots of the supernatant solution were taken for measurement of DNA concentration by the method of Burton (3) using calf thymus DNA as a standard. The remainder of the supernatant solutions were neutralized with KOH and chromatographed on Cellex-D anion-exchange resin; then, deoxynucleotide fractions collected, and radioactivity therein was determined as described above for RNA samples.

RESULTS

Typical gradient polyacrylamide gel profiles of peptides synthesized in the rat liver in vitro protein-synthesizing system as measured by the incorporation of [3H]leucine are shown in Chart 1. No difference was detected in the peptides synthesized in systems derived from either 18-hr partially hepatectomized control livers or from partially hepatectomized, 6-TG-treated livers.

However, when tyrosine was substituted for leucine as the radioactive amino acid used in the rat liver in vitro protein-synthesizing system, the peptide profiles on polyacrylamide gels shown in Chart 2 were obtained; in these studies as well as for those described in Chart 1 using leucine as the measure of proteins synthesized, both the polysomes and the cell sap were derived from the same liver. The data show that in one particular region (3 to 5 cm), corresponding to approximately M.W. 30,000, the synthesis of peptides was significantly depressed in the in vitro protein-synthesizing system derived from 6-TG-treated regenerating liver, relative to that from control regenerating liver. The average area under the affected peaks from nine 6-TG-derived systems was 52% of the average control area from 8 scans (p < 0.005). The reason for the amino acid specificity toward leucine and tyrosine in the action of 6-TG is unknown; presumably, it reflects the amino acid composition of the newly synthesized peptides, since the profiles of peptides synthesized in the presence of tyrosine and leucine differ markedly with respect to peak position and height.

![Chart 1](image-url)

Chart 1. Gradient polyacrylamide gel electrophoretic scans of products derived from the rat liver in vitro protein-synthesizing system using [3H]leucine as the tracer. 6-TG (○) was injected i.p. into rats 12 hr after partial hepatectomy at a dose of 40 mg/kg body weight. The control group (●) received an equivalent volume of 0.9% NaCl solution. At 18 hr after partial hepatectomy the rats were sacrificed, and the liver polysomes and cell sap were isolated. The polysomes and cell sap were added to an in vitro system containing pyruvate kinase, [3H]leucine, ATP, GTP, phosphoenolpyruvate, and an amino acid mixture lacking leucine as described under "Materials and Methods." Following incubation, peptides were subjected to electrophoresis on 4 to 30% linear gradient polyacrylamide gels. The gels were divided into 2-mm sections, and the radioactivity in each segment was determined. Carbonic anhydrase (molecular weight of 34,000) used as a standard migrated 4.1 cm.
In an effort to determine whether the polysome component or the cell sap component was responsible for the observed decrease by 6-TG in the synthesis of a peptide or peptides containing tyrosine, an experiment was performed in which polysomes from 18-hr control regenerating liver were added to an in vitro protein-synthesizing system containing cell sap from 18-hr 6-TG-treated regenerating liver. A polyacrylamide gel profile from this experiment is shown in Chart 3 (bottom). Profiles of the product(s) synthesized in the presence of polysomes from control regenerating liver and cell sap from drug-treated regenerating liver indicate that the 30,000-M.W. region was present apparently in unaltered form (compare to control profile; Chart 3, top).

The converse experiment, that is, the use of polysomes from 18-hr 6-TG-treated regenerating liver with cell sap from 18-hr control regenerating liver, was performed (Chart 4, top); the 6-TG gel profiles clearly demonstrated the induced inhibitory effect (Chart 4). From these experiments, it was concluded that the decrease in tyrosine-containing peptides synthesized in the in vitro system derived from 6-TG-treated regenerating liver was due to the polysome component rather than to the cell sap component.

To determine whether the observed effect might be due to a decrease in the quantity of specific mRNA's in polysomes from the livers of 6-TG-treated animals, a wheat germ in vitro protein-synthesizing system was used which allowed the addition of an equal quantity of poly(A)$^+$ RNA from the livers of 6-TG-treated or control rats to the incubation mix. The peptides synthesized in vitro were separated on gradient polyacrylamide gels, and representative duplicate radioactivity profiles of gels from preparations run with control and 6-TG poly(A)$^+$ RNA are shown in Chart 5. The areas under the affected peaks from 6-TG-derived systems were 30% greater than the areas under the peaks from control systems ($p < 0.005$), indicating that more total peptides were being synthesized from poly(A)$^+$ RNA from 6-TG-treated rats than from control poly(A)$^+$ RNA.

That this effect might be due to the presence of 6-TG in mRNA was shown by the finding that 6-TG was incorporated into all species of RNA as a nucleotide (Table 1). Much more 6-TG was incorporated into poly(A)$^+$ RNA than was incorporated into poly(A)$^-$ RNA under these conditions. This effect was particularly striking in the nucleus where poly(A)$^+$ RNA incorporated 50 times more 6-TG than did poly(A)$^-$ RNA. 6-TG was also incorporated into the DNA of regenerating rat liver. As expected, at 18 hr after partial hepatectomy, the liver DNA contained only $31 \pm 8$ pmoles of 6-TG in nucleotide form per mg of DNA. At 24 hr after partial hepatectomy, however, during peak synthesis of DNA in regenerating liver, DNA contained $494 \pm 55$ pmoles of 6-TG nucleotide per mg of DNA. Quantities of 6-TG equal to or greater than those incorporated into the nucleic acids were associated with these macromolecules, particularly poly(A)$^+$ RNA, demonstrating the need for isolation of 6-TG nucleotide from both DNA and RNA to insure that the antimetabolite was incorporated as part of the structure of these molecules. The dose of 6-TG used in these studies would appear to be reasonable, since the degree of incorporation into RNA correlates well with the cytotoxic level reported by Tidd and Paterson (18).

**DISCUSSION**

The previous report (4) demonstrated that 6-TG had major effects on the 1st wave of partial hepatectomy-induced en-

[Chart 2: Gradient polyacrylamide gel electrophoretic scans of products derived from the rat liver in vitro protein-synthesizing system using [H]$^+$tyrosine as the tracer. The data were obtained by procedures identical to those described in Chart 1 except that [H]$^+$tyrosine was used instead of leucine as the radioactive amino acid, and the cold amino acid mix lacked tyrosine instead of leucine.]

[Chart 3: Gradient polyacrylamide gel electrophoretic scans of products from a recombined rat liver in vitro protein-synthesizing system in which polysomes from untreated partially hepatectomized animals and cell sap from 6-TG-treated rats were used. The data were obtained by procedures identical to those described in Chart 2, except that in both cases polysomes were obtained from control partially hepatectomized rat liver and cell sap from either 6-TG-treated or untreated regenerating liver.]
zyme synthesis and DNA replication in regenerating rat liver and that the biosynthesis of poly(A)+RNA was depressed in 6-TG-treated regenerating liver before the synthesis of DNA. To provide evidence for a connection between these 2 phenomena, an in vitro protein-synthesizing system was used which allowed a close inspection of the translational process. A rat liver system was chosen for the initial studies because evidence is available which indicates that the initiation factors are species and, possibly, tissue specific (1, 19). The 2 major separable components of this system are the polysomes and the cell sap. The polysome portion consists of ribosomes, mRNA, bound tRNA, and bound factors. The cell sap component contains the majority of the tRNA, the aminoaacyl-tRNA synthetases, and the free factors (i.e., EF1, EF2, and R).

Gray and Rachmeler (6) have reported that the acceptor activity of tyrosyl-tRNA in E. coli is susceptible to the action of 6-TG and have equated this sensitivity with the incorporation of the analog into these tRNA molecules. For this reason, radioactive tyrosine was chosen as one of the labeled precursors to be used in the in vitro protein-synthesizing system. In addition, since leucine had been used to assess the effects of 6-TG on total protein synthesis in vivo (4), radioactive leucine was also used in the in vitro protein-synthesizing system. The profiles of peptides labeled in the presence of radioactive tyrosine and those labeled in the presence of radioactive leucine differed markedly, implying that different peptides (or groups of peptides) are being represented. These peptides appear to contain quite different amounts of these 2 amino acids, and some of the peptides high in tyrosine content are sensitive to the action of 6-TG, whereas those containing more leucine are not. Thus, in profiles of tyrosine-containing peptides synthesized in vitro from 6-TG-derived systems, a decreased tyrosine incorporation occurred into 1 particular region with no apparent shifting of the position of the peaks in that region. This result lends support to the concept, derived from studies in vivo (4), that the protein synthetic machinery per se is not altered by 6-TG, since at least 1 group of peptides (i.e., those high in leucine content) are apparently synthesized in the same fashion in the in vitro protein-synthesizing systems derived from the livers of 6-TG-treated and control rats.
At 12 hr after partial hepatectomy, rats were given 6-³⁵S[TG (3 mCi/m mole) at a dose of 40 mg/kg body weight. Six hr later, rats were sacrificed, and their liver RNA fractions were isolated and hydrolyzed in 0.3 N KOH. The neutralized hydrolysates were chromatographed on Cellex-D anion-exchange columns to separate 6-TG from its nucleotide, as described under "Materials and Methods."

The polysome component of the in vitro protein-synthesizing machinery appears to be responsible for the inhibitory effect of 6-TG seen in profiles of high-tyrosine-containing peptides synthesized in vitro. This implies that the ribosomes, the mRNA, or the bound factors are being affected by 6-TG, rather than the tRNA as reported for E. coli by Gray and Rachmeler (6). Although 6-TG, incorporated into certain tRNA molecules, may alter their amino acid acceptor activity, this effect does not appear to be significant in the in vitro protein-synthesizing system used in these experiments. Data presented in the previous paper (4), on the effects of 6-TG on the synthesis of poly(A)⁺ RNA in regenerating liver, indicate that the most likely site of action of 6-TG on the polysomes responsible for inhibition of protein synthesis in vitro is the mRNA.

Since the time at which 6-TG was administered to rats corresponded to a time after partial hepatectomy when a 2nd burst of RNA synthesis occurs (see, e.g., Ref. 16), it seemed reasonable to assume that at least some of the analog was incorporated into this newly synthesized RNA. This assumption was indeed true as demonstrated by the finding that 6-TG was incorporated into RNA at this time to a significant extent. Under the conditions used, the poly(A)⁺ RNA incorporated significantly more 6-TG per mg of RNA than did the poly(A)⁻ RNA. In addition, the poly(A)⁺ RNA fraction exhibited a greater response to the action of 6-TG in terms of diminished incorporation of radioactive orotic acid and decreased Amax than did the poly(A)⁻ RNA (4).

In the regenerating liver, like other cell systems, significantly more 6-TG was found in the RNA fraction as compared to the DNA fraction, although 6-TG nucleotide was found in both DNA and RNA. Although the method used to measure 6-TG incorporation into nucleic acids eliminates the contribution of binding of the base or nucleoside to the DNA and RNA, it does not differentiate between labeled drug present in internucleotide linkage from any 6-TG nucleotide bound to the nucleic acid fractions.

The results obtained by use of a wheat germ in vitro protein-synthesizing system indicate a possible additional effect of 6-TG on mRNA. Because the wheat germ transla-

### Table 1

**Incorporation of 6-³⁵S[TG into RNA of regenerating liver**

<table>
<thead>
<tr>
<th>RNA source</th>
<th>RNA fraction</th>
<th>pmoles/mg²</th>
</tr>
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<tbody>
<tr>
<td>Nucleus</td>
<td>Poly(A)⁻</td>
<td>142 ± 57 (4)²</td>
</tr>
<tr>
<td></td>
<td>Poly(A)⁺</td>
<td>37 ± 11 (6)²</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Poly(A)⁻</td>
<td>142 ± 57 (4)²</td>
</tr>
<tr>
<td></td>
<td>Poly(A)⁺</td>
<td>37 ± 11 (6)²</td>
</tr>
</tbody>
</table>

² Poly(A)⁺ and poly(A)⁻ RNA were isolated from total nuclear and cytoplasmic RNA on polyuridylate-Sepharose as described previously (4).

³ Incorporation of 6-TG is expressed as pmoles 6-TG occurring as the nucleotide per mg of RNA ± S.E.

⁴ Numbers in parentheses, number of animals in each determination.

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REFERENCES


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