Inhibition of Ribonucleic Acid Polymerase by 5-Hydroxyuridine 5'-Triphosphate*

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SUMMARY

5-Hydroxyuridine triphosphate was synthesized and its effectiveness as substrate for deoxyribonucleic acid-dependent ribonucleic acid polymerase was determined. The analogue was incorporated into RNA to a very low extent as compared to naturally occurring nucleoside triphosphates, and it replaced uridine triphosphate to a much greater extent than any of the other nucleoside triphosphates. The ability of the analogue to replace UTP in RNA synthesis was greatest at pH 7 and decreased markedly with increasing pH values. This behavior was related to the low pK, of 5-hydroxyuridine as compared to uridine.

5-Hydroxy-UTP strongly inhibited synthesis of RNA and acted as a competitive inhibitor of UTP in the polymerase reaction. This inhibitory effect was lowest at pH 7.0 and increased with increasing pH values to a maximum at pH 9.0. Thus, in contrast to the data on analogue incorporation, the inhibitory effect of the analogue is predominant when the pyrimidine exists in ionized form.

The nucleoside analogue exerted its strong inhibitory effect only when it existed as the triphosphate. 5-Hydroxy-UDP and 5-hydroxy-UMP inhibited incorporation of UMP into RNA only slightly as compared to 5-hydroxy-UTP.

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The uridine derivative 5-hydroxyuridine, undergoes most of the reactions of uridine, including conversion to 5-hydroxyuridine diphosphoglucoce and minor incorporation into RNA (1). The inhibitory effect of 5-hydroxyuridine on nucleic acid metabolism (2) and bacterial, viral, and tumor growth (3-5) has been explained in part by the demonstration that one of the phosphorylated derivatives, 5-hydroxyuridine 5'-monophosphate, strongly inhibits orotidyl acid decahydrogenase (1). Additional sites of inhibition are suggested by the demonstration that 5-phosphorylated analogues, 5-hydroxy-UTP and 5-hydroxyuridine triphosphate, were incorporated into RNA only slightly as compared to 5-hydroxy-UTP.

In the present report the results of these studies with the use of a polymerase isolated from Escherichia coli are described.

Incorporation of 5-HO-UMP into RNA and inhibition of UMP incorporation into RNA by 5-HO-UTP are described. Differences in these effects produced by changes in pH are discussed in relationship to the influence of the 5-hydroxyl substituent of the pyrimidine ring.

EXPERIMENTAL PROCEDURE

Materials—UTP-2-14C and ATP-8-W were purchased from Schwarz BioResearch. Calf thymus DNA, ATP, GTP, CTP, UTP, and spermidine-HCl were obtained from Calbiochem.

The E. coli cells used were harvested as described by Forth et al. (8) or were obtained as frozen cells harvested in early log phase from General Biochemicals.

Preparation and Properties of 5-HO-Urd Derivatives—5-HO-UTP was prepared by a modification of the method described by Ueda (9). A slight excess of bromine (5 ml of bromine and bromine water) was added to 500 mg of UTP (0.80 mmole) in 2 ml of water at 0°. After removal of excess bromine, 7 ml of pyridine were added, and the solution was maintained at 37° for 24 hours. The yellow solution was adjusted to pH 8.5 with 0.1 N NH₄OH and passed through a column (2 × 40 cm) of DEAE-cellulose (bicarbonate). Pyridine was removed by washing with water, and the products were eluted with a 0.2 M triethylammonium bicarbonate gradient. The major products, 5-HO-UTP and 5-Br-UTP, emerged from the column in the order mentioned, but they were not always completely separated. 5-HO-UTP was recognized by its characteristic A₂₆₀: A₂₈₀ ratio of 1.0 at pH 12 and 1.8 at pH 2.0, whereas the corresponding ratios for 5-Br-UTP are 1.3 and 1.5. Usually, the first one-third of the total optical density units eluted from the column in the nucleoside triphosphate fractions was found to be 5-HO-UTP free of 5-Br-UTP as ascertained by descending paper chromatography in ethanol-0.5 M ammonium acetate, pH 7.5. (Rₖ values: 5-HO-UTP, 0.20; 5-Br-UTP, 0.30.) Fractions containing mixtures of 5-Br-UTP and 5-HO-UTP were combined and separated by repeating the ion exchange chromatography on DEAE-cellulose (bicarbonate) as described above. The combined 5-hydroxyuridine diphosphate; 5-HO-UTP, 5-hydroxyuridine triphosphate; Br-, bromo.
5-HO-UTP fractions were lyophilized, dissolved in a small quantity of water, and converted to the sodium salt by passing through a column of Dowex 50 resin (Na⁺ form). This solution was lyophilized and kept at −20°C until used. The yield was about 23%.

5-HO-UTP-2⁻¹⁴C, 5-HO-UDP, and 5-HO-UMP were prepared similarly starting from UTP-2⁻¹⁴C, UDP, and UMP, respectively, except that 5-HO-UMP was purified by chromatography on Dowex 1-formate resin.

The pH₅₀ value of 5-HO-Urd was 7.8 as determined by electrophoretic titrations with a Radiometer type TTT11e autotitrator and a Radiometer Titirgraph. Insufficient 5-HO-UTP was available for determination of its corresponding pH₅₀ value, but it may be assumed that this value is not significantly different from that of the nucleoside (10, 11).

Criteria for 5-HO-UTP Purity—The presence of a 5-hydroxyuracil moiety was verified by the production of a purple color with ferric chloride (12). Acid-labile phosphorus was determined as inorganic orthophosphate (13) following treatment with 1 N HCl at 100°C for 15 min. Acid-stable phosphorus was calculated by subtracting acid-labile phosphorus from total phosphorus formed on ashing. The molar ratio of acid-labile phosphorus to acid-stable phosphorus was 2.1. Characterization as a nucleoside triphosphate was also established by the fact that the 5-HO-UTP migrates to approximately the same position as UTP or 5-Br-UTP on electrophoresis at 2500 volts in 0.075 M sodium acetate, pH 4.0.

The probable contaminants in the 5-HO-UTP preparations are 5-Br-UTP and UTP. The presence of these impurities was excluded by use of descending paper chromatography with the ethanol-ammonium acetate solvent system. The product was shown to be completely separated from 5 Br UTP or UTP when the chromatogram was allowed to develop for 30 hours. The 5-HO-UTP used in the experiments migrated as a single ultraviolet-absorbing area which produced a purple color with ferric chloride solution. No ultraviolet-absorbing material in the area corresponding to 5-Br-UTP or UTP was detected.

5-HO-UTP-⁴⁷Ca was chromatographed on paper with unlabeled 5-HO-UTP as described above. About 99% of the radioactivity was found to be associated with the ultraviolet-absorbing region corresponding to 5-HO-UTP, and about 1% migrated as UTP or 5-Br-UTP on the chromatogram. The possibility that these contaminants contributed to the low level of incorporation of radioactive activity into RNA was excluded by identification of 5-hydroxyuridine 2'(3')-phosphate-¹⁴C as the only radioactive constituent in an RNA hydrolysate. In addition, the strong inhibitory effect of 5-HO-UTP upon the incorporation of UMP (and presumably 5-Br-UMP, since it substitutes for UMP in the polymerase reaction (10)) would be expected to prevent incorporation of isotope from a 1% contamination of either UTP or 5 Br UTP.

Detection of 5-HO-Urd 2'(3')-Monophosphate in RNA Hydrolysate—RNA was isolated (14) from a 10-ml reaction mixture incubated under conditions described in Experiment 3, Table II. Carrier 5-HO-Urd 2'(3')-monophosphate (1) was added to the alkaline hydrolysate (14), and the acid-soluble components were separated by paper chromatography in an ethanol-ammonium acetate solvent system as described previously. Radioactivity was associated only with the ultraviolet-absorbing band corresponding to 5-HO-Urd 2'(3')-monophosphate. Radioactivity was not detected in areas corresponding to 5-Ho-Urd 2'(3')-monophosphate or Urd 2'(3')-monophosphate (Rₚ values of the nucleotides: 5-HO-Urd 2'(3')-monophosphate, 0.42; 5-Br-Urd 2'(3')-monophosphate, 0.50; Urd 2'(3')-monophosphate, 0.50).

Preparation and Assay of RNA Polymerase—DNA-dependent RNA polymerase was prepared from E. coli W by Procedure B described by Furth, Hurwitz, and Anders (14) and was stored at −20°C in 50% glycerol (15). Incorporation of the isotope from radioactive nucleoside triphosphates into the acid-insoluble product was used as a measure of RNA polymerase activity. The reaction mixture, 0.5 ml, contained 40 µM labeled ribonucleoside triphosphate; the other three ribonucleoside triphosphates, each at 80 µM; 1 mM MgCl₂; 2 mM MnCl₂; 50 mM Tris buffer, pH 7.5; 2 mM mercaptoethanol; 70 µg of calf thymus DNA (0.9 optical density at 260 mµ); and enzyme. Prior to use, the enzyme was diluted with a solution containing 2 mg of crystalline bovine serum albumin per ml and 1 mM mercaptoethanol. In some experiments, spermidine at 2 mM was used (16). After incubation for 20 min at 38°C, the reaction was stopped by addition of 2 ml of 5% trichloracetic acid at 0°C. The filters were placed in aluminum planchets and dried with gentle heat under an infrared lamp. Radioactivity was measured in a Micromil end window, gas flow counter. Data given in the figures and tables, except for those given in Table II, were calculated after subtraction of blank values obtained when DNA or enzyme was omitted from the reaction mixtures. The blank values varied from 20 to 26 cpm. The enzyme used contained from 500 to 1000 units of activity (14). Each table and graph represent data obtained from a single enzyme preparation.

RESULTS AND DISCUSSION

Incorporation of 5-HO-UMP into RNA—The extent to which 5-HO-UTP replaced UTP in the synthesis of RNA by DNA-dependent RNA polymerase is shown in Fig. 1. Incorporation of the analogue was about 5% as compared to a control containing UTP-²¹⁴C, GTP, ATP, and CTP. This inefficient utilization of 5-HO-UTP is consistent with the previous finding that 5-HO-Urd is incorporated into RNA of Ehrlich ascites cells to a minor extent even though a facile conversion of the nucleoside to 5-HO-UTP occurs (1). This behavior is in contrast to UTP, ribothymidine 5'-triphosphate, 5-fluorouridine triphosphate, and 5-Br-UTP, which are utilized by RNA polymerase with an efficiency of 30% or more when one of these derivatives replaces UTP (10, 17, 18).

Kahan and Hurwitz (10) have correlated dissociation constants of purines and pyrimidines with the capacity of corresponding nucleoside triphosphate analogues to replace their respective natural substrates. Their data support the concept that bases bearing dissociated acidic radicals are not incorporated into polynucleotides with RNA polymerase even though they possess hydrogen-bonding groups at the same positions as the natural purines and pyrimidines they replace. Thus, the dissociation constants of pyrimidine analogues directly determine their effectiveness as substrates for RNA synthesis. Since the pH₅₀ of 5-HO-Urd is 7.8, the low level of incorporation of 5-HO-UMP into RNA at pH 7.5 can be explained, in part, by the failure of the polymerase to utilize the ionized form of the analogue. This conclusion is substantiated by the data of Table I, which show
TABLE I

Effect of pH on incorporation of UMP and 5-HO-UMP into RNA

Each incubation mixture contained 20 mM UTP-14C or 20 mM 5-HO-UTP-14C; ATP, GTP, and CTP, each at 80 µM; and 50 mM Tris buffer at pH 7.5, 8.0, 9.0, or 9.5. The pH values of the incubation mixtures are indicated in the table. Incubation was carried out for 20 min at 38°. Other conditions were as described in Fig. 1.

<table>
<thead>
<tr>
<th>Nucleoside triphosphate added</th>
<th>Nucleotide incorporation (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP, GTP, CTP, UTP-14C........</td>
<td>pH 7.0  1.75    2.02    2.17  1.52   1.28</td>
</tr>
<tr>
<td>ATP, GTP, CTP, 5-HO-UTP-14C...</td>
<td>pH 7.5  0.16    0.00    0.07  0.02   0.02</td>
</tr>
</tbody>
</table>

that 5-HO-UMP is incorporated into RNA optimally at pH 7.0 and not at all when the analogue exists predominately in ionized form at pH 8.6 or pH 9.0. In contrast, UMP was incorporated optimally at about pH 8.0, and was utilized with greater than 88% maximum efficiency at pH 9.0.

Since it was desirable to obtain RNA containing a sufficiently high concentration of 5-HO-UMP for its detection in the polymerase, the incubation time was increased to 1 hour at pH 7.0. At these conditions, the analogue was incorporated into RNA with an efficiency of about 20%, as compared to a control containing UTP-14C (Fig. 2). Detection of radioactive 5-HO-Urd 2'-3'-phosphate in an alkaline hydrolysate (14) of the enzymatically synthesized RNA showed that the analogue was incorporated into polynucleotide by the polymerase.

Conditions (pH 7.0; incubation time, 1 hour) which allowed maximal incorporation of the analogue into RNA were also used to compare the degree of stimulation of RNA synthesis produced by 5-HO-UTP-14C in the absence of each of the four natural nucleoside triphosphates. In the absence of UTP, the analogue was incorporated into RNA with an efficiency of about 20%, as compared to UMP-14C incorporation (Table II, Experiments 1 and 3). Similar results were obtained with ATP-14C as the labeled substrate. Incorporation of isotope from 5-HO-UTP-14C in the absence of ATP, GTP, or CTP was less than 5%, as compared to a control (Table II). Thus, of the four natural substances, the analogue replaced UTP most efficiently.

The data (Table II) also show that the requirements for RNA synthesis by RNA polymerase used in these experiments are identical with those reported previously (14, 16). Incorporation of isotope from nucleoside triphosphates into acid-insoluble product did not occur when RNase was included in the reaction mixture. Radioactivity incorporated into an acid-insoluble product formed in the complete system became acid-soluble by treatment with RNase or alkali.

Inhibition of UMP-14C Incorporation into RNA by 5-HO-UTP —The inhibitory effect of 5-HO-UTP on incorporation of UMP-14C into RNA is shown in Fig. 3. The presence of an equimolar concentration of 5-HO UTP and UTP-14C depressed incorporation of UMP-14C into RNA by more than 70%. A 4-fold molar excess of 5-HO-UTP produced almost complete inhibition. In contrast, inhibitory effects were slight in the presence of 5-HO-UMP or 5-HO-UDP (Table III). Thus, it is apparent that the nucleoside analogue must exist as the triphosphate to exert a strong inhibitory effect.

A more detailed investigation of the nature of this inhibitory effect of 5-HO-UTP compared initial rates of UMP incorporation
TABLE II
Capacity of HO-UTP to replace UTP, CTP, ATP, or GTP in RNA synthesis

Each incubation mixture, 0.5 ml, contained 80 μM of each of the following nucleoside triphosphates indicated to be present in each experiment: ATP, GTP, CTP, 5-HO-UTP, UTP-2-W (6.1 × 10^4 cpm per μmole), ATP-8-W (6.5 × 10^4 cpm per μmole), and 5-HO-UTP-2-i% (1.1 × 10^5 cpm per μmole). Tris buffer (50 mM) at pH 7.0 was used. Incubation was carried out for 1 hour at 38°C. Other conditions were as described in Fig. 1. Experiments 10, 11, and 12 were carried out at pH 7.5 with a different preparation of RNA polymerase.

<table>
<thead>
<tr>
<th>Experiment and nucleoside triphosphate added</th>
<th>Nucleotide incorporated (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. UTP*, ATP, GTP, CTP</td>
<td>4.12</td>
</tr>
<tr>
<td>2. As in Experiment 1 minus DNA or enzyme</td>
<td>0.02</td>
</tr>
<tr>
<td>3. 5-HO-UTP*, ATP, GTP, CTP</td>
<td>0.86</td>
</tr>
<tr>
<td>4. 5-HO-UTP*, ATP, GTP, UTP</td>
<td>0.07</td>
</tr>
<tr>
<td>5. 5-HO-UTP*, ATP, CTP, UTP</td>
<td>0.19</td>
</tr>
<tr>
<td>6. 5-HO-UTP*, CTP, GTP, UTP</td>
<td>0.20</td>
</tr>
<tr>
<td>7. As in Experiment 3 minus enzyme</td>
<td>0.03</td>
</tr>
<tr>
<td>8. As in Experiment 3 minus DNA</td>
<td>0.03</td>
</tr>
<tr>
<td>9. As in Experiment 3 plus RNase</td>
<td>0.05</td>
</tr>
<tr>
<td>10. ATP*, UTP, GTP, CTP</td>
<td>2.16†</td>
</tr>
<tr>
<td>11. As in Experiment 10 minus UTP</td>
<td>0.16†</td>
</tr>
<tr>
<td>12. ATP* plus CTP, GTP, 5-HO-UTP</td>
<td>0.34†</td>
</tr>
</tbody>
</table>

* 1Ci-Labeled nucleoside triphosphate.
† Incubations were carried out for 20 min.

5-HO-UTP inhibited incorporation of isotope from UTP-14C into RNA to a greater extent than UTP inhibited utilization of 5-HO-UTP-14C. Incorporation of 5-HO-UMP-14C into RNA was inhibited only 28% by an equimolar concentration of UTP (Table IV). These data suggest that the analogue has a strong affinity for polymerase or a polymerase-DNA complex (19).

The influence of pH upon 5-HO-UMP incorporation into RNA, in a manner which reflects the low pKₐ of 5-HO-Urd as compared to Urd, led to studies of the effect of pH on the inhibition by 5-HO-UTP. The results show that the inhibitory effect of 5-HO-UTP on UMP-14C incorporation into RNA increased with increasing pH values (Table V). At pH 9.0, 5-HO-UTP decreased UMP incorporation into RNA to about 20% of the values obtained in the absence of analogue. At pH 7.0, UMP was incorporated to about 58% of the control at the same molar ratio (1:2) of analogue to UTP-14C.

TABLE III
Effect of 5-HO-UMP, 5-HO-UDP, and 5-HO-UTP on RNA synthesis

The conditions of the reaction mixture were the same as described in Fig. 1, except that any one of 5-hydroxyuridine mono-, di-, or triphosphates were present and their concentrations were varied as indicated. UTP-2-14C (80 μM) and Tris buffer at 8.0 (50 mM) were used.

<table>
<thead>
<tr>
<th>Addition of analogues</th>
<th>Synthesis rate in presence of nucleotide analogues* (μmoles)</th>
<th>% control</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HO-UMP</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5-HO-UDP</td>
<td>94</td>
<td>91</td>
</tr>
<tr>
<td>5-HO-UTP</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>0</td>
<td>81</td>
<td>82</td>
</tr>
<tr>
<td>20</td>
<td>73</td>
<td>73</td>
</tr>
</tbody>
</table>

* Control in the absence of 5-HO-Urd derivatives incorporated 1.7 μmoles of UTP into RNA. This value was taken as 100% RNA synthesis.

FIG. 3. Effect of 5-HO-UTP on incorporation of UMP-14C into RNA. The reaction mixtures were the same as described in Fig. 1 except that 80 μM UTP-2-14C was present and the concentration of 5-HO-UTP was varied as indicated. Tris buffer (50 mM) at pH 7.5 or 8.0 was used.

in the presence of 40 μM 5-HO-UTP and various concentrations of UTP-14C. Analysis of the data according to Lineweaver and Burk (Fig. 4) suggests that the inhibition is competitive under these experimental conditions. Results similar to those shown in Fig. 4 were obtained with 20 μM 5-HO-UTP and a different enzyme preparation. The Kₘ calculated for UTP was 1.2 × 10⁻³ M, a value similar to those reported previously (16, 18).

FIG. 4. Plot of the reciprocal of the rate of UMP-14C incorporation (V) against the reciprocal of the molar concentration of UTP (S) in the presence and absence of 5-HO-UTP. The conditions were as described in Fig. 1 except that the concentration of UTP-2-14C was varied as indicated and 5-HO-UTP was present at 40 μM concentration.
Since 5-HO-Urd is not stable at high pH values, a control experiment was conducted to determine whether or not the increase in the inhibitory effect of 5-HO-UTP on RNA synthesis with increasing pH values was due to degradation to inhibitory products. No change in absorptivity of light at 260 nm or 260 nm occurred when a solution of 5-HO-Urd was maintained at room temperature in a buffer at pH 9 for 18 hours. Thus, degradation was excluded, since it was established previously that 5-HO-UMP is converted to compounds which do not absorb light at 260 nm after treatment with alkali (1).

In addition to the usual complexities of enzyme-catalyzed reactions, RNA synthesis by DNA-dependent RNA polymerase is complicated by the requirement for hydrogen bond formation, as well as dependence upon the simultaneous presence of four substrates for enzyme binding. Therefore, more than the usual number of mechanisms can be invoked to explain the inhibitory effect of 5-HO-UTP on RNA synthesis in the presence of UTP, CTP, ATP, and GTP. It does not appear likely that the inhibitory effect of 5-HO-UTP is due primarily to its ability to act as an alternate substrate. First, the analogue is a very inefficient substitute for UTP in RNA synthesis (Fig. 1), whereas it acts as a strong inhibitor of UMP incorporation into RNA (Fig. 3). Second, if 5-HO-UTP inhibits UMP incorporation primarily as an alternate substrate, it would be expected that inhibition would be more pronounced at the optimum pH for 5-HO-UMP incorporation. As can be seen from the data of Table V, the reverse is true. The inhibitory effect of 5-HO-UTP, at two different concentrations, as increased as the pH was raised, the greatest inhibition occurring at pH values above the pKa of 5-HO-Urd. Similarly, Fig. 3 shows a consistently greater degree of inhibition at pH 8.0 than at pH 7.5 at five different concentrations of analogue. Thus, although the influence of the 5-hydroxyl substituent on the pKa of the pyrimidine accounts, in part, for the low level of incorporation at the pH optimum of RNA polymerase, the inhibitory effect of 5-HO-UTP is not related directly to this inductive effect. Quantitative studies on the relationship between inhibitory effects and the concentration of the ionized form of 5-HO-UTP were not carried out; however, the data indicate that the ionized form is the more effective inhibitor.

The behavior of 5-HO-UTP in the polymerase reaction is distinctly different from other nucleoside triphosphate analogues which have been investigated. Kahan and Hurwitz (10) have reported that 6-azauridine triphosphate does not permit significant incorporation of ribonucleotides into RNA in the absence of any one of the natural substrates. However, in contrast to 5-HO-UTP, 6-azauridine triphosphate does not inhibit RNA synthesis when it is added to reaction mixtures containing all four natural substrates. Other nucleoside analogues, such as γ-UTP (17, 18), ribothymidine 5'-triphosphate (10), and 5,6-dihydro-UTP (20), are more efficiently utilized for RNA synthesis than is 5-HO-UTP. Unlike 5-HO-UTP, the inhibitory effect of these derivatives in the presence of all four natural substrates can be explained largely by their ability to act as alternate substrates for UTP. These differences, in context with the inability of poly-5-HO-UMP to direct phenylalanine polymerization, suggest that the 5-hydroxyuracil moiety may form a hybrid base pair with RNA or DNA bases, conceivably involving the 5-hydroxyl substituent. Whatever the detailed mechanism of the inhibitory effect may be, the data are consistent with a conclusion that the analogue has a strong affinity for RNA polymerase or a polymerase-DNA complex (19). The resulting complex does not readily participate in polyribonucleotide synthesis and inhibits RNA synthesis in the presence of all four natural nucleoside triphosphates.

### Enzymatic Sites of Inhibition Produced by 5-HO-Urd

The metabolism of 5-HO-Urd and known inhibitory effects of phosphorylated derivatives of the nucleoside analogue (1, 6) may be summarized as shown in Scheme 1, in which OMP represents orotidine 5'-phosphate.

![Scheme 1](http://www.jbc.org/)
The present finding that 5-HO UTP inhibits incorporation of UTP by DNA-dependent RNA polymerase provides another major explanation for the inhibition of RNA synthesis (2) by 5-HO-Urd in Ehrlich ascites cells. Although 5-HO-UMP strongly inhibits decarboxylation of orotidine 5'-phosphate, the concentration of 5-HO-UTP in whole cells incubated in the presence of 5-HO-Urd is high as compared to 5-HO-UMP. Thus, inhibition of either the polymerase or the orotidine 5'-phosphate decarboxylase reaction may conceivably predominate depending upon environmental conditions.

The demonstration that 5-hydroxyuracil analogues are incorporated to a minor extent into RNA also explains the impairment of protein synthesis by the analogue (1, 2). It is known that poly-5-HO-UMP is an inefficient (7) or completely ineffective (6) polynucleotide in its ability to direct phenylalanine polymerization. This is in contrast to other analogues which are incorporated into RNA (7, 10, 17, 21, 22). Thus, even though incorporation of 5-HO-UMP into RNA is not extensive (1), a small percentage of the analogue present in messenger RNA may have an inhibitory effect on protein synthesis.

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