Regulation of Mammalian Deoxyribonucleotide Biosynthesis by Nucleotides as Activators and Inhibitors*

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SUMMARY

The effects of several nucleoside triphosphates as activators and inhibitors of the enzymatic reduction of ribonucleoside triphosphates to deoxynucleotides was studied. The enzyme was a partially purified preparation of the ribonucleotide reductase system from the Novikoff ascites rat tumor, with dithioerythritol used as a model reducing agent. The reduction of cytidine diphosphate and uridine diphosphate to the corresponding deoxyribonucleotides required activation of the enzyme by adenosine triphosphate; reduction of guanosine diphosphate required activation by low concentrations of deoxyxymidodine triphosphate with ATP giving further stimulation; and reduction of ADP required activation by low concentrations of dGTP or dTTP. Reduction of GDP was inhibited by dGTP and dATP; reduction of GDP was inhibited by dGTP and dATP; and reduction of ADP was inhibited by dATP. Only slight inhibition by dCTP was seen. The results are interpreted in terms of metabolic control of the biosynthesis of deoxynucleotides by these nucleoside triphosphates serving as regulatory effectors.

The fact that deoxyribonucleotides are present in most tissues only in very small amounts (1) has led to the suggestion that the step in which they are synthesized would be a suitable locus for the regulation of deoxyribonucleic acid synthesis (2, 3). Reichard, Canellakis, and Canellakis (3), in a study of such regulation in a chick embryo extract, found that formation of the nucleotides of deoxyctydine and deoxyguanosine by reduction of ribonucleotides was inhibited by purine deoxyribonucleotide triphosphates, and that deoxyguanosine nucleotide formation was stimulated about 75% by deoxythymidine triphosphate. The previously reported requirement for adenosine triphosphate to constitute an almost absolute requirement for the reaction. The previously reported requirement for adenosine triphosphate for the reduction of pyrimidine ribonucleotides has also been reinvestigated and found to be an activation effect. A part of this work has been reported orally (4).

This enzyme preparation, in the presence of a suitable activator, magnesium ion and a disulfhydryl reducing agent, is able to convert each of the four common ribonucleoside diphosphates (ADP, CDP, GDP, and uridine diphosphate) to the corresponding deoxyribonucleotide without rupture of the glycoside linkage. The exact mechanism of the reaction and the fate of the oxygen atom are not known. The physiological reducing agent from rat tissue, presumably a protein (4), has not been completely characterized; in most of these experiments the model compound dithioerythritol was used as the reducing agent.

MATERIALS AND METHODS

dUTP was prepared by deamination of dCTP with nitrous acid and was purified on Dowex 1-formate. Other nucleotides were commercial products. CDP labeled with 3H was prepared in this laboratory. It was used at a specific activity of 1 to 5 x 10^6 cpm per pmole. ADP-8-14C, GDP-8-14C, ATP-8-14C, and UDP-2-14C were purchased from Schwarz BioResearch and diluted to about 4 x 10^4 cpm per pmole.

The ribonucleotide reductase preparation was made from Novikoff rat hepatoma extract by precipitation at pH 5 and chromatography on DEAE-cellulose essentially as was described previously (4). It contained a significant amount of nucleoside diphosphate kinase. Twelve different batches were used in these experiments, with specific activities (for reduction of CDP), ranging from 6 to 34 pmammole per mg of protein per 30 min.

The standard incubation mixture, adapted from that used previously (4), contained, in a total volume of 0.12 ml, 1 pmole of potassium phosphate, pH 7; 0.5 pmole of magnesium acetate; 0.005 pmole of ferric chloride; and 0.75 pmole of dithioerythritol. Standard amounts of substrate and activator were either 0.02 pmole of CDP and 0.25 pmole of ATP; 0.05 pmole of UDP and 0.25 pmole of ATP; or 0.05 pmole of GDP and 0.013 pmole of TTP, and sometimes 0.05 pmole of ATP. Inhibitors were included as described in the individual experiments. In a few experiments, TPNH and the thioredoxin system from E. coli (4) or the comparable partially purified system from the Novikoff tumor or reduced lipoic acid.
were used instead of dithioerythritol, and, occasionally, Tris-chloride buffer was used instead of phosphate. These changes are noted in the graphs and tables.

Reduction of $^{14}$C-labeled ribonucleotides was determined by hydrolysis of the product and measurement of the label in the deoxynucleoside. At the end of the incubation with the $^{14}$C-labeled nucleotides, the reaction was stopped by heating for 2 min in boiling water, about 0.2 μmole of carrier deoxynucleoside was added, and the nucleotides were dephosphorylated by successive incubation for 15 min each with 10 μl of a potato apyrase preparation (7) and 5 μl (about 2.5 units) of bacterial alkaline phosphatase (Worthington BAP-C, diluted 1:2) with 5 μl of 0.5 M Tris, pH 8.5. The mixture was heated again for 2 min and centrifuged, and the nucleotides and deoxyribonucleoside were separated by paper chromatography of a 50-μl sample on Whatman No. 1 paper in the borate system of Reichard (8). For better separation of adenine from deoxyadenosine, the ethanol concentration was decreased to one-third the usual amount in the experiments on ADP reduction. The spots were cut out and counted directly in a Packard Tri-Carb liquid scintillation counter in 2,5 diphenyloxazole-1,4-bis[2 (5 phenyl oxazolyl)]benzene-toluene solution. Reduction of $^{32}$P-CDP was assayed as described previously (4). Results are expressed as millimicromoles of deoxynucleotide formed per incubation tube in 30 min unless stated otherwise.

**RESULTS**

**Activation Effects**

**Reduction of GDP**—Fig. 1 shows the effect of different concentrations of dTTP, in the presence and absence of ATP, on the reduction of GDP to deoxyguanosine nucleotides by the purified nucleotide reductase and dithioerythritol. Low concentrations of dTTP were absolutely required for the reduction; less than 10% of the maximum reduction of GDP was observed in the absence of added dTTP. In the absence of ATP, the concentration of dTTP required for maximum activation was about 0.01 mM. Certain concentrations of ATP augmented the action of dTTP but did not stimulate in the absence of dTTP. In the presence of 2 mM ATP (which is optimal for reduction of the pyrimidine nucleoside diphosphates), the optimum concentration of dTTP was increased by a factor of 100. Excess dTTP or excess ATP inhibited reduction of GDP in both cases. The data in Fig. 1 were obtained in a number of separate experiments, and so the activity is expressed in terms of percentage of the maximum for each experiment. Fig. 2 gives a more direct comparison from a single experiment and shows again that ATP can either stimulate or inhibit the reduction of GDP, depending on the dTTP concentration. At 0.1 mM dTTP, addition of 0.4 mM ATP stimulated maximally while 2 mM ATP inhibited. At higher concentrations of dTTP (not shown), 2 mM ATP was
**Table I**

Effectiveness of various nucleotides as activators of GDP reduction

<table>
<thead>
<tr>
<th>Activator</th>
<th>Deoxyguanosine formed (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.01</td>
</tr>
<tr>
<td>dTTP, 0.008 mM</td>
<td>2.76</td>
</tr>
<tr>
<td>dTTP, 0.08 mM</td>
<td>2.62</td>
</tr>
<tr>
<td>dTDP, 0.008 mM</td>
<td>2.44</td>
</tr>
<tr>
<td>dTDP, 0.08 mM</td>
<td>2.64</td>
</tr>
<tr>
<td>dTMP, 0.008 mM</td>
<td>0.57</td>
</tr>
<tr>
<td>dUTP, 0.008 mM</td>
<td>1.12</td>
</tr>
<tr>
<td>dUTP, 0.08 mM</td>
<td>1.70</td>
</tr>
<tr>
<td>dCTP, 0.002 mM</td>
<td>0.12</td>
</tr>
<tr>
<td>dATP, 0.008 mM</td>
<td>0.08</td>
</tr>
<tr>
<td>dGTP, 0.002 mM</td>
<td>0.07</td>
</tr>
<tr>
<td>dGTP, 0.006 mM</td>
<td>0.10</td>
</tr>
<tr>
<td>ATP, 0.42 mM</td>
<td>0.11</td>
</tr>
<tr>
<td>ATP, 0.42 mM, + dTTP, 0.08 mM</td>
<td>4.24</td>
</tr>
</tbody>
</table>

In the absence of ATP, less than 0.1 μmole of dTTP was sufficient to activate the formation of 2.96 μmole of dGDP, thereby proving that transfer of deoxyribose did not play an important role.

Table I shows that even in the absence of ATP, dTDP was almost as effective as dTTP. At low concentrations, dUTP could replace dTTP as activator, but at higher concentrations (not shown), it inhibited more strongly than dTTP. Neither dGTP nor dATP replaced dTTP as activator; both inhibited the reduction in the presence of dTTP, at levels of about 0.04 and 0.004 mM, respectively. A very slight reduction of GDP (about 5% of maximum) was observed when dCTP was substituted for dTTP.

**Reduction of ADP**—The reduction of ADP required activation by either dGTP or dTTP, with dGTP being much more effective. Fig. 3 shows the activating effects of different concentrations of dGTP, dGDP, and dTTP. In the absence of activator, no reduction was obtained in this experiment (compare Fig. 4). In the presence of 0.01 mM dGTP as activator the reduction was maximal; dTTP at higher concentration was about one-third as effective as dGTP. No further stimulation could be obtained by adding dTTP to dGTP (not shown). When labeled ATP was used instead of ADP, it was reduced, but less rapidly than was ADP.

**Reduction of CDP and UDP**—The reduction of the pyrimidine ribonucleoside diphosphates required activation by 1 to 2 mM ATP. Table II shows that no other nucleotide tested had comparable activity, except perhaps for ADP. Slight activation was observed with very low levels of dATP, but with increased dATP, inhibition was seen instead. The reduction of CDP was stimulated slightly by GTP, dTTP, and dUTP, but the activity with these nucleotides was only 8 to 20% of that seen with ATP.

More recent experiments showed that GTP at levels of 0.1 to 1.0 mM gave about half the activity obtained with 0.01 mM dGTP. This is probably not due to contamination with dGTP.
Table II
Effectiveness of various nucleotides as activators of CDP and UDP reduction

Each incubation tube contained, in 0.12 ml, 1 μmole of phosphate buffer, pH 7; 0.5 μmole of magnesium acetate, 0.75 μmole of dithioerythritol; 0.05 μmole of UDP-14C (Column I) or 0.08 μmole of CDP-14P (Column I); and approximately 0.13 mg of nucleotide reductase. (A more active enzyme preparation was used in the UDP reduction than in the CDP reduction experiment.)

<table>
<thead>
<tr>
<th>Activator</th>
<th>Concentration</th>
<th>Deoxynucleotide formed</th>
<th>L. UDP</th>
<th>II. GDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>1.25</td>
<td>3.65</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>1.25</td>
<td>1.70</td>
<td>2.50</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>1.25</td>
<td>0.58</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>UTP</td>
<td>1.25</td>
<td>0.39</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>dTTP</td>
<td>0.0016</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dCTP</td>
<td>0.0035</td>
<td>0.47</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>dATP</td>
<td>0.0003</td>
<td>0.30</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

It has been found that the nucleotide reductase of Lactobacillus leichmanii acts on CTP rather than on CDP (9). If the rat tumor reductase also required CTP, the effect of ATP might be explained by phosphorylation of CDP. However, the previously reported (4) results were confirmed; with equal amounts added and under standard conditions, CDP was reduced 30% faster than CTP. Moreover, the addition of ATP stimulated the reduction of CTP to the same degree as the reduction of CDP.

An experiment to measure the actual degree of interconversion of di- and triphosphates during incubation with the enzyme showed that, in the absence of ATP, 90% of the labeled CDP was recovered unchanged after 20 min. In the presence of 2 mM ATP, or when labeled CTP was used with or without ATP, an equilibrium distribution of about 30% diphasphate and 70% triphosphate was reached after incubation for 5 to 10 min. Less than 3% of the nucleotide was recovered as the monophosphate. Despite the interference by phosphokinases, it is apparent that CDP is the preferred substrate and that ATP stimulates by some mechanism other than phosphorylation of CDP.

**Effect of Activators on Kinetic Parameters**—Fig. 4 shows the effects of the activators on the activity as a function of substrate concentration. The results must be regarded as only approximate because the kinases in the enzyme preparation altered the actual concentrations of diphosphates from the initial concentrations (see above). Additionally, at the lowest substrate concentrations, a significant percentage of substrate was consumed during the fully activated reaction.

For these and several other experiments, the apparent Michaelis constants and maximum velocities were calculated by computer according to the program of Cleland (10), which yields 0.25 mg. In the figure for reduction of GDP, broken lines and open symbols indicate no ATP, while solid lines and solid symbols represent the results with ATP present. ATP concentration was 0.42 mM. TTP concentrations were 0.005 mM for Line 1, 0.075 mM for Lines 3 and 4, and 0.27 mM for Lines 5 and 5. The highest reaction velocity seen in the absence of TTP was less than 5% of the lowest velocity shown.

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Fig. 4. Reductions of CDP, UDP, GDP, and ADP with respect to effects of substrate concentration. Lineweaver-Burke plots of the reciprocal of initial substrate concentration (millimolar concentration) with respect to the reciprocal of reaction velocity (millimicromoles of deoxynucleotide produced in 30 min). Standard incubation conditions were used except for substrates and activators as indicated on the figure; enzyme amounts were, for reduction of ADP, 0.27 mg; CDP, 0.18 mg; GDP, 0.2 mg; UDP,
The figures represent the concentration of inhibitor required to give 50% inhibition of the reduction of each substrate. Results for GDP reduction are with 0.04 mM dATP as activator, and for ADP are with 0.04 mM dGTP as activator.

Inhibition by dATP

This experiment was done under standard conditions except with the use of dihydrolipoate as reducing agent. The enzyme preparation contained about 3 mg of protein per ml. The level of dATP was 0.2 mM in all cases.

**TABLE III**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Concentration of inhibitor for 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dATP</td>
</tr>
<tr>
<td></td>
<td>mM</td>
</tr>
<tr>
<td>CDP</td>
<td>0.01</td>
</tr>
<tr>
<td>UDP</td>
<td>0.005</td>
</tr>
<tr>
<td>GDP</td>
<td>0.004</td>
</tr>
<tr>
<td>ADP</td>
<td>0.009</td>
</tr>
</tbody>
</table>

* Little or no inhibition.

Table IV

**TABLE IV**

Inhibition by dATP of CDP reduction at three levels of enzyme

This experiment was done under standard conditions except with the use of dihydrolipoate as reducing agent. The enzyme preparation contained about 3 mg of protein per ml. The level of dATP was 0.2 mM in all cases.

**TABLE V**

Effect of incubation time on inhibition by dATP of CDP reduction

Experiment I was carried out in Tris buffer with the tumor "thioredoxin equivalent" system as reducing agent; Experiment II was with Tris buffer and dihydrolipoate as reducing agent. The level of dATP was 0.2 mM in all cases.

not only the best values of $K_m$ and $V_{max}$, but also estimates of their standard errors.

In the cases of reduction of UDP and CDP, the presence of ATP increased markedly the affinity of the enzyme for the substrate, as indicated by a decrease of at least 10-fold in the apparent Michaelis constant. For CDP, the ranges of apparent $K_m$ values were from 0.12 ± 0.03 to 0.45 ± 0.22 mM for four experiments in the absence of ATP, from 0.013 ± 0.0017 to 0.0159 ± 0.0048 mM for five experiments with 0.1 to 0.3 mM ATP, and from 0.015 ± 0.006 to 0.047 ± 0.005 mM in seven experiments with 2.1 mM ATP. The averages weighted according to the reciprocal of the square of the standard error were 0.18, 0.0122, and 0.0223 mM, respectively. When CTP was used as substrate, the apparent Michaelis constant (averaged from two experiments) was 0.46 mM without ATP and 0.039 mM with 2.1 mM ATP. For UDP the average $K_m$ values from two experiments were 5.044, and 0.067 mM for 0.0, 0.35, and 2.1 mM ATP, respectively. The slight increase in apparent Michaelis constant with the highest level of ATP may be due to the conversion of diphosphate to triphosphate.

In addition to affecting the $K_m$, ATP also affected the maximum velocity of CDP reduction. In a typical experiment, the $V_{max}$ in the absence of ATP was 0.09 ± 0.024 mmole/30 min; with 2.1 mM ATP it was increased to 1.04 ± 0.05, and with 2.1 mM ATP it was further increased to 1.96 ± 0.08. Similar changes were observed in five other experiments, although in most cases the low rates of the completely unactivated reaction make the extrapolation to $V_{max}$ somewhat ambiguous. One of two experiments with UDP as substrate showed a doubling of $V_{max}$ when the ATP level was increased from 0.35 to 2.1 mM, while the other experiment showed only a 20% increase. In neither experiment was the extrapolation to $V_{max}$ in the absence of ATP sufficiently accurate to allow conclusions to be drawn.

In the case of ADP, the addition of dGTP as an activator clearly increased the affinity of the enzyme for the substrate. Average apparent Michaelis constants for ADP were 4.4 mM without dGTP and 0.084 mM with 0.0125 mM dGTP for four experiments. There was little or no change in the maximum velocity.

The case of GDP reduction is more complex, since both dTTP and ATP are required for maximum activity. With no activator present, activities were usually too low for measurement of $K_m$; in one experiment a value of 0.6 ± 0.1 mM was obtained. With dTTP alone, at concentrations of 0.007 and 0.075 mM, the average apparent $K_m$ values for GDP were 0.065 and 0.027 mM, respectively. The addition of 0.4 mM ATP increased these apparent $K_m$ values to 0.14 and 0.063 mM, respectively, possibly by the phosphorylation of GDP, but at the same time approximately doubled the maximum velocities. The optimum concentration of dTTP was also changed (see above). There was no stimulation by ATP in the absence of dTTP. It may be tentatively concluded that dTTP is required for efficient binding of GDP; the effect of ATP seems to be primarily on the maximum velocity.

**Inhibition Effects**

The deoxynucleoside triphosphates which were not activators for the reduction of a particular substrate generally inhibited the reduction. These inhibitions were measured at the standard substrate and activator concentrations given under "Materials and Methods." Two or more separate experiments were done with each substrate, each experiment including four concentrations of dATP, dGTP, dTTP, and dUTP, and two concentrations of dCTP. The inhibitor levels were chosen to cover the range from 25 to 75% inhibition. The observed activities in the presence of inhibitor were expressed as percentage of the control activity in each experiment and were plotted against the logarithm of the inhibitor concentration (see Fig. 6). The shape of the inhibition
curves was consistent with that given by the Hunter and Downs equation (11) for noncompetitive inhibition or competitive inhibition at constant substrate concentration. From the graphs, the concentration of each inhibitor giving 50% inhibition of the reduction of each substrate was determined. The results are summarized in Table III. It is apparent that the reduction of each substrate showed a different pattern of inhibition. Reduction of CDP was inhibited strongly by dATP and dGTP, and to a lesser degree by dUTP and dTTP. Reduction of UDP was inhibited by dATP and dGTP, and the reduction of ADP was inhibited by dATP.

The effects of certain changes in the incubation conditions on the inhibition were investigated, primarily with the use of the combination of CDP as substrate and dATP as inhibitor. Table IV shows that the inhibition was independent of the amount of enzyme; Table V shows that it was not affected by the time of incubation; and Table VI shows that it was independent of the reducing agent used.

Decreased inhibition by dATP and dGTP was, however, observed under conditions of partial inhibition by excess iron salts. Such inhibition by iron occurred inadvertently in several experiments, in which incubation mixtures buffered with Tris-chloride were used, because of the presence of iron in some batches of ATP. When Tris buffer is used, a very narrow margin exists between stimulatory (0.04 mM) and inhibitory (0.08 mM) levels of iron. The addition or substitution of phosphate, with which the iron presumably is in complex, prevents inhibition by FeCl₃ at levels up to 0.125 mM. This is 3 times the amount which is routinely included in the incubation mixture to give maximum stimulation of the nucleotide reductase (4). The 50% inhibitory concentrations of dATP and dGTP which were given in the preliminary report (5) were inadvertently determined under conditions of inhibition by iron and were considerably higher than those shown in Table III.

Another condition affecting the degree of inhibition by dATP and dGTP of CDP reduction proved to be the ATP concentration. This is illustrated in Fig. 5, where the effects of increasing concentration of ATP on the uninhibited reaction and the reaction inhibited by dATP and dGTP are shown. It is clear that ATP reversed the inhibition by the purine deoxyribose triphosphates. In another experiment (not shown), no reversal of the inhibition by dATP was obtained by increasing the concentration of the substrate, CDP, from 0.20 to 0.83 mM. Similar results (not shown) were obtained for reduction of ADP, where the activator, dGTP, partially reversed inhibition by dATP.

No reversal of inhibition by increased concentration of activator could be shown in the case of reduction of GDP. The concentration of dTTP did not affect the degree of inhibition by dATP, and the presence of ATP appeared to increase rather than reverse the inhibition by dATP and dGTP, especially at low inhibitor concentrations (Fig. 6); this we attribute tentatively to a slight activity of dATP and dGTP as activators in place of ATP.

**TABLE VI**

**Effect of reducing agent on inhibition by dATP of CDP reduction**

Dihydrolipoate and dithioerythritol were compared in one experiment, and thioredoxin and dithioerythritol in a second experiment.

<table>
<thead>
<tr>
<th>dATP</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dihydrolipoate</td>
</tr>
<tr>
<td>mM</td>
<td>%</td>
</tr>
<tr>
<td>0.0038</td>
<td>70</td>
</tr>
<tr>
<td>0.0088</td>
<td>50</td>
</tr>
<tr>
<td>0.025</td>
<td>25</td>
</tr>
<tr>
<td>0.083</td>
<td>15</td>
</tr>
</tbody>
</table>

* Control activity, 2.01 mmoles per tube.
* Control activity, 2.84 mmoles per tube.
* Control activity, 2.57 mmoles per tube.
* Control activity, 0.66 mmmole per tube.

**FIG. 5.** Effect of inhibitors on the requirement for ATP in the reduction of CDP. Activity is expressed as percentage of the maximum observed in the uninhibited series for each of the three experiments. ○—○, not inhibited; O—O, plus 0.0005 mM dGTP; X—X, plus 0.008 mM dATP.

**FIG. 6.** Inhibition by dGTP of the reduction of GDP. O, without ATP; ·, with 0.42 mM ATP.

**DISCUSSION**

It was previously reported (12) that the tumor nucleotide reductase system catalyzed the formation of deoxyribose (assayed...
inhibition of a highly purified nucleotide reductase system from rat embryo tissue have revealed no major differences between results of Holmgren, Reichard, and Thelander (13) and more recent experiments. Preliminary experiments with reduction of CDP and GDP by an enzyme preparation from tissues or organisms is not yet known. Preliminary experiments had a specific activity about seven times higher than before, permitting the use of smaller amounts of enzyme and thus presumably smaller amounts of contaminating deoxyribonucleotide activators. However, only 0.001 mM deoxyribose was found in the unincubated control in the earlier study, which would not represent sufficient dTTP to activate reduction of GDP in the presence of 2 mM ATP. It is conceivable that ribonucleotide contaminations were converted enzymatically to activators or that sufficient ATP was destroyed to permit activation by the dTTP present.

Whether these activations and inhibitions are relevant to other tissues or organisms is not yet known. Preliminary experiments with reduction of CDP and GDP by an enzyme preparation from rat embryo tissue have revealed no major differences between this preparation and the enzyme from Novikoff tumor. The results of Holmgren, Reichard, and Thelander (13) and more recent results of Larsson and Reichard (14) on the activation and inhibition of a highly purified nucleotide reductase system from Escherichia coli were quite similar to those reported here. In that system, however, more nucleotides were active as activators and fewer as inhibitors. Thus the activations and inhibitions are another point of general similarity but nonidentity between the mammalian and E. coli nucleotide reductase systems. This general similarity suggests that the same type of regulatory system may be found in other organisms as well.

The variation in activators and inhibitors for the reduction of the four substrates is so great as to suggest the existence of four separate enzymes. However, Larsson and Reichard (14) have found that a single enzyme system from E. coli reduces all four substrates by the use of different activators. A decision on this question with regard to the tumor enzymes must await further purification, now being attempted.

The Lineweaver-Burk plots of activity with respect to substrate concentration must be interpreted cautiously; the presence of kinases in the enzyme preparation and the consumption of substrate during the reaction make the actual substrate concentration uncertain. However, the changes in the $K_m$ produced by the activators are so marked that it is clear that the activators affect primarily the binding of the substrate by the enzyme or enzymes, as well as, in some cases, the maximum velocity.

The most probable function of the activators and inhibitors discussed here would be that of regulatory effectors (15). A function of the activators as cosubstrates appears unlikely, as is discussed above. That they function as coenzymes appears equally unlikely because the different nucleotide activators (which are not readily and reversibly oxidized and reduced) all stimulate a similar reaction, namely the removal of the 2'-oxygen atom from ribose. The activators do bind specifically to the enzyme; that the binding is reversible is indicated by the fact that the enzyme is prepared in the unactivated state, although it must have been exposed to activators in vivo. All the activators, except ATP as activator of the reduction of GDP, clearly exert their effect by modifying the affinity of the enzyme for its substrate and thus may be classified as positive effectors (15).

The effect of the inhibitors is clearly not reversal by mass action, since they are not the immediate products of the reduction reaction and since the reduction reaction is irreversible under these conditions. It has not yet been rigorously shown whether all the inhibitors act by competing with the activators for an activation site or whether some may act as analogues of the substrate in competition for the substrate site. It has been indicated in three cases (inhibition by dATP or dGTP of the reduction of CDP, and inhibition by dATP of the reduction of ADP) that the inhibitor is competitive with the activator to some degree, and in the first of these cases the inhibition is independent of substrate concentration. Thus $d$ATP does appear to function as a negative effector (15) in the reduction of CDP, and it is possible that the other inhibitors may be similarly classified.
Although it is tempting to postulate that these effectors act by allosteric mechanisms (10), no proof regarding the relative locations of binding sites and effects on conformation of the enzyme or subunits thereof is available at this time.

A regulatory function of these activators and inhibitors may, however, be visualized. All are natural metabolites; the activators (ATP, dTTP, and dGTP) would function as positive effectors in initiation and maintenance of the series of reductions while the inhibitors (dTTP, dCTP, and dATP) are terminal products and would serve as negative effectors in restricting the rate of synthesis of the deoxynucleotides. Fig. 7 summarizes this complex series of inhibitions and activations and postulates a sequential series of steps, controlled by the various regulatory effectors, leading to the full complement of deoxynucleotide triphosphates. The reduction of CDP and UDP is activated by ATP; if the other enzymes needed for the further biosynthesis of dTTP are present, the resulting dTTP will activate reduction of GDP, and the dGTP produced will in turn activate reduction of ADP. If the final product, dATP, is not consumed, its accumulation will inhibit all four reductions.

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REFERENCES
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