MODELS OF THE REGULATION OF RIBONUCLEOTIDE REDUCTASE AND THEIR EVALUATION IN INTACT MAMMALIAN CELLS

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I. INTRODUCTION

Models of the structure and mechanisms of catalysis and regulation of ribonucleotide reductase (ribonucleoside diphosphate reductase, EC 1.17.4.1) based on enzymological studies have been extremely influential in discussions and investigations of deoxyribonucleotide metabolism, the toxicity of naturally occurring deoxyribonucleosides, deoxyribonucleoside analogues and drugs affecting deoxyribonucleotide metabolism, and the regulation of DNA synthesis by the concentrations and rates of synthesis of deoxyribonucleoside triphosphates.

The first model of ribonucleotide reductase and its regulation was presented in 1966,[1] but as studies of this enzyme have progressed, additional models have been published. Although these studies have used ribonucleotide reductase purified from both bacterial and mammalian sources, the resulting models have been identical or similar in many respects, and most investigators have in practice tended to view them all as identical or interchangeable. A more detailed study of these models, however, reveals some very significant differences among them, differences which ought to affect the interpretation of studies of deoxyribonucleotide metabolism and deoxyribonucleoside toxicity in intact cells. This review considers the 11 major models of ribonucleotide reductase action and regulation and indicates both their similarities and differences.

A relatively small number of studies also have been carried out to determine how the reduction of ribonucleotides is regulated in intact mammalian cells; these usually have been influenced by or related to one or another model of ribonucleotide reductase. These investigations will also be reviewed here, and the present state of this question will be summarized.

II. MODELS OF RIBONUCLEOTIDE REDUCTASE AND ITS REGULATION

Ten of the eleven models to be described were based completely or in part on kinetic studies. One, however, was based entirely on binding and other physiochemical

Table 1
MODEL 1: MOORE AND HURLBERT[1]

Substrate	Effects of nucleotides				
	dTTP	dCTP	dGTP	dATP	ATP
CDP	I	0	I[a]	I[a]	S^R
UDP	I	0	I	I	S^R
GDP	S^R	0	I	I	S
ADP	S^R	0	S_R	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement.

[a] Inhibition competitively reversed by ATP.

measurements, and such data entered into some of the other models either explicitly or implicitly.

Most investigators have presented the results of their studies of ribonucleotide reductase in terms of various schemes or drawings; these interpretations are what are here called "models". It is important to note, however, that in most cases additional pertinent data were reported in the various publications, but not incorporated into the models themselves.

For the present purposes, we have summarized all of the models based on kinetic studies in a uniform, tabular format (Tables 1 to 10). The schemes based on binding and other physiochemical studies, however, are presented in a variety of diagrammatic forms (Figures 1 to 5). In each case, other important information given in each study will be summarized in the accompanying text.

A. Model 1

In 1966, Moore and Hurlbert[1] published the first model depicting the control of ribonucleotide reductase, based on studies of a partially purified preparation from Novikoff rat hepatoma cells. Their model is summarized in Table 1.

Other findings which are either not obvious from the model or which were not included in the model are as follows:

1. dTTP was required for GDP reduction to occur. The rate of reduction in the absence of dTTP was less than 10% of the rate in the presence of dTTP.
2. Either dGTP or dTTP was required for ADP reduction to occur, with dGTP being much more effective. In the absence of activator, no reduction occurred.
3. In order for reduction of pyrimidines to occur, 1 or 2 m*M* ATP was required. The rate of reduction without ATP was less than 4% of the rate with ATP.
4. The inhibition of CDP reduction by dATP or dGTP was reversed by ATP.
5. ATP was reduced less rapidly than ADP. At equal concentrations, CDP was reduced 30% faster than CTP.
6. The enzyme preparation contained nucleoside diphosphate kinase activity, which had the effect of lowering substrate concentrations when nucleoside triphosphates especially ATP, were present. No phosphatase activity was detected, however.

B. Model 2

In 1966 Larsson and Reichard[2] published the results of kinetic studies with partially purified ribonucleotide reductase from *Escherichia coli.* The enzyme was essentially free

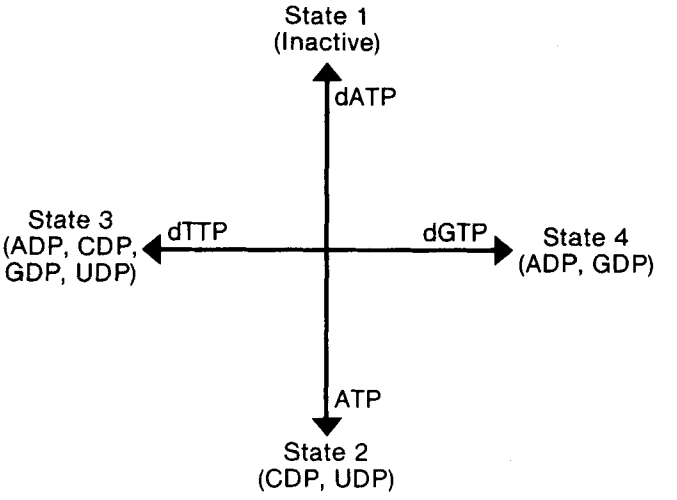

FIGURE 1. Model of Larsson and Reichard.[2] Schematic interpretation of allosteric effects on ribonucleotide reduction. (From Larsson, A. and Reichard, P., *J. Biol. Chem.*, 241, 2540, 1966. With permission.)

Table 2
MODEL 2: LARSSON AND REICHARD[2]

	Effects of nucleotides				
Substrate	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
CDP	S(−ATP) I(+ATP)	0	0	I	S′
UDP	S(−ATP) I(+ATP)	0	0	I	S′
GDP	S	0	S	I	0
ADP	S	0	S	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect, r = partial requirement.

of pyrophosphatase and kinase activities. Many of their findings are summarized in the two schemes shown in Figure 1 and Table 2. Figure 1 depicts a model for the conformational changes induced in ribonucleotide reductase by allosteric effectors, while Table 2 represents Reichard's first attempt to suggest how his emzymological findings might be relevant to the physiological regulation of this enzyme.

Larsson and Reichard did not state that this model applies to mammalian cells, but did say that ". . . a striking parallelism is observed with the results of Moore with enzymes from Novikoff hepatoma".

Other results of this study which should be noted are as follows:

1. ATP stimulated CDP and UDP reduction, and the rate of reduction in the absence of ATP was approximately 20% of the rate in its presence.
2. dTTP stimulated CDP and UDP reduction in the absence of ATP, but inhibited their reduction in the presence of ATP.
3. dGTP, in the presence of ATP, inhibited CDP and UDP reduction. This result

was not included in the model and was not studied further although it agrees with the results of Moore and Hurlbert.[1]

4. UTP was the only ribonucleoside triphosphate which gave a stimulation of GDP reduction comparable to the stimulation produced by dTTP or dGTP. Although 1 m*M* UTP was required for maximum stimulation (100 times the concentration of dTTP or dGTP required for maximum stimulation), this is within the range of UTP concentrations found in intact cells.
5. dCTP caused a substantial stimulation of GDP reduction. For example, a maximum tenfold stimulation of GDP reduction by dTTP or dGTP was achieved at a concentration of 10 μM. This concentration of dCTP produced a sevenfold stimulation, while a ninefold stimulation was observed with 50 μM dCTP. Although the stimulation of GDP reduction by dTTP and dGTP was included in the allosteric model, the stimulation caused by dCTP was not.
6. Evidence was obtained that different types of binding sites might exist for ATP and dTTP.
7. Some of the data suggested that one enzyme catalyzed the reductions of all four substrates.
8. UDP was a competitive inhibitor of CDP reduction and vice versa. This result was obtained in the presence of ATP which, according to the model, should make the enzyme specific for pyrimidine nucleotide substrates ("pyrimidine specific").
9. In the presence of dTTP, GDP competitively inhibited CDP reduction. In the presence of ATP, which is predicted to make the enzyme pyrimidine specific, GDP inhibited CDP reduction. According to the model, in the presence of ATP, GDP and CDP are reduced by different allosteric states of the enzyme; therefore, one would not expect them to compete for reduction.
10. The enzyme did not reduce GMP and was 5% as active with GTP as with GDP.

C. Model 3

In 1969, Brown and Reichard[3] published the results of binding studies with purified ribonucleotide reductase from *E. coli;* no kinetic studies were included. They incorporated many of the results of their study into the model shown in Figure 2.

This model is considerably more elaborate than the one presented in 1966 (Table 2), which was formulated without the aid of binding data. Although other data had suggested the existence of different types of allosteric binding sites, this was the first time that strong evidence for their existance was presented. For the sake of simplicity the scheme presented (Figure 2) shows only one l-site and one h-site per molecule, but in fact there were two per molecule. The term "h-site" refers to a "high affinity site", while "l-site" refers to a "low affinity site" for the binding of dATP. The dissociation constant of dATP from the h-site was 50 n*M* while this value for the l-site was 210 n*M*. Other results which should be noted are as follows:

1. h-Sites had a high affinity for dATP, but also bound dTTP, dGTP, and ATP.
2. The removal of one half the dATP bound to the h-sites required a 20-fold excess of dTTP or a 5000-fold excess of ATP.
3. l-Sites had a low affinity for dATP, some affinity for ATP, and no affinity for dTTP or dGTP.
4. The enzyme was relatively inactive in the absence of effectors, in agreement with Moore and Hurlbert's results with Novikoff hepatoma ribonucleotide reductase.[1] This differs from the previous conclusion of Holmgren et al. that "The ATP requirement was not absolute and was more pronounced at the lower CDP concentration."[4] This difference may only be a matter of degree, since no numbers were presented.

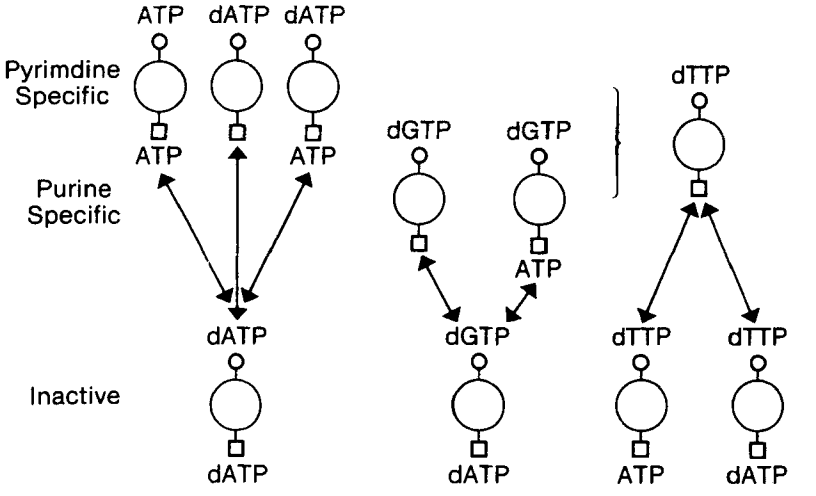

FIGURE 2. Model of Brown and Reichard.[3] Scheme for different forms of protein B1 of ribonucleotide reductase. The h-sites (specificity sites) are represented by circles and l-sites (activity sites) by squares. For convenience only one set of each class is depicted. The arrows show transitions between active and inactive forms. (With permission Brown, N. C. and Reichard, P., *J. Mol. Biol.*, 46, 39, 1969. Copyright by Academic Press, Inc. (London) Ltd.)

5. With one exception, all the inhibited states of the enzyme had dATP bound to the l-sites. The exception occurred when dTTP was bound to the h-sites and ATP to the l-sites. In contrast, the model of Moore and Hurlbert indicates that this state would preferentially reduce GDP, since they found that dTTP stimulated GDP reduction in the presence or absence of ATP, although less dTTP was required for maximum stimulation in the absence of ATP.
6. The data indicate that the l-sites probably regulate the level of activity of the enzyme, while the h-sites regulate substrate specificity.

D. Model 4

In 1976, von Doblen and Reichard[5] published further binding studies with ribonucleotide reductase from *E. coli,* this time dealing with substrate binding; some kinetic experiments were also done. The study was complicated by the fact that the reductase preparation was contaminated with kinase activity which, in the presence of nucleoside triphosphates, converted the added diphosphate substrates to triphosphates. Therefore, various nucleoside triphosphate analogues were used as allosteric effectors. In kinetic studies with the reductase it was found that these analogues affected the reaction in ways similar, but not identical, to the natural effectors. Thus it was found that a 10-fold higher concentration of dAMP-P-(CH_2)-P could substitute for dATP, a 6-fold higher concentration of dTMP-P-(CH_2)-P could substitute for dTTP, and a 50-fold higher concentration of dGMP-P-(NH)-P or a 3-fold lower concentration of dGMP-P-P-(S) could substitute for dGTP. None of the analogues tested could substitute for ATP. A second complication was that in order to prevent utilization of the substrates, only the B1 subunit was used in the detailed binding studies. Subunit B2 was found to bind neither substrates nor effectors, while B1 bound both, but showed no catalytic activity by itself. The use of the B1 subunit alone raises questions concerning the relevance of the results both for the holoenzyme and for the whole cell, especially since von Doblen and Reichard concluded that the catalytic site is constructed from B1 and B2 together. The major

Table 3
MODEL 4: VON DOBLEN AND REICHARD[5]

	Effects of Nucleotides			
Substrate	**dTTP**	**dCTP[a]**	**dGTP**	**dATP**
CDP	S		S[b]	S
UDP	S		0	S
GDP	S		S	I
ADP	S		S	I

Note: These experiments were performed in the absence of ATP or an ATP analogue. I = inhibitory, S = stimulatory.

[a] Not included in model.
[b] Effect on enzyme activity, but not substrate binding, was measured.

findings of this study are described below, and the results of the kinetic studies are summarized in Table 3.

1. The dTTP analogue and the dGTP analogue each lowered the dissociation constant (K_{diss}) for the binding of GDP to subunit B1, but neither effector competed with GDP for binding. These conditions stimulated GDP reduction by the holoenzyme. Low concentrations of the dATP analogue did not affect GDP reduction and at higher concentrations, which strongly inhibited GDP reduction, GDP binding was only slightly decreased.
2. The dATP analogue and the dTTP analogue each lowered the K_{diss} for the binding of CDP to subunit B1 and also stimulated the reduction of CDP.
3. At low concentrations the dTTP and the dGTP analogues each increased the binding of ADP to subunit B1 (i.e., the K_{diss} was lowered). These conditions also stimulated ADP reduction. The dATP analogue had no effect on ADP binding at low or high concentrations. This is not consistent with the model of Brown and Reichard[3] (Model 3) which predicts that low concentrations of dATP will bind to the specificity sites and make the enzyme pyrimidine specific, whereas high concentrations will bind to both the specificity and activity sites and inhibit the enzyme completely.
4. S-Shaped binding curves were not observed, indicating lack of cooperativity for the binding of allosteric effectors.
5. CDP and GDP competed for the same sites of B1 in the presence of the dTTP analogue. This result agrees with that of Larsson and Reichard[2] in 1966.
6. It was not possible to demonstrate simple competition between ADP and GDP, although the presence of one substrate decreased the binding of the other.
7. Scatchard plots indicated two binding sites each for CDP and GDP. However, these plots suggested at least four binding sites for ADP, although it is possible that the ADP was binding to some effector sites as well as substrate sites.

E. Model 5

Reichard published the next model of the regulation of ribonucleotide reductase in 1978 in a review.[6] The essential ingredients of the model are described using the following three schemes. Figure 3 depicts the physical make-up of the enzyme, showing the subunit construction and the location of the allosteric effector binding sites and of the substrate

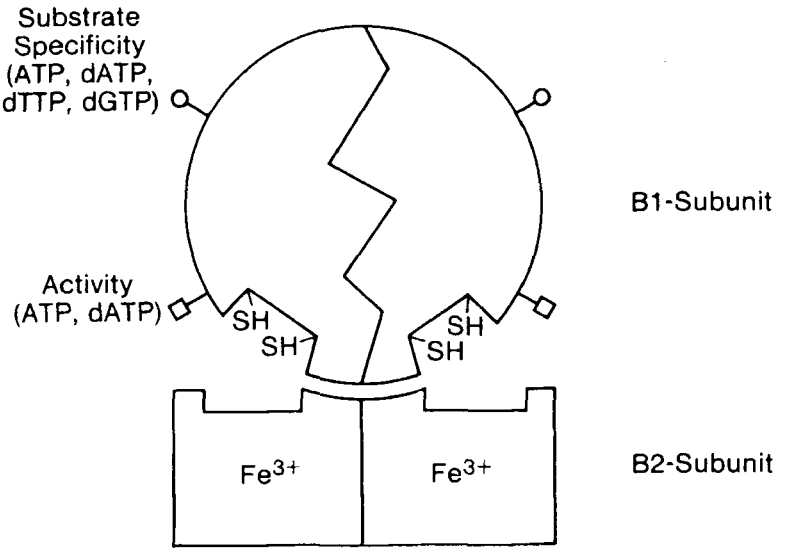

FIGURE 3. Model of Reichard.[6] Model of *E. coli* ribonucleotide reductase. (From Reichard, P., *Fed Proc. Fed. Am. Soc. Exp. Biol.*, 37, 9, 1978. With permission.)

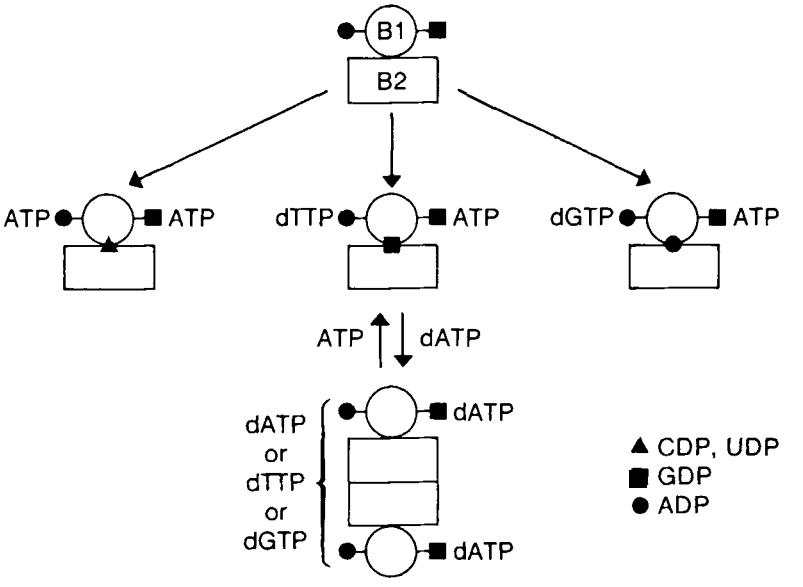

FIGURE 4. Model of Reichard.[6] Main allosteric forms of ribonucleotide reductase. Binding of ATP to "activity sites"(■) results of catalytically active forms of the enzyme. The substrate specificity of these forms is determined by binding of effectors to "specificity sites"(●). Binding of dATP to "activity sites" gives an inactive dimer. (From Reichard, P., *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 37, 9, 1978. With permission.)

binding sites. Figure 4 shows the different allosteric forms of the *E. coli* reductase, while Table 4 shows the model presented for the regulation of ribonucleotide reduction in cells.

It is important to note that there are substantial differences between the model presented in 1969[3] and that presented in 1978,[6] although no data were presented to explain the changes. The differences in the models are as follows:

Table 4
MODEL 5: REICHARD[6]

Substrate	Effects of nucleotides				
	dTTP	dCTP	dGTP	dATP	ATP
CDP	I	0	0	I	S
UDP	I	0	0	I	S
GDP	S	0	0	I	S
ADP	0	0	S	I	S

Note: I = inhibitory, S = stimulatory, 0 = no effect.

1. In the 1969 model (Figure 2), the l-sites (activity sites) could either be occupied by ATP or dATP, or left unoccupied. All the states with the l-sites unoccupied were active. In the 1978 model the l-sites were always occupied, either by ATP or dATP.
2. In the 1969 model, dATP, ATP, dGTP, or dTTP competed for the h-sites (specificity sites) on the active forms of the enzyme. In the 1978 model, dATP was not included as a competitor for the h-sites on the active forms of the enzyme, but was included as an h-site competitor on the inactive form of the enzyme; however it was not really important which effector occupied the specificity sites on the inactive form of the enzyme. The omission of dATP as a competitor for the specificity sites on the active enzyme is inconsistent with data which show that dATP competes well for the h-sites; for example, the K_{diss} for dATP was 50 n*M* and for dTTP was 300 n*M*.[3] Also, in the 1969 model occupation of the h-site by dATP caused the enzyme to reduce pyrimidines specifically.
3. The final difference in the models involves the activity of the enzyme when dTTP occupies the h-sites and ATP occupies the l-sites. In the 1969 model this form of the enzyme was inactive, whereas in the 1978 model this form specifically reduced GDP.

One further point concerning the 1978 model is that Reichard used it to explain the results of experiments in mammalian cells in which deoxyribonucleotide pool size changes had occurred, even though the model was based on experiments with *E. coli* ribonucleotide reductase.

One possible explanation for the differences between the 1969 and the 1978 models is that the latter may have been influenced by the results of experiments using intact cells. For example, using the 1969 model, one would predict that an increase in dTTP in intact cells could cause either an increase or a decrease in dCTP, depending on the ATP concentration. However, in 1973 Bjursell and Reichard[7] published results demonstrating that treatment of Chinese hamster ovary cells with thymidine resulted in an 18-fold increase in dTTP and a 30-fold decrease in dCTP. Results such as these may have influenced the modification of the earlier model to conform more closely to the available intact cell data.

F. Model 6

In 1979 Eriksson et al.[8] published the results of a study of the allosteric regulation of calf thymus ribonucleotide reductase. This was the first thorough study of the regulation of a purified mammalian ribonucleotide reductase since the work of Moore and Hurlbert in 1966.[1] The enzyme preparation used in the study of Eriksson et al. contained no detectable phosphatase activity and only trace amounts of kinase activity. Their model is summarized in Table 5, and the main results of their study are as follows:

Table 5
MODEL 6: ERIKSSON ET AL.[8]

	Effects of nucleotides				
Substrate	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
CDP	I	0	I	I	S^R
UDP	I	0	I	I	S^R
GDP	S^R	0	I	I	S^a
ADP	0	0	S^R	I	S^b

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement.

[a] ATP was an activator only in the presence of dTTP.
[b] ATP was an activator only in the presence of dGTP.

1. The reduction of ribonucleotides required the presence of a positive allosteric effector. No deoxyribonucleotide formation could be detected in the absence of an effector, even with high ribonucleotide concentrations.
2. The reduction of CDP specifically required ATP, with optimal concentrations of 2 to 5 m*M*. Other triphosphates, e.g., dTTP, dGTP, dATP, and dCTP, did not substitute for ATP.
3. In the presence of 1 m*M* ATP, CDP reduction was inhibited by dATP, dTTP, and dGTP, but not by dCTP. dATP was the most effective inhibitor, while inhibition by dTTP and dGTP was not evident at high CDP concentrations, but became pronounced at CDP concentrations close to the K_m (30 μM).
4. The reduction of UDP required ATP; again, optimum concentrations were 2 to 5 m*M*.
5. In the presence of 1 m*M* ATP, UDP reduction was inhibited 50% by 5 μM dATP or by 100 μM dTTP or dGTP.
6. Reduction of GDP required dTTP while other triphosphates (e.g., dATP, dGTP, or dCTP) had no effect as positive effectors. (In the presence of dTTP, ATP was an activator.)
7. GDP reduction was inhibited 50% by 1 μM dATP, in the presence of dTTP or dTTP plus ATP, or by 50 μM dGTP.
8. The reduction of ADP specifically required dGTP. ATP, dATP, dTTP, and dCTP showed no effect as positive effectors. dGTP plus ATP gave a twofold higher activity than dGTP alone. (Chang and Cheng have found that for purified human ribonucleotide reductase, GTP and dGTP serve equally well as activators for ADP reduction.[9])
9. ADP reduction was inhibited 50% by 50 μM dATP in the presence of dGTP plus ATP, or by 300 μM dTTP. These results differ from Moore and Hurlbert's results with Novikoff hepatoma reductase in which dTTP stimulated ADP reduction. Larsson and Reichard's 1966 model showed a stimulation of ADP reduction by dTTP but Reichard's 1978 model did not.
10. The presence of dTTP at 20 μM and ATP at 1 m*M* allowed the reduction of both CDP and GDP. This disagrees with Brown and Reichard's 1969 model in which these conditions produced an inactive enzyme. It also disagrees with Reichard's 1978 model in which these conditions produced a GDP-specific enzyme.
11. GDP and CDP competed for the same catalytic site. This experiment was carried out in the presence of dTTP and ATP which allowed reduction of both GDP and

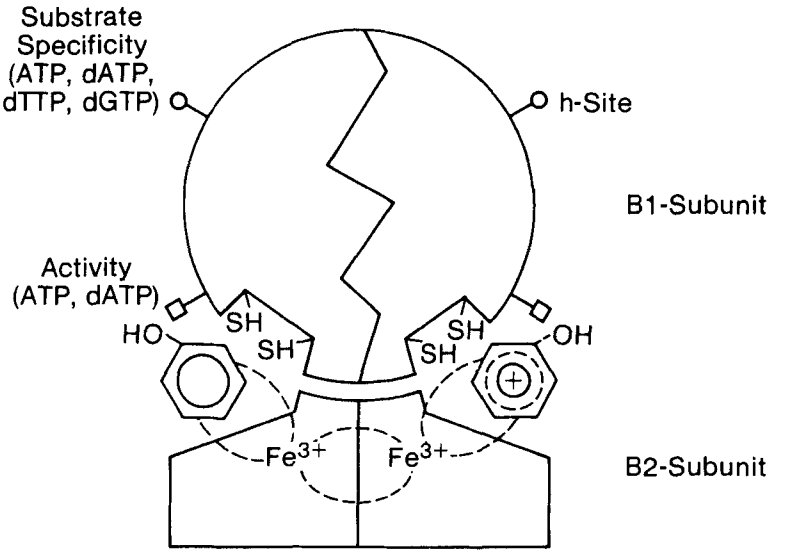

FIGURE 5. Model of Thelander and Reichard.[10] Model of ribonucleotide reductase from *E. coli*. (Reproduced with permission from the *Annual Review of Biochemistry*, Vol. 48. Copyright 1979 by Annual Reviews, Inc.)

CDP. A Lineweaver-Birk plot showed competitive inhibition of CDP reduction by GDP. The fact that GDP and CDP competed for reduction is evidence that one enzyme state reduced both substrates and not that two states existed, one specific for CDP and one for GDP.

12. The reductase was 10% as active with CTP as substrate as with CDP with each compound at 500 μM.

The model of Eriksson et al is very similar to Reichard's 1978 model, although the following important difference should be noted; Reichard's model did not show inhibition of CDP, UDP, or GDP reduction by dGTP, while the model of Eriksson et al. did. It should also be noted that both models represent a selection of the data; for example, the model of Eriksson et al. did not include inhibition of ADP reduction by dTTP although they found this to occur.

G. Model 7

In 1979 Thelander and Reichard[10] published another model for the regulation of ribonucleotide reductase in intact cells. This model was presented in a review and it appears to be a composite of Reichard's 1978 model for the *E. coli* enzyme[6] and that of Eriksson et al. for the calf thymus ribonucleotide reductase.[8] The authors state: "*in vitro*, the activity and substrate specificity of the reductases from *E. coli* and mammalian sources behave similar (sic) towards allosteric effectors. The data can be integrated into a scheme that links ribonucleotide reduction to DNA synthesis." Thus it appears that this new model is meant to describe the behaviour of both *E. coli* and calf thymus ribonucleotide reductases. This model is shown in Figure 5 and Table 6.

H. Model 8

In 1979, Chang and Cheng published studies on ribonucleotide reductase purified from cultured human lymphoblast cells (Molt-4F).[9,11,12] They did not use their results to formulate a model; however we have summarized their data on the effects of

Table 6
MODEL 7: THELANDER AND REICHARD[10]

	Effects of nucleotides				
Substrate	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
CDP	I	0	I	I	S
UDP	I	0	I	I	S
GDP	S	0	I	I	S
ADP	0	0	S	I	S

Note: I = inhibitory, S = stimulatory, 0 = no effect.

Table 7
MODEL 8: CHANG AND CHENG[9,11,12]

	Effects of nucleotides				
Substrate	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
CDP	I	I(+ATP) S(−ATP)	I(+ATP) S(−ATP)	I	S^R
UDP	I	I	I	I	S^R
GDP	S^R	S^R	S^R	I	S^a
ADP	I	0	S^R	I	0

Note: The inhibitory effects of nucleotides were determined in the presence of a positive allosteric effector, at a concentration of 2.5 m*M*. The effector was ATP for CDP and UDP reduction, GTP for ADP reduction, and dTTP for GDP reduction. The stimulatory effects of nucleotides, at a concentration of 5 m*M*, were determined in the absence of other nucleotides. I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement.

[a] ATP stimulated GDP reduction to 25% of the rate produced by dTTP.

deoxyribonucleoside triphosphates and ATP on nucleotide reduction in Table 7. Other pertinent data are given below:

1. The ratio of ADP to CDP reductase activity was constant throughout the purification procedure, which is consistent with the hypothesis that one enzyme catalyzes both reactions.
2. The rate of reduction of CDP, UDP, and GDP in the absence of an activating nucleoside triphosphate was less than 7% of the activity in the presence of the best activator. No reduction of ADP was detected in the absence of an activator. The best activators for the reduction of pyrimidines, GDP, and ADP were ATP, dTTP, and GTP, respectively.
3. In the absence of ATP, dGTP and dCTP stimulated the rate of CDP reduction to 19 and 25% of maximum, respectively, whereas in the presence of ATP, dGTP and dCTP reduced the rate of CDP reduction to 10 and 70% of maximum, respectively.
4. In the presence of 2.5 m*M* ATP, the reduction of UDP was substantially

inhibited by every nucleoside triphosphate tested, at concentrations of 2.5 m*M*. The effects of the nucleotides, expressed as the percentage of the maximum activity in the presence of ATP alone, were as follows: GTP (25%), CTP (1%), dATP (0%), dUTP (1%), dTTP (1%), dGTP (0%), and dCTP (22%). The effect of UTP was not determined. When considering enzymological data on the effects of ribonucleotides on ribonucleotide reductase, one must remember that in intact cells the concentrations of ribonucleoside triphosphates are 10- to 200-fold higher than those of deoxyribonucleoside triphosphates.[13] The inhibitory effect of dCTP on UDP reduction is also noteworthy, in that is has not been observed in other enzyme preparations, either from mammalian cells or from *E. coli*, and most models have assigned no regulatory role to dCTP.

5. Both GTP and dGTP, at a concentration of 5 m*M*, were equally effective activators of ADP reduction.
6. Although maximum activation of GDP reduction was achieved with dTTP, several other nucleotides stimulated the reaction. Their effects, expressed as a percentage of the maximum enzyme activity in the presence of dTTP alone, were as follows: dUTP (95%), dGTP (86%), UTP (46%), dCTP (43%), ATP (27%), and CTP (25%).
7. Reduction of each ribonucleoside diphosphate was noncompetitively inhibited by each of the other substrates. This is especially interesting in the case of UDP and CDP, since both reductions are activated by ATP. If ATP converted the enzyme to a pyrimidine-specific state and a single site catalyzed both reactions, as proposed by Reichard for the *E. coli* enzyme, then one would expect CDP and UDP to compete directly for reduction.
8. No phosphatase or nucleoside kinase activity was detected in the enzyme preparation.

Chang and Cheng's data demonstrate that, when assayed singly or in pairs, all eight nucleoside triphosphates alter the activity of purified ribonucleotide reductase. This complicates the picture considerably, since other models have included at most four allosteric effectors. Also, these data illustrate the difficulty of developing a model of such a complex enzyme solely from enzymological data. It is not possible to assay the enzyme under physiological conditions, that is, in the presence of all eight (or more) allosteric effectors and all four substrates.

I. Model 9

Although most of the models for the regulation of deoxyribonucleotide synthesis in vivo or in intact cells have come from Reichard's group, other models have been proposed which are based to varying degrees on either Reichard's models or on Moore and Hurlbert's model. The contributions of these "second generation" models to the ribonucleotide reductase story are sufficiently important to justify discussing their development separately from the evaluation of Reichard's models.

One such model was proposed by Werkheiser et al.[14] in 1973 (Table 8). It was based very loosely on both the 1969 model of Brown and Reichard[3] and the model of Moore and Hurlbert.[1] The following details of the model should be noted:

1. The model was an open steady-state system such that the concentrations of CDP, UDP, and ADP were constant, and the incorporation of nucleotides into DNA was an irreversible process.
2. The synthesis of dCTP, dTTP, dATP, and dUMP was regulated by feedback inhibition of ribonucleotide reductase, which is represented as three separate enzymes.

Table 8
MODEL 9: WERKHEISER ET AL.[14]

	Effects of Nucleotides					
Substrate	**dUMP**	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
CDP			I			
UDP	I	I				
GDP						*
ADP	I				I	

Note: I = inhibitory.

* Not included in model.

3. The metabolism of guanine nucleotides was not included in the model.
4. Ribonucleotide reductase had a constant V_{max} for each substrate.

Obviously, there are large differences between this model and any of the models proposed by Reichard and Moore and Hurlbert. The major differences are as follows:

1. The model of Werkheiser et al. did not include stimulation of ribonucleotide reductase, either by deoxyribonucleotides or by ATP.
2. This model did not include the reduction of GDP, nor the effects of dGTP on other reductions.
3. This model included only one interaction between different families of deoxyribonucleotides.
4. It included a feedback inhibition role for dCTP and dUMP.
5. One final important difference among the models is that of Werkheiser et al. treated ribonucleotide reductase as three separate enzymes and hence did not include inhibition of reduction by substrate competition for a single catalytic site; the models of Reichard and Moore and Hurlbert at least implicitly included this feature.

Werkheiser et al. recognized these differences and stated that the true pattern of feedback inhibition was probably quite different from their model. Nevertheless, they used their model to predict the effect of pairs of drugs on DNA synthesis and therefore on growth rate. All the drugs used were specific inhibitors of deoxyribonucleotide or DNA synthesis. When they compared their results with the results obtained by Grindey and Nicol[15] in cultured L1210 cells, it was concluded that "the over-all agreement is good and suggests that this model does in some sense mimic actual events in the intact cell." They also noted that the deoxyribonucleotide pool size changes predicted by their model agreed with some results of other researchers using intact cells. It is interesting that although this model is very simple and quite different from Reichard's models, it still appears to mimic the situation in intact cells.

Nicholini et al.[16] have used the model of Werkheister et al. to analyze the effects of combinations of the antimetabolites hydroxyurea, fluorodeoxyuridine, arabinosyladenine, arabinocytosine, and methotrexate on deoxyribonucleoside triphosphate concentrations. Their study was limited to computer modeling and unlike the study by Werkheiser et al. did not include a comparison of the results generated by the computer model with results obtained in cultured cells.

Table 9
MODEL 10: GRINDEY ET AL.[17]

Substrate	Effects of nucleotides					
	dUMP	dTTP	dCTP	dGTP	dATP	ATP
CDP	I	I	0	I	I	0
UDP	I	I	0	I	I	0
GDP	0	S	0	I	I	0
ADP	0	0	0	S	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect.

J. Model 10

In 1975, Grindey et al.[17] published a much more complicated version of their previous model; this is summarized in Table 9. This model resembles more closely the models of Moore and Hurlbert and Reichard than did the 1973 model of Werkheiser et al. The major differences between this new model and Reichard's models are as follows: (1) the model of Grindey et al. represented the reductase as four separate enzymes; (2) the model included dCMP deaminase, which was stimulated by dCTP and inhibited by dTTP; and (3) ATP did not act as a general activator of all reductions or as a specific activator of pyrimidine reduction.

As with the 1973 model of Werkheiser et al., this model was used to predict the effects of pairs of drugs on DNA synthesis and thus on cell growth. The results compared very well with the results obtained in cultured L1210 cells by Grindey and Nichol.[15] In a few cases this new model predicted drug effects more accurately than the original model of Werkheiser et al., but in most cases there was little room for improvement. Therefore, both very simple and more complicated models were consistent with data from intact cells.

K. Model 11

A very comprehensive mathematical model of cellular metabolism was published by Jackson in 1980.[18] The entire model included 63 reactions which were grouped under the headings of "folate dependent reactions", "reactions of nucleotide metabolism" and "reactions involving membrane transport and activation of drugs". The portion of the model dealing with ribonucleotide reduction is summarized in Table 10. This part of the model is based on the 1966 model of Moore and Hurlbert, although the following differences should be noted:

1. Jackson included one result of Moore and Hurlbert which they had not included in their model. That is, he defined low and high concentrations of dTTP as activating and as inhibitory, respectively, for GDP reduction, whereas Moore and Hurlbert included dTTP only as an activator.
2. Jackson represented ribonucleotide reductase as three separate enzymes, thus omitting substrate competition, whereas Moore and Hurlbert included substrate competition.
3. Jackson omitted UDP reduction as a source of dTTP. All dTTP was thus derived via dCMP deamination.
4. He did not include ATP either as a general activator of all reductions or as a specific activator of pyrimidine reduction.
5. One final point which clearly distinguishes this model from either the Moore and Hurlbert model or that of Grindey et al. is that GDP and ADP reduction obey

Table 10
MODEL 11: JACKSON[18]

Substrate	Effects of nucleotides				
	dTTP	dCTP	dGTP	dATP	ATP
CDP	I	0	I	I	0
UDP					[a]
GDP	S/I[b]	0	I	I	0
ADP	S	0	S	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect.

[a] Not included in model.
[b] dTTP was defined as an activator at low concentrations and an inhibitor at high concentrations.

Michaelis-Menton kinetics. Thus at substrate concentrations which are similar to or less than the K_m, the rate of the reaction was proportional to the substrate concentration. For simplicity, the CDP concentration was maintained in a steady state. This is the first model in which the law of mass action plays a role in regulating ribonucleotide reduction.

Jackson used this model to predict deoxyribonucleotide pool sizes and cell growth rates, in cultured N1S1 cells, following drug treatment. The qualitative agreement of the model with the data from intact cells was good but the quantitative agreement was only fair. In two of the tests the model was qualitatively incorrect, and the author pointed out that this could be a result of errors either in the rate equation itself or in the kinetic parameters of the reductase as determined by Moore and Hurlbert.

L. Conclusions

Models 1 to 8 can be described as "first-generation" models in that they are based mainly on enzymological studies of ribonucleotide reductase. Models 9 to 11 are "second-generation" models in that they are based to varying degrees on the "first-generation" models and also on the results of studies in intact cells. Finally, there are other published models which are not discussed here that claim to be derived directly from "first-generation" models, but in fact are misrepresentations of these.

In order to compare the differences and similarities among the 11 models described above more easily, Tables 11 to 14 consider each of the four ribonucleotide reduction reactions separately. As these tables illustrate, the models often disagree on the effects of a given nucleotide effector on a particular reaction; however there are exceptions. For example, with two exceptions, all the models agree that dCTP has no effect on the reduction of any of the substrates, although Larsson and Reichard's data using purified *E. coli* ribonucleotide reductase show that dCTP can stimulate GDP reduction.[2] Also, ATP is not an inhibitor of any of the reactions, while dATP is always an inhibitor of ADP and GDP reduction and usually an inhibitor of CDP and UDP reduction as well. In the majority of the models, dTTP is an inhibitor of CDP and UDP reduction and a stimulator of GDP reduction, but only an inhibitor of ADP reduction in one case. Finally, dGTP is almost always a stimulator of ADP reduction. Nevertheless, the amount of disagreement among these models demonstrates the need for further study.

In future studies it will be important to determine if the differences noted in the various investigation summarized above are intrinsic features of the ribonucleotide reductases from different biological sources or are due, at least in part, to experimental conditions.

Table 11
EFFECTS OF NUCLEOTIDES ON CDP REDUCTION

	Nucleotide				
Model	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
1	I	0	I	I	S^R
2	I (+ATP) S (−ATP)	0	0	I	S^r
4	S	N.D.	S	S	N.D.
5	I	0	0	I	S
6	I	0	I	I	S^R
7	I	0	I	I	S
8	I	I (+ATP) S (−ATP)	I (+ATP) S (−ATP)	I	S^R
9	0	I	0	0	0
10	I	0	I	I	0
11	I	0	I	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement, r = partial requirement, N.D. = not determined.

Table 12
EFFECTS OF NUCLEOTIDES ON UDP REDUCTION

	Nucleotide				
Model	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
1	I	0	I	I	S^R
2	S (ATP^-) I (ATP^+)	0	0	I	S^r
4	S	N.D.	0	S	N.D.
5	I	0	0	I	S
6	I	0	I	I	S^R
7	I	0	I	I	S
8	I	I	I	I	S^R
9	I	0	0	0	0
10	I	0	I	I	0
11	*	*	*	*	*

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement, r = partial requirement, N.D. = not determined, * = UDP not included in model.

The latter might include the presence of interferring enzymes, basic assay procedures, purity of radioactive and nonradioactive reagents, etc. Recent studies have concluded that certain deoxyribonucleoside-resistant cultured cell lines contain mutant ribonucleotide reductases with altered sensitivity to nucleotide effectors.[32] Although these systems need more detailed characterization, they do suggest that it would not be surprising if ribonucleotide reductases from different tissues or species also might vary in their properties. If such were the case, then attempts to generalize about the modes of regulation of this enzyme would be even more fraught with uncertainty than now seems to be the case.

M. Questions Remaining

Two enzymological questions remain to be clarified regarding ribonucleotide

Table 13
EFFECTS OF NUCLEOTIDES ON GDP REDUCTION

	Nucleotide				
Model	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
1	S^R	0	I	I	S
2	S	0	S	I	0
4	S	N.D.	S	I	N.D.
5	S	0	0	I	S
6	S^R	0	I	I	S
7	S	0	I	I	S
8	S^R	S^R	S^R	I	S
9	•	•	•	*	•
10	S	0	I	I	0
11	S/I[a]	0	I	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement, N.D. = not determined, * = not included in model.

[a] a = dTTP was defined as an activator at low concentrations and an inhibitor at high ones.

Table 14
EFFECTS OF NUCLEOTIDES ON ADP REDUCTION

	Nucleotide				
Model	**dTTP**	**dCTP**	**dGTP**	**dATP**	**ATP**
1	S^R	0	S^R	I	0
2	S	0	S	I	0
4	S	N.D.	S	I	N.D.
5	0	0	S	I	S
6	0	0	S^R	I	S
7	0	0	S	I	S
8	I	0	S^R	I	0
9	0	0	0	I	0
10	0	0	S	I	0
11	S	0	S	I	0

Note: I = inhibitory, S = stimulatory, 0 = no effect, R = absolute requirement, N.D. = not determined.

reductase and its regulation. The first involves the complexity of the reaction, with its four ribonucleoside diphosphate substrates, four deoxyribonucleoside diphosphate products, the four deoxyribonucleoside triphosphates which actually or potentially are allosteric effectors, and ribonucleoside triphosphate effectors, especially ATP. Previous enzymological studies have dealt with only one or two of these factors at a time, but eventually it will be important to study the effect of varying the concentration of each single substrate and effector when all the other components are present at physiological concentrations.

A second question has to do with the generally held belief that the reduction of all four ribonucleoside diphosphates is carried out by a single enzyme. This traditional view has recently been challenged by several investigators. For example, Cory and Mansell observed changes in the ratio of CDP to ADP reductase activities during purification of ribonucleotide reductase from Ehrlich ascites tumor cells.[19,20] Considering the difficulties involved in assaying this enzyme in the presence of contaminating enzymes, this result is

not conclusive. Also, Eriksson et al. found that during the purification of calf thymus ribonucleotide reductase the ratio of CDP to GDP reductase activity was almost constant in the different fractions, and no separation of the different reductase activities occurred.[8]

Cory and Mansell have also found that the CDP and ADP reductase activities showed different sensitivities to dimethylformamide, dimethylsulfoxide, and to the periodate-oxidized derivative of AMP[19] and proposed the existence of either separate reductases for ADP and CDP or different substrate binding sites on the same enzyme. In addition, treatment of purified ribonucleotide reductase from Ehrlich ascites tumor cells with pyridoxyl phosphate-sodium borohydride, Triton X-100, or blue dextran caused significant changes in the ratio of the four activities.[21] Cory also studied competition among the substrates for reduction by the purified reductase and found that UDP had no effect on CDP reduction under conditions which activated the enzyme for both substrates.[21] Chang and Cheng, using ribonucleotide reductase purified from cultured Molt-4F cells, have found that UDP and CDP act as noncompetitive inhibitors of the reduction of one another, under conditions in which the reductase is active toward both substrates.[11] The results of Cory[21] and Chang and Cheng[11] are in contrast to the behavior of the purified *E. coli* reductase, in which CDP and UDP compete directly for reduction.[2]

Theiss and Fisher observed that treatment of cultured L5178Y cells with hydroxyurea inhibited the conversion of tritiated uridine to thymidine nucleotides but not to deoxycytidine nucleotides.[22] They suggested that the existence of separate reductases for CDP and UDP would explain the data.

Finally, Peterson and Moore measured the activities of CDP and ADP reductase in synchronized Chinese hamster cells.[23] They found that CDP and ADP reductase activities were 8-fold and 2.5-fold higher, respectively, in S phase than in G_1 phase. Lewis et al. measured the CDP and GDP reductase activities in synchronized Chinese hamster cells and found tenfold and twofold higher CDP and GDP reductase activities, respectively, in S phase than in G_1 phase cells.[24]

The questions raised by these various observations clearly need to be sorted out.

Finally, future models of the regulation of deoxyribonucleotide concentrations in intact cells will have to consider the role of deoxycytidylate deaminase as well as ribonucleotide reductase, as the deaminase is also under allosteric regulation. Both enzymes were indeed included in the model of Jackson, but in no other models.

III. EVALUATION OF MODELS IN INTACT MAMMALIAN CELLS

The models of ribonucleotide reductase and its regulation that have been considered above were formulated as aids to understanding the deoxyribonucleotide metabolism of intact cells, whether by prediction or by retrospective analysis. Although it is widely believed that the enzymological models are satisfactory images of the situation in intact cells, this point has in fact not received serious critical evaluation. There has been a tendency to accept the models as valid, in part because of their elegance and complexity, and in part because methods by which they can be tested have only recently become available and are still not in routine use. In addition, the fact that there are a number of somewhat different models, and not a single model, has not been taken into account in considering studies using intact cells. Against these negative points it must be admitted that at least some observations of deoxyribonucleotide metabolism in intact cells are indeed in accord with at least one or another of the enzymological models. This section considers the most complete and most relevant studies of deoxyribonucleotide metabolism and their relationship to the several enzymological models.

A. Experimental Approaches

Four types of evidence have been or may be adduced to support the idea that one or another enzymological model of ribonucleotide reductase regulation is indeed applicable to deoxyribonucleotide synthesis in intact mammalian cells. These are as follows.

1. Toxicity of Purine and Pyrimidine Deoxyribonucleosides

The toxicity of thymidine, deoxyadenosine, deoxyguanosine, etc. often is "explained" on the basis of models of ribonucleotide reductase regulation. Thus *if* the added deoxyribonucleoside is converted to the triphosphate, *if* the concentration of the triphosphate is elevated, *if* the additional triphosphate inhibits ribonucleotide reductase in the cell, *if* the synthesis of another deoxyribonucleoside triphosphate is blocked as a result, and *if* the concentration of this triphosphate becomes limiting for DNA synthesis (or if DNA synthesis is inhibited due to an imbalance in concentrations of different triphosphates), then one could conclude that such toxicity provides evidence for the models described above.

At least in the case of purine deoxyribonucleoside toxicity (see Reference 25), this full chain of events has not been demonstrated, although parts of it have. In lieu of this all one can conclude is that deoxyribonucleoside toxicity is consistent with these models (or at least potentially consistent because of differences among them). At the present time observations of toxicity by themselves constitute no proof whatsoever of the models.

2. Protection Against Deoxyribonucleoside Toxicity by Deoxycytidine

In many cases, purine and pyrimidine deoxyribonucleoside toxicity can be prevented by simultaneous addition of deoxycytidine and sometimes of other deoxyribonucleosides as well (alone or in combinations) (see Reference 25). Again, if the toxicity is in the first place in accord with one or another model of ribonucleotide reductase regulation, as described above, then concentrations of dCTP may fall and this may lead to inhibition of DNA synthesis. The effect of the added deoxycytidine, *if* it replenishes dCTP pools and thereby circumvents the inhibition of ribonucleotide reductase, would be in agreement with some or all of the models described above.

Again, however, this whole chain of events has not been demonstrated, and alternative explanations are possible. All one can conclude is that deoxycytidine protection is consistent with the models. At the present time, therefore, observations of deoxycytidine protection by themselves constitute no proof of the models.

3. Synthesis of Radioactive Deoxyribonucleotides from Labeled Ribonucleotides

To elevate the concentrations of individual deoxyribonucleoside triphosphates in a controlled and quantifiable fashion, and then to study the rates of reduction of all four radioactive ribonucleoside diphosphates in intact cells, would provide the most direct and clear-cut evidence for or against any of the models of ribonucleotide reductase regulation. Unfortunately, this approach is technically difficult to do well, and it has been attempted only in a few cases[26] (See Reference 25); the results so far have not been conclusive.

4. Concentrations of Deoxyribonucleoside Triphosphates

Relatively recent improvements in the methodology of deoxyribonucleotide measurements have led to several studies in which the concentration of one deoxyribonucleoside triphosphate has been altered (usually increased) by one or another technique, the concentrations of one or all of the other triphosphates have been measured, and the results obtained have been compared with one or another of the models of ribonucleotide reductase regulation described above.

5. Complications and Assumptions

Although some useful data has been gathered in studies such as those mentioned above, a number of real or potential complications often have not been taken into account.

1. The differences among the various enzymological models of ribonucleotide reductase regulation usually have neither been recognized, nor the consequences of having multiple models faced
2. In order for these data to be used to test the model, the data themselves should be evaluated using the following criteria:
   A. All the deoxyribonucleoside triphosphate pools should actually be measured. Often it is assumed that, for example, addition of deoxyadenosine or deoxyguanosine to cells results in an increase in dATP or dGTP, respectively.[26] This is not necessarily true, and alternative pathways of metabolism also need to be considered
   B. All the ribonucleoside triphosphate pools should also be measured. ATP plays an important role in the ribonucleotide reductase models developed by Reichard by regulating both the general activity of the enzyme and the substrate specificity. Therefore, any test of the model must consider the possible effects of changes in ATP. Also, the possibility that changes in the other ribonucleoside triphosphate pools affect the deoxyribonucleotide pools should be considered
   C. In long-term experiments under growth-inhibitory conditions the cell cycle distribution of the population should be measured because shifts may occur, and cells in different phases of the cell cycle have different deoxyribonucleotide pool sizes.[27,35] Changes in cell volume should be measured as well
3. As mentioned above, the most commonly used approach to the evaluation of models of the regulation of ribonucleotide reduction using intact cells is to measure concentrations of the deoxyribonucleoside triphosphates when the pool size of one or another of these is deliberately perturbed. This approach, however, really involves an important underlying assumption, namely that deoxyribonucleoside triphosphate concentrations directly and accurately reflect the rates of ribonucleotide reduction and this process only. In order for this to be true, three conditions must be met:
   A. The rates of deoxyribonucleoside diphosphate phosphorylation must be equal to or greater than the rates of ribonucleotide reduction
   B. Deoxyribonucleotide catabolism must not compensate for changes in the rate of ribonucleotide reduction
   C. Deoxyribonucleotide consumption, via DNA synthesis or via catabolism, must be constant and must be sufficient to cause a triphosphate pool to decline if its synthesis is inhibited. Obviously if the rate of deoxyribonucleotide consumption responded immediately to decreases in the deoxyribonucleotide pools, then large drops in pool size would not be observed.

These basic assumptions have not been tested directly, although there is some evidence available that pertains to two of them. Assumption A seems reasonable in view of the fact that the deoxyribonucleoside diphosphate concentrations are a fraction of the triphosphate concentrations in cells, indicating that the equilibria favor the triphosphates. Furthermore, radioactive precursors such as [^{3}H]thymidine and [^{3}H]deoxycytidine are readily phosphorylated to the triphosphate level, with less than 10% of the label being present as diphosphates.

We were unable to find conclusive published evidence relating to assumption B, and further study is required.

There is good evidence that assumption C is justified. Bjursell and Reichard have found that although the rate of DNA synthesis in S-phase CHO cells declined as the size of the dCTP pool declined during thymidine treatment, DNA synthesis continued even when the dCTP pool had decreased by 87%.[7] Also, CHO cells continue to multiply and therefore to synthesize DNA, even when the dCTP pool is 3% of control values.[36]

B. The Experimental Evidence

With these caveats in mind, it is now possible to review and evaluate critically the available experimental evidence that relates measurements of deoxyribonucleoside triphosphate concentrations to models of ribonucleotide reductase regulation. In the following discussion, the model of Thelander and Reichard[10] has been taken as the basis of comparison.

Lowe and Grindey[28] found that in L1210 cells treated with thymidine an increase in dTTP corresponded to a decrease in dCTP, but the dCTP concentration leveled off at approximately 70% of control values and remained constant up to the maximum value of dTTP achieved (500% of control). They found that a dGTP concentration of 150% of control corresponded to a dTTP concentration of 300% of control. Further increases in dTTP, up to 500% of control, had no further effect on dGTP. Although these results appear to agree with the model, it should be noted that these experiments were long term, used growth inhibitory conditions, and ribonucleoside triphosphate concentrations were not measured.

Grindey et al.[29] obtained similar results with CCRF-CEM cells treated with thymidine. They found that a dTTP concentration of 800% of control corresponded to a dCTP concentration of 58% of control, a dGTP concentration of 500% of control, a dATP concentration of 380% of control, and an ATP concentration of 245% of control. Unfortunately the increases in ATP and dATP make interpretation of the results difficult because Reichard's more recent ribonucleotide reductase models state that it is the ratio of ATP to dATP that determines the overall activity of the enzyme, but also an increase in ATP is predicted to increase pyrimidine reduction.[6,11] Pool size measurement in these experiments were made after 45 hr under growth-inhibitory conditions.

Tattersall et al.,[30] using phytohemagglutin-stimulated human lymphocytes treated with thymidine, found that a dTTP concentration of 600% of control corresponded to a dCTP concentration of 65% of control, a dGTP concentration of 118% of control, and a dATP concentration of 0% of control. These results do not agree well with the model. The drastic decrease in the dATP concentration in particular is not explained by the model. With deoxyadenosine treatment they found that a concentration of dATP of 570% of control corresponded to a dTTP concentration of 22% of control and dCTP and dGTP concentrations of 0% of control. These results agree with the model. Treatment with deoxycytidine increased the dCTP pool to 890% of control and had no effect on the other deoxyribonucleoside triphosphate pools. Again, these results agree with the model which assigns no regulatory role to dCTP. The treatment time in these experiments was 1 hr; therefore significant cell synchronization could not have occurred. Ribonucleotide concentrations were not measured.

Lowe et al.,[31] using L5178Y cells treated with deoxyadenosine plus deoxycoformycin, found that dATP concentrations of 400% of control corresponded to dCTP concentrations of 45% of control and dGTP and dTTP concentrations of 100% of control. Ribonucleoside triphosphate pools were unchanged. These results do not agree with the model which predicts an increase in dATP to inhibit reduction of all substrates. These data were obtained using short-term incubations under growth-inhibitory

conditions. The cell cycle distribution, as measured by flow cytofluorometry, had not changed significantly.

Ullman et al. have used a mutant mouse T-lymphoma cell line which is resistant to the cytotoxic and growth-inhibitory effects of deoxyadenosine, deoxyguanosine, and thymidine to test the Thelander-Reichard model for ribonucleotide reductase.[32] They concluded that their data were in almost complete agreement with the model, the one exception being that pyrimidine reduction was not sensitive to inhibition by dATP but GDP reduction was. This is an important finding because the Thelander-Reichard model states that ATP and dATP compete for the same activity site and that when dATP is bound the enzyme is completely inactive.

Finally, two recent studies by the present writers may be mentioned; both used cultured Chinese hamster ovary cells. In one case the relationship between ribonucleotide concentrations and those of the corresponding deoxyribonucleoside triphosphates were evaluated, both when ribonucleotide concentrations were elevated and when they were lowered below control values.[33] In both cases, corresponding changes in deoxyribonucleotide concentrations were observed, although the magnitude of the change varied from one nucleotide family to another.

The simplest explanation for these results is based on mass action: the rate of ribonucleotide reduction is dependent on the concentration of the substrates, the corollary being that under these conditions other regulatory mechanisms, including those proposed in the models considered here, are inoperative or of less importance than substrate concentrations. Most of the results obtained were found to be inconsistent with the predictions of the model of Thelander and Reichard (Model 7, above). However, these experiments were not designed rigorously to test any of the models for the regulation of ribonucleotide reductase, but they definitely do support the more modest conclusion that factors other than or in addition to allosteric effects are reasonably important in the regulation of ribonucleotide regulation in intact cells.

In the second study the concentrations of individual deoxyribonucleoside triphosphates were elevated and lowered by manipulation with drugs or naturally occurring deoxyribonucleosides, and concentrations of ribo- and deoxyribonucleotides were measured.[34] The results were consistent with the predictions of the Thelander-Reichard model that dTTP inhibits CDP reduction and stimulates GDP reduction and that dGTP inhibits CDP reduction. However, the results are contrary to the hypothesis that dGTP inhibits UDP reduction and stimulates ADP reduction and that dATP inhibits CDP reduction, and hence this particular model does not seem to apply totally under the conditions used.

C. Conclusions

Some experimental results appear to be consistent with the predictions of one or another model of the regulation of ribonucleotide reductase based on enzymological studies. However, not all of the data fit these models, and the basis and significance for these deviations are not yet known. The extent to which any of the models accurately portrays the actual mode of regulation of ribonucleotide reductase in intact cells really has yet to be determined. It seems questionable whether measurements of deoxyribonucleoside triphosphate concentrations under various concentrations will be sufficient to resolve the many questions that remain and to provide the critical evaluation of the various models that is required for further progress to be made in this field. It is hoped that other approaches, especially measurements of movement of radioactivity from ribonucleotides to both soluble and DNA deoxyribonucleotides, will be employed and will provide clearer evidence.

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