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Mathematical Simulation of the Interaction of Drugs That Inhibit Deoxyribonucleic Acid Biosynthesis

W. C. WERKHEISER,[1] G. B. GRINDEY, AND R. G. MORAN

Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14203

C. A. NICHOL

Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709

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SUMMARY

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A mathematical model was devised which could satisfactorily simulate the effects of combinations of inhibitors of DNA biosynthesis on the growth of L1210 leukemic cells *in vitro*. The model represents an open steady-state system regulated by a network of feedback controls. The behavior of the model was surprisingly insensitive to variations in the values of its 15 parameters, implying that it was the basic shape of the network which was responsible for the simulation. The presence of any one (or two) of the inhibitors caused pronounced changes in the concentrations of *all* the intermediate compounds in the resulting new steady state. Thus, in such a closely regulated system, the effects of a perturbation in one region will not be localized, but will be manifested throughout the entire system. Furthermore, it was predicted that such feedback regulation could lead to perplexing experimental results; for instance, extensive inhibition of a pathway within such a network could result in an increased, decreased, or even unchanged pool size of the product of this pathway. It was also observed that the inhibitor-induced process of passing from the original steady state to a new steady state was complex. Concentrations of intermediates need not change smoothly from one state to the other, but may overshoot, transiently move in a direction opposite to the final concentration, or undergo a damped oscillation. Attempts to deduce the effect of an inhibitor on such a system by consideration of the initial effects therefore may be misleading. Finally, experiments using the model indicated that the intensity of interaction of drugs in combination, whether antagonistic or synergistic, increased as the degree of inhibition of the system increased. Thus a combination which was mildly synergistic when studied at 50% inhibition became intensely so at 90% inhibition. This unexpected prediction has significance in the evaluation of studies in combination chemotherapy.

INTRODUCTION

A recent report by Grindey and Nichol has described the effects of combinations of inhibitors of DNA biosynthesis on the growth of L1210 leukemic cells *in vitro* (4). The patterns of interaction observed were

[1] Deceased, September 1, 1972.

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quite unexpected, and all attempts to explain them by reference to current concepts of *sequential* (*5*), *concurrent* (*6*), and *complementary* (7) inhibition were unsuccessful (4). Further efforts led to the development of a model which consisted of an open steady-state system of metabolic interconversions catalyzed by drug-sensitive Michaelis-Menten enzymes and regulated by feedback inhibition. Investigation of this model by mathematical simulation showed encouraging agreement with experimental data and yielded provocative predictions accessible to experimental confirmation.

DESCRIPTION OF MODEL

The enzymes involved in the synthesis of DNA *de novo* form a unidirectional network for its synthesis from the four ribonucleoside diphosphates. The kinetic behavior of several of these enzymes is modulated by feedback activation and inhibition by a variety of the intermediate compounds within this system. In order for this network of reactions to permit a steady flow of deoxynucleoside triphosphates into DNA, it is necessary for it to approximate an open steady-state system in which nucleoside diphosphates provide a source of deoxynucleotides at one end and the growing DNA polymer acts as a sink at the other. It seems reasonable to assume that the nucleoside diphosphate precursors remain at a constant concentration, unaffected by events within the network, since the total flux into DNA is certainly small compared with the other reactions these compounds undergo. This brief description of the main features of DNA biosynthesis is incomplete in many aspects; yet its complexity is already too great to warrant attempts to investigate its behavior mathematically.

To reduce the complexity of the system to be simulated, we decided to include only the enzymes directly affected by the inhibitors previously studied by Grindey and Nichol (4). Enzymes of the "salvage" pathways were not considered, since the experimental data to be simulated were obtained in the absence of an exogenous source of purines or pyrimidines. Feedback paths were introduced as necessary, both to maintain stability of the system and to adjust the model to mimic the experimental data. Feedback inhibition was considered to be of a simple competitive type as would occur in the limiting case of a K-type Monod-Wyman-Changeux model of allosteric interaction (8) for an enzyme with only one subunit.

The drugs whose action was to be simulated are shown in Table 1, together with citations to the relevant literature on their sites of action. On this basis, IQ-1[2] was represented as a noncompetitive inhibitor of ribonucleoside diphosphate reductase; FUdR, as a competitive inhibitor of thymidylate synthetase; and ara-A and ara-C, as inhibitors of DNA polymerase competitive with dATP and dCTP, respectively. The metabolic activation required in the case of the latter three compounds was not included in the model. The case of MTX is more complex. In its role as an inhibitor of dihydrofolate reductase, its presence results in depletion of the tetrahydrofolate pools which provide the second substrate for thymidylate synthetase. The effect of this reduction in cofactor concentration on the enzyme velocity is qualitatively similar to that predicted for an inhibitor that is noncompetitive with respect to the first substrate, dUMP. MTX was therefore represented as a noncompetitive inhibitor of this enzyme. Since the interaction of MTX and ara-C was unchanged in the presence of deoxyadenosine, a condition which reverses the purine-inhibitory effect of MTX (4), it was felt justified to ignore this action of this drug in the construction of the model.

The final model is shown in Fig. 1 and the symbols are described in Table 2. No provision was made to include guanine nucleo-

versity of New York at Buffalo. A portion of this work has been submitted (by G. B. G.) to the Faculty of the State University of New York at Buffalo in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Preliminary accounts of this work have appeared (1–3).

[2] The abbreviations used are: IQ-1, 1-formylisoquinoline thiosemicarbazone; FUdR, 5-fluorodeoxyuridine; ara-A, 9-β-D-arabinofuranosyladenine; ara-C, 1-β-D-arabinofuranosylcytosine; MTX, methotrexate.

TABLE 1

Primary sites of action for drugs employed by Grindey and Nichol (4)

Drug	Primary site of action	Assumed type of inhibition	References
IQ-1	Ribonucleoside diphosphate reductase	Noncompetitive	9–11
MTX	Dihydrofolate reductase [5,6,7,8-tetrahydrofolate: NAD(P) oxidoreductase, EC 1.5.1.3]	Complex (see text)	12–14
FUdR	Thymidylate synthetase	Competitive	15
Ara-A	DNA polymerase	Competitive	16–17
Ara-C	DNA polymerase	Competitive	16–20

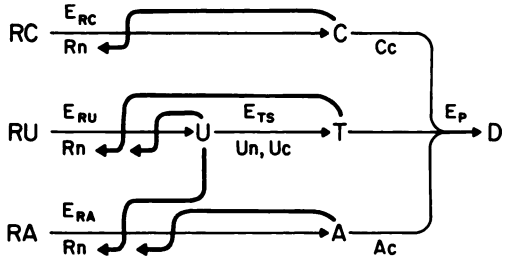

FIG. 1. *Simplified physical model used in construction of mathematical model*

The symbols are defined in Table 2. Metabolic conversions are designated by thin lines; feedback inhibition by intermediates, by heavy lines.

tides in the model, since none of the drugs employed has a direct effect upon them *in vivo*. The synthesis of the intermediates *C*, *T*, *A*, and *U* is regulated by feedback inhibition upon the first enzyme in their biosynthetic pathways. In addition, *U* also inhibits the synthesis of *A*. In this open system the concentrations of substrates *RC*, *RU*, and *RA* are constant and the product, *D*, is irreversibly removed from the system. The inhibitor *Rn* is defined as a strict noncompetitive inhibitor of E_{RC}, E_{RU}, and E_{RA}. *Un* and *Uc* are noncompetitive and competitive inhibitors of E_{TS}. *Cc* and *Ac* are inhibitors of E_P, competitive with *C* and *A*, respectively.

In formulating a mathematical model based on this rudimentary physical model, we have made the simplest assumptions possible. All enzymes were assumed to operate according to a simple Michaelis-Menten formulation, and the concentrations of all substrates and inhibitors are represented in terms of specific concentration, i.e., molar concentration divided either by Michaelis constant (K_m) or by inhibition constant (K_i) (21). The five feedback constants are defined as the ratio K_m/K_i, where K_m applies to the compound as a substrate and K_i applies to the compound as a feedback inhibitor. E_{RC}, E_{RU}, and E_{RA} were assumed to have constant maximal velocities with respect to each substrate, since no rate law describing the effector-dependent specificity of ribonucleoside diphosphate reductase has yet been put forward. In deriving an equation for DNA polymerase (E_P), it was assumed that, during the insertion of a single nucleotide residue, the enzyme displays simple Michaelis-Menten kinetics with respect to the appropriate deoxynucleoside triphosphate and that the product, *D*, is a random trimer of *A*, *C*, and *T*. With these assumptions, the velocity of a single step is given by Eq. 1. Since the time to synthesize the polymer is the sum of the times required to introduce each precursor, Eq. 2 gives the differential equation representing this process.

$$v = \frac{V_s}{1 + 1/s} \qquad (1)$$

$$dt = \frac{1/V_{PC}(1 + 1/C) + 1/V_{PT}(1 + 1/T) + 1/V_{PA}(1 + 1/A)}{3} dD \qquad (2)$$

V_{PC}, V_{PT}, and V_{PA} are the maximal velocities of the steps introducing *C*, *T*, and *A*, respectively, and the divisor 3 is a statistical factor which recognizes that incorporation of each component comprises only one-third of the over-all reaction. Figure 2 shows the system of algebraic equations which describe the properties of the individual enzymes of the model. Figure 3 presents the system of

TABLE 2

Symbols used in model

For definitions of the symbols, see the text.

Symbol	Counterpart
E_{RC} , E_{RU} , E_{RA}	Ribonucleoside diphosphate reductase
E_{TS}	Thymidylate synthetase
E_P	DNA polymerase
RC	CDP
RU	UDP
RA	ADP
D	DNA
U	dUMP
C	dCTP
T	dTTP
A	dATP
Rn	IQ-1
Un	MTX
Uc	FUdR 5′-monophosphate
Cc	Ara-C 5′-triphosphate
Ac	Ara-A 5′-triphosphate
Kcc	Parameter describing feedback of *C* upon its own synthesis
Ktt	Parameter describing feedback of *T* upon its own synthesis
Kaa	Parameter describing feedback of *A* upon its own synthesis
Kuu	Parameter describing feedback of *U* upon its own synthesis
Kua	Parameter describing feedback of *U* upon synthesis of *A*
V_{RC} , V_{RU} , V_{RA}	Individual maximal velocities
V_{TS} , V_{PC} , V_{PT} , V_{PA}	Individual maximal velocities
v_{RC} , v_{RU} , v_{RA} , v_{TS} , v_P	Velocities of individual reactions
v	Steady-state velocity

For definitions of the symbols, see the text.

$$\nu_{RC} = \frac{V_{RC}}{\left[1+\frac{1}{RC}(1+C\cdot Kcc)\right]\left[1+Rn\right]}$$

$$\nu_{RU} = \frac{V_{RU}}{\left[1+\frac{1}{RU}(1+U\cdot Kuu+T\cdot Ktt)\right]\left[1+Rn\right]}$$

$$\nu_{RA} = \frac{V_{RA}}{\left[1+\frac{1}{RA}(1+U\cdot Kua+A\cdot Kaa)\right]\left[1+Rn\right]}$$

$$\nu_{TS} = \frac{V_{TS}}{\left[1+\frac{1}{U}(1+Uc)\right]\left[1+Un\right]}$$

$$\nu_P = \frac{3}{\left[\frac{1}{V_{PC}}\left[1+\frac{1}{C}(1+Cc)\right]+\frac{1}{V_{PT}}(1+\frac{1}{T})+\frac{1}{V_{PA}}\left[1+\frac{1}{A}(1+Ac)\right]\right.}$$

FIG. 2. *Equations representing individual enzymes of mathematical model*

Each reaction step is considered to be catalyzed by a Michaelis-Menten enzyme with no back reaction. Drugs inhibit by either a strictly competitive (*Uc*, *Cc*, *Ac*) or noncompetitive (*Rn*, *Un*) mechanism. Feedback control results from competitive inhibition of the reactions by intermediates (see Fig. 1).

$$\frac{dC}{dt} = \nu_{RC} - \nu_P \qquad \frac{dT}{dt} = \nu_{TS} - \nu_P$$

$$\frac{dU}{dt} = \nu_{RU} - \nu_{TS} \qquad \frac{dA}{dt} = \nu_{RA} - \nu_P$$

$$\nu = \nu_{RC} = \nu_{RU} = \nu_{RA} = \nu_{TS} = \nu_P$$

FIG. 3. *Differential equations and steady-state conditions representing mathematical model*

differential equations whose solution describes the transient behavior of the model in approaching a steady state. In the steady state the concentrations of the intermediates *C*, *U*, *T*, and *A* are constant, implying that each differential equation is to be set equal to zero. This has the consequence that the velocities of all the enzymes in the model are identical in the steady state, as shown at the bottom of Fig. 3. Insertion of this steady-state velocity, *v*, in the equations of Fig. 2 permitted their simultaneous solution by

the maximum neighborhood algorithm of Marquardt (22). Solution of the simultaneous differential equations of Fig. 3, for investigation of the transient behavior of the model, was performed with the use of the digital simulation language MIMIC.

SIMULATION OF EFFECTS OF DRUG COMBINATIONS

For the basic model the 15 parameters (seven maximal velocities, five feedback inhibition constants, and the specific concentrations of the three prime substrates) were all arbitrarily assigned the value unity. This choice, the validity of which will be discussed below, made it possible to solve the system of nonlinear algebraic equations of Fig. 2 analytically.

The strategy used in simulating the cell culture experiments of Grindey and Nichol (4) can be described as follows. The uninhibited steady-state velocity, v, and the normal concentrations of the four intermediates, C, U, T, and A, are obtained by solving the set of equations (Fig. 2) with the concentrations of exogenous inhibitors set to zero. Since Grindey and Nichol chose 50% inhibition as the level of effect in their experiments, the velocity, v, is set to 50% of the steady-state value and, for each inhibitor, the equations are solved for the new values of the concentrations of intermediates and for the concentration of drug corresponding to this level of effect (ED_{50}).

To study the effect of combinations of two drugs, the velocity was once again set to one-half the steady-state value, the concentration of one inhibitor was set to a fraction of its ED_{50}, and the equations were solved for the concentrations of the second inhibitor and the various intermediate compounds. The resulting data were used to construct isobols[3] corresponding to those presented by Grindey and Nichol (4). The results are presented in Fig. 4, where the points represent the experimental data (4) and the lines show the behavior of the mathematical model. The over-all agreement is good and suggests that this model does in some sense mimic actual events in the intact cell. Certain comparisons emphasize the significance of that agreement. When MTX was studied in combination with either ara-C or ara-A, strong antagonism occurred. When FUdR was combined with ara-C, the interaction was also antagonistic. However, when FUdR was combined with ara-A, only additive or slightly antagonistic interactions were seen in both the actual and simulated experiments. Since both arabinosides inhibit the same enzyme, this finding was unexpected and illustrates the fidelity of the modeling. A similar illustration is provided by the mild antagonism with IQ-1 and MTX and the mild synergism with IQ-1 and FUdR seen in both types of experiments.

EFFECTS OF PARAMETER VARIATIONS

The arbitrary assignment of parameter values mentioned earlier was cause for concern about the generality of the model. Since little information is available which could be used to provide realistic estimates of these parameters, the effect of increasing or decreasing each in turn was used in an effort to assess the stability of the model. The equations of Fig. 2 were therefore programmed for a digital computer, and the entire set of experiments was calculated with each parameter set separately to 0.1 and to 10.0. The 30 experiments yielded 270 interaction patterns. In many instances the intensity of the interaction was changed by parameter variation, but in only 16 cases was there a change in the *type* of interaction. In four of these cases it was clear that the quantitative change in parameter value re-

[3] The isobol is an effective condensation of the dose-response curves obtained in studies of the inhibitory effects of pairs of drugs (6). A suitable level of effect (50% inhibition of growth in this instance) is selected and the concentrations of the two inhibitors, both singly and in combination, which result in this end point are read from the dose-response curves. The isobol is then constructed by plotting the concentration of one drug against the corresponding concentration of the other drug which results in the desired level of effect. For convenience in comparing replicate experiments, concentrations of each drug are plotted as a fraction of the concentration of that drug required to yield the desired end point by itself. Points on the falling diagonal connecting unity on the ordinate with unity on the abscissa indicate additivity; points below or above this line represent synergism or antagonism, respectively.

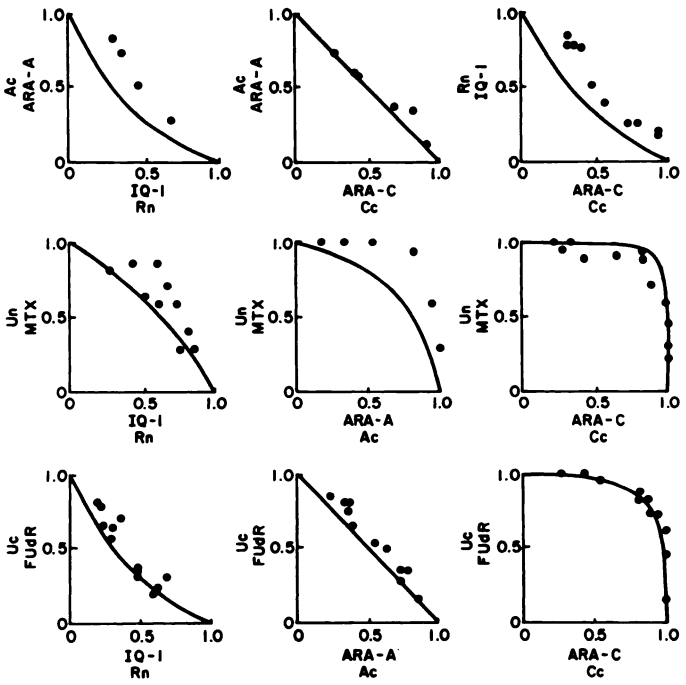

FIG. 4. *Isobols*[3] *showing interactions of pairs of drugs on growth of L1210 as observed in vitro* (*4, 23*) *(points) and on rate of DNA synthesis as predicted by the model (solid lines)*

sulted in a qualitative change in the structure of the model. In the remaining 12 (4.4%), no explanation for the changed behavior was evident. Therefore the correspondence of the model with the experimental data appears to be primarily a function of the *structure* of the model and only secondarily of the particular parameter values used to evaluate it. These conclusions are similar to those of Garfinkel (24), who stated in regard to a model of brain metabolism that "the behavior of the model is generally not very sensitive to changes in parameters; a drastic change in behavior usually requires the introduction of a new idea."

VARIATION OF DRUG INTERACTIONS WITH LEVEL OF EFFECT

An end point of 50% inhibition of cell growth was used in the experiments shown in Fig. 4. Additional calculations were performed with the basic model in which the end point ranged from 5 to 95% inhibition. The results are shown in Table 3, which represents the interactions obtained at several effect levels. For this purpose the isobols[3] illustrating the interactions are replaced by a number indicating the equal fraction of effective doses, the EFED (see footnote, Table 3), which characterizes their shapes. In all cases it was found that additive combinations were unchanged by variation of level of effect while the intensity of either synergism or antagonism was increased with increasing degrees of inhibition. Thus a mildly synergistic interaction observed in an experiment carried out using an end point of 50% inhibition would be predicted to be intensely synergistic if used in animals at maximally effective dosage levels. This prediction lends itself to experimental verification.

EFFECTS OF INHIBITORS ON CONCENTRATIONS OF INTERMEDIATES

Recent studies on the effects of metabolic perturbations on the pool sizes of DNA precursors have assumed an increasing importance in investigations on the regulation of DNA biosynthesis. It was therefore of interest to examine the behavior of the con-

centrations of intermediates in the model. The influence of ED_{50} concentrations of inhibitors of each enzyme in the basic model on the pool sizes of the four intermediate compounds is shown in Table 4. It is apparent that the presence of *any* one inhibitor can cause the concentrations of *all* the intermediates to change significantly, in some instances by as much as 8–9-fold.

TABLE 3

Enhancement of inhibitor interactions as a function of effect level

Pair of inhibitors	Equal fraction of effective doses[a]		
	ED_{10}	ED_{50}	El_{90}
Ac + *Cc*	0.50	0.50	0.50
Ac + *Uc*	0.50	0.50	0.50
Rn + *Cc*	0.50	0.40	0.21
Rn + *Ac*	0.48	0.39	0.21
Rn + *Uc*	0.49	0.39	0.21
Rn + *Un*	0.52	0.56	0.58
Un + *Ac*	0.54	0.66	0.84
Uc + *Cc*	0.61	0.84	0.97
Un + *Cc*	0.65	0.91	0.99

[a] An equation describing any symmetrical isobol can be written $(1/F_A - 1)\ (1/F_B - 1) = K$, where F_A and F_B are the fractions of the effective doses of drugs A and B, respectively. The point at which F_A equals F_B always lies on the rising diagonal of the graph. This point, where equal fractions of the effective doses (EFED) of the two drugs give the desired level of effect, represents a convenient and characteristic parameter to describe the shape of any isobol. An EFED of 0.5 defines additivity, while values between 0 and 0.5 indicate synergism and values between 0.5 and 1, antagonism. The magnitude of the deviation from 0.5 is a measure of the intensity of the interaction.

Thus a given inhibitor not only reduced the concentration of the product of the inhibited reaction, as might be expected, but also had dramatic and unpredictable effects throughout the entire network.[4] Examination of the pool size changes occurring in the parameter variation studies cited above emphasized this unpredictability. For example, lowering the value of *Kuu*, the parameter which describes the feedback of *U* on its own synthesis to 0.3 or 0.1, while holding the remaining parameters at unity, resulted in the concentration of *T* being *increased* by the presence of *Un* or *Uc*, with no appreciable change in any of these drug interactions. This unexpected finding of an increase in the concentration of the product of an inhibited reaction in these theoretical studies is of particular interest in relation to the observation of Baumunk and Friedman (26) that neither MTX nor FudR, at concentrations causing about 93% inhibition of deoxycytidine incorporation into DNA, had any significant effect on the concentration of TTP.

Since the value of the feedback parameter *Kuu* was found to affect the direction of change of the pool size of *T* in the presence of *Un* or *Uc*, the relationship between *Kuu* and the concentrations of intermediates *A* and *T* was more extensively examined. As can be seen in Fig. 5, the uninhibited steady-state pool size of *A* or *T* was relatively unaffected by the value assigned to this feedback parameter. However, in the presence of an ED_{50} of *Un*, *A* or *T* could increase, decrease, or, indeed, remain constant, depending

[4] This prediction has been more thoroughly treated in ref. 25.

TABLE 4

Effects of inhibitors on steady-state concentrations of intermediates

Inhibitor	*C*		*U*		*A* and *T*[b]	
	Concentration[a]	Per cent of control	Concentration	Per cent of control	Concentration	Per cent of control
None	0.94		0.52		0.42	
Rn	0.37	39	0.21	40	0.12	29
Un or *Uc*	3.88	413	3.74	719	0.14	33
Ac or *Cc*	3.88	413	0.21	40	3.67	874

[a] Concentrations are given in arbitrary units.

[b] The equality of *A* and *T* in this table is a consequence of the particular parameter values used and is not intrinsic to the model.

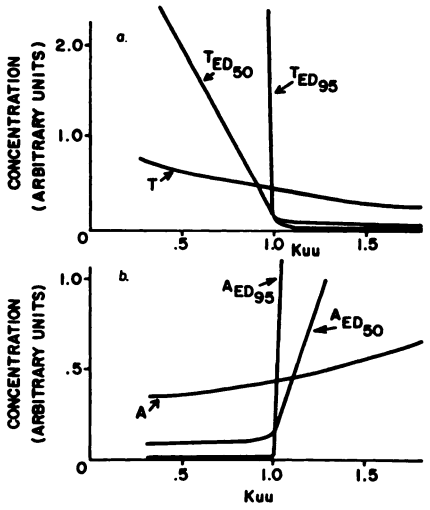

FIG. 5. *Effect of variation of feedback parameter Kuu on concentrations of intermediates T (a) and A (b) in uninhibited steady state (T, A), and in the presence of a 50% ($T_{ED_{50}}$, $A_{ED_{50}}$) or 95% ($T_{ED_{95}}$, $A_{ED_{95}}$) inhibitory concentration of a noncompetitive inhibitor of enzyme E_{TS} (Un)*

As *Kuu* increases, the intermediate *U* becomes a more powerful inhibitor of its own synthesis.

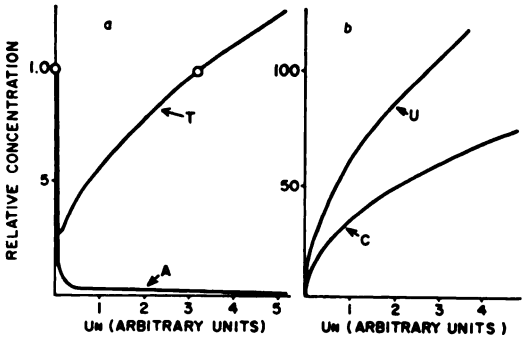

FIG. 6. *Steady-state concentration of intermediates as a function of Un*

The pool sizes have been normalized so that the uninhibited steady-state concentration is 1.0. The presence of 3.55 units of *Un* results in an inhibited steady state in which the rate of synthesis of product *D* is inhibited by 95% but *T* is present in the same concentration as in the uninhibited steady state (O). Notice that the ordinates in Fig. 6a and b differ by a factor of 100.

upon the value of *Kuu*. When a 95% degree of inhibition is considereed, a situation which closely resembles that of the experiments of Baumunk and Friedman (26), the changes in the pool sizes of *A* and *T* are predicted to be even more dramatically dependent upon this parameter. It is notable that, at either degree of inhibition, there is a corresponding value of *Kuu* that results in the same concentration of *T* in control and inhibited states. A re-examination of the interactions seen in Fig. 4, using either of these values of *Kuu*, showed the same pattern of interaction for all pairs of inhibitors.

The value of *Kuu* which resulted in an unchanged pool size of *T* at the 95% level of inhibition was now used to calculate the effects of increasing *Un* (and hence increasing degrees of inhibition) on the pool sizes of intermediates. As shown in Fig. 6, the concentration of *T* in the inhibited steady state is predicted to be decreased at any degree of inhibition less than 95%, to be identical with control at this level of inhibition and actually to exceed control concentrations at more extensive inhibition. At levels of inhibition greater than 95% (Fig. 6), the pool sizes of *C* and *T* are greater than those observed in the uninhibited steady state. Thus the decreased concentration of *A* (Fig. 6a) must be limiting the rate of synthesis of the product *D*, and hence the flux through the over-all steady-state system. In fact, since *C* is not limiting at any level of inhibition, and since inhibition is not correlated with *T*, the degree of inhibition in this model is apparently best explained not by a lack of availability of the product of the inhibited reaction (*T*) but rather by a severely decreased pool of another intermediate (*A*) within this system (Fig. 6a). When the time course of the concentrations of intermediates was examined, it was found that the transients induced by exposure to an ED_{95} of drug *Un* were qualitatively the same as those seen in Fig. 7 for an ED_{50} dose. *Un* initially caused a decrease in *T*, but the feedback control resulted in a readjustment of the concentration of intermediates that was far from intuitively obvious and eventually resulted in steady-state inhibition because of an insufficient pool of *A*. From these results it is apparent that the regulatory controls built into this model are responsible for the unpredictable effects of inhibitors on pool sizes of intermediates. It would appear

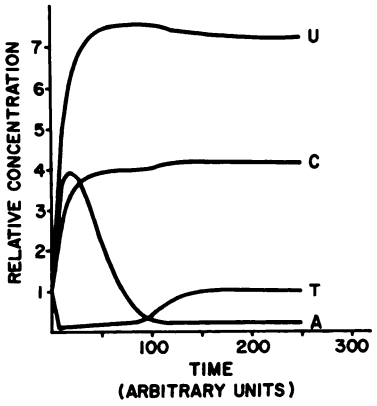

FIG. 7. *Changes in pool sizes as a function of time following introduction of a 50% inhibitory concentration of Un to a simulated tissue originally in the normal steady state*

dangerous, then, to attempt to predict effects of an inhibitor on the pool sizes in any regulated system (e.g., cellular metabolism) merely by considering the expected initial direction of change. Yet this is the intuitive approach one would make in qualitative efforts to predict the behavior of such a system. It has been suggested by von Bertalanffy (27) that this phenomenon of "false start" is a general characteristic of open systems.

That the unexpectedly complex time course of changes in metabolite concentrations which was observed in the model also occurs in real systems is shown by the equally complex behavior reported recently by Smith and Holmes (28) in studies on the regulation of a arginine biosynthesis in *Blastocladiella emersonii*. These authors found that, following an instantaneous change in the arginine concentration in the medium, the concentrations of the various arginine precursor pools initially responded in a relatively slow manner, followed by a period of rapid fluctuations leading to the new steady-state levels. In a similar vein, in a study on genetic selection, a phenomenon which parallels DNA synthesis in the intensity of its regulation, Kirkman (29) concludes that the "genes . . . are capable of assuming intermediate distributions that might not be anticipated from either the rate of the process or the final distribution of the genes."

DISCUSSION

The model used in these studies bears little direct resemblance to the actual network of DNA biosynthesis. Not only have most of the paths in the real system been eliminated, but the pattern of feedback regulation is probably quite different. Indeed, the structure of the model implies that dCTP is a regulatory effector of ribonucleotide reductase, although this is known not to be true. It is therefore perhaps surprising that the model so well mimics reality. Since the model is so insensitive to parameter variations, it seems probable that the important feature is its over-all shape or topology. The presence or absence of particular reactions or feedback paths would then be of importance only if the resultant model had a different fundamental topology. It is of interest that it was necessary to introduce an asymmetry in the model in the form of the feedback of U on the synthesis of A. It is probable that another asymmetry would have worked as well. In fact, the kinetics of ribonucleoside diphosphate reductase detailed by Brown and Reichard (30) for the *Escherichia coli* enzyme and by Moore and Hurlbert (31) for the Novikoff hepatoma enzyme both imply multiple asymmetrical feedback loops in the regulation of these enzymes.

It seems from this work that the effects of combinations of drugs on a tightly regulated, open network of reaction paths such as occurs in the synthesis of DNA *can* be explained by the type of model presented here. No claim is made that this model represents the real system in any of its details, but it seems likely that the principles of its construction are reasonable. This conclusion is strengthened by confirmation of the prediction based on the model that a single perturbation of the system can cause profound transient and steady-state changes in the concentrations of *all* the intermediates in such a system (Fig. 7). Indeed, such changes have been experimentally observed in the concentrations of precursors not only of DNA (32, 33) but also of RNA (34) and amino acids (28).

An opportunity is provided by a model such as this to perform easily and quickly

experiments that might be difficult and time-consuming to carry out in the laboratory. When one of these experiments leads to a provocative prediction, the effort to carry out the corresponding experiment in the laboratory is warranted. The experiment shown in Table 3 on the influence of the level of effect on the intensity of drug interaction provides an illustration of this approach. In fact, simulations that do not lead to new experiments in the laboratory have little value; there should be a continuing interplay between model and experiment throughout the life of the model.

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