THE DISPOSITION OF ARA-C AND ITS METABOLITES: A PHARMACOKINETIC SIMULATION

PREPARED UNDER GRANTS FROM THE NATIONAL INSTITUTES OF HEALTH

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AUGUST 1975**

Rand
SANTA MONICA, CA. 90406

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PREFACE

Rand's program of research in the biosciences, currently supported by the National Institutes of Health and by The Rand Corporation after initial Air Force support, is committed to the development of quantitative medicine and biology. Mathematical models of biology, computer methods and technology applied to the life sciences, and methodological aids to physician decisionmaking are the principal study areas of the program. The present report discusses means of improving the predictability of leukemia and cancer treatment by incorporating computer-based models of drug disposition and cellular growth into a decision-aiding methodology.

This report should be of interest to those physicians and others who are interested in quantitative medicine, and to biochemists, pharmacologists, oncologists, and other investigators who are engaged in experimental, clinical, and analytic studies of cancer chemotherapy.

This work is dedicated to the memory of Dr. Myron Karon, a gifted hematologist, who, as a Rand Consultant, shared with the authors his interest, creativity, and tremendous enthusiasm for improving the quantitative basis of chemotherapy.

SUMMARY

Rand's objective under the sponsorship of the National Institutes of Health* is to improve the biological precision of chemotherapy in the treatment of leukemia and cancer. Our rationale is that mathematical models and computer simulations based on the biology of disease and its treatment can develop a clinically meaningful predictive approach to chemotherapy.

Antitumor drugs are powerful, but they are not specific to malignant cells. They affect normal cells as well. Relatively small differences in chemical structure, dose, method of administration, and treatment intervals can lead to profound effects on the outcome of therapy. Fortunately, much can be learned from laboratory tests on experimental animals and cells grown in tissue culture. Experimental models of the human clinical situation are selected that share important characteristics with clinical therapy. Indications of major side effects and toxicity, and indications of powerful anticancer and antileukemia activity, are the key specific results of these experimental studies. They also provide the scientific foundations for clinical progress. However, there are few direct biologically plausible rules for comparing these experimental results with the clinical situation, or for comparing or predicting clinical response of individual patients.

As part of our exploration of a decision-aiding methodology for use during the treatment of leukemia, we are investigating the feasibility of a computer-based Leukemia-Therapy Simulator. The present work is a result of that investigation as applied to experimental rather than clinical therapeutics.

Different animals, organs, and cell types have highly specific physical and biochemical properties that alter their response to therapy. One aspect of these differences, the growth characteristics of cells both untreated and treated, is the theme of cell kinetics. We have recently summarized current progress in this field from the point of view

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of mathematical models, computer simulation, and biological similitude.*
Cell-kinetic descriptions of both normal and malignant cells are to be included in the Leukemia-Therapy Simulator.

Another aspect of this range of biological variability, the altered disposition and time history in critical body organs of the active forms of a drug, constitutes the subject of pharmacokinetics.

In this report, we describe a computer model of the pharmacokinetics of anticancer agents, and apply it to a specific drug, cytosine arabinoside (ara-C). This drug is a major factor in the improved survival rate for adult leukemia, but we believe that further improvements can be made in its utilization.

The basic model applies to any species--however, it is only for mice that enough measurements, on an organ-by-organ basis, have been made to permit the detailed verification of the modeling approach and predictions. For this reason, we concentrate on the application to the experimental therapeutics of mouse leukemia.

The model predictions are shown to be in agreement with recent measurements for both blood levels of ara-C and tissue levels of ara-CTP as a function of time. Since ara-CTP is the metabolite of ara-C that is known to kill dividing cells or severely inhibit their rate of DNA synthesis, this agreement with ara-CTP measurements is highly significant. It is shown that ara-CTP is present in tissue, after a single injection of ara-C, for times that are much longer than those estimated from ara-C levels alone. For a single high dose applied to mice, significant effects on cellular growth are predicted to occur for 26 hr, whereas ara-C levels are negligible within 6 hr. Our study also elaborates on the need for further measurements of certain enzyme levels (phosphatase) that are the determinants of the long ara-CTP halving time. These results suggest new interpretations and new protocols for the treatment of mouse leukemia. On-going Rand work on the Leukemia-Therapy Simulator is aimed at relating cell growth and animal survival to tissue levels of ara-CTP, for both mice and cells in culture, and

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* J. Aroesty, T. L. Lincoln, N. Z. Shapiro, and G. Boccia, "Tumor Growth and Chemotherapy: Mathematical Methods, Computer Simulations, and Experimental Foundations," *Math. Biosciences*, 17, 1973, p. 243.

will be published shortly. In addition, we are attempting to relate these experimental findings and model predictions to the treatment of human disease.

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I. INTRODUCTION

The objective of cancer chemotherapy is to eliminate, or at least to suppress, a population of neoplastic cells without undue toxicity to the host. Pharmacokinetic simulation seeks to put this effort on a firm rational basis based on the physiology, molecular biology, and biochemistry of drug distribution and metabolism. As a result of such simulations, we should be able to calculate tissue-specific drug exposures that would make available to the chemotherapist the same kind of dosimetry that is already available to the radiologist. We are studying the pharmacokinetics of cytosine arabinoside (ara-C) as one example of this approach. We have chosen ara-C because of its theoretical interest, the amount of experimental data at hand, and its demonstrated clinical fruitfulness.

The pioneering work in the simulation of the multicompartment pharmacokinetics of anticancer agents is by Bischoff, Dedrick, and their co-workers.⁽¹⁻³⁾ Our work extends theirs to include the simulation and prediction of intracellular metabolites and enzyme kinetics within those target cells that are the site of the ultimate cytotoxic and cytostatic effects. The next step, to be discussed in a later report, is to relate intracellular concentrations to cell death and the inhibition of DNA synthesis. In this way, a realistic approach to the prediction and interpretation of optimal schedules is being developed.

In this study^{*} we report on pharmacokinetic baseline simulations that also enable us to interpret the experimental and clinical impact of ara-C. We wish to show the consistency of experimental data about the pharmacology of ara-C by applying our models to the detailed measurements of Chou et al.⁽⁴⁾ for the DBA hybrid mouse.

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* Abbreviations: ara-C, 1- β -D-arabinofuranosylcytosine; ara-CMP, ara-C monophosphate; ara-CDP, ara-C diphosphate; ara-CTP, ara-C triphosphate; dCR, deoxycytidine; CR, cytidine, dCTP, deoxycytidine triphosphate; ara-U, 1- β -D-arabinofuranosyluracil; ara-UMP, ara-UDP, ara-UTP, respectively, the ara-U mono-, di-, and triphosphates; ATP, adenine triphosphate; ~DNA~, replicating DNA; ~[DNA - dCMP]~, replicating DNA after addition of deoxycytidine nucleotide.

Blood levels of ara-C have already been measured in mice, rabbits, and man as a function of dose and route of administration. Typical of these measurements are the results of Borsa et al.⁽⁵⁾ shown as crosses in Fig. 1. These data correspond to blood levels of ara-C found in mice after a single i.p. injection of 200 mg/kg. This is less than a tenth of the single LD₁₀ dose cited by Skipper et al.⁽⁶⁾ The line through the data points is the ara-C decay curve predicted by our version of

Fig. 1—Pharmacokinetic simulation of ara-C and splenic ara-CTP

the Bischoff-Dedrick pharmacokinetic model. The prediction is made using standard values of organ size and blood flow, available *in vitro* values of kinase and deaminase levels, and clearances based on the 1-hour drug nucleoside levels in blood measured by Chou.⁽⁴⁾ While this agreement between prediction and measurements is exemplary,* Dedrick et al.⁽¹⁻³⁾ have also verified that the basic pharmacokinetic model is an accurate predictor of ara-C and ara-U levels in blood and other tissues.

However, the active intracellular form of ara-C is the triphosphate, ara-CTP, not ara-C itself. Ara-CTP has its impact by blocking DNA synthesis and possibly by introducing cytosine arabinoside moieties into the DNA coding sequences. At high intracellular concentrations, ara-CTP is cytocidal; and at much lower concentrations, there is still a pronounced cytostatic effect. It is important, then, for the therapeutic utilization of pharmacokinetic data on ara-C, to determine the corresponding ara-CTP concentration within target cells. Does it follow closely the levels of ara-C within blood, as in curve A of Fig. 1? or does it continue to increase even while blood levels of ara-C are decreasing and ultimately decay with a long half time as in curve B of Fig. 1? Estimates of the minimum cytotoxic concentrations of ara-CTP ($.1\mu\text{M}$), and the concentration that inhibits 50 percent of DNA synthesis ($.1\mu\text{M}$), are also shown in Fig. 1.

If the levels of phosphatase are high, then curve A is valid, the duration of the cytocidal and cytostatic effects can be estimated directly from the blood levels of ara-C, and a single pulse dose of 200 mg/kg of ara-C does not inhibit cell growth for more than 4 or 5 hr. If the levels of phosphatase are low, then curve B is valid, and the situation is both different and more complex. The effective duration of activity is much longer, i.e., between 12 and 16 hr, with corresponding implications for schedule and dosage studies. In this case, ara-C blood levels do not reflect the effective duration of drug action against target cells.

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* It is appropriate to use the natural logarithm of the concentration, $\ln c$, in the analysis of the goodness of fit to the data. The standard error of the prediction of $\ln c$ is 16 percent of the mean value of the measurements of $\ln c$.

The main body of this report will deal with the application of available information on the enzyme chemistry of ara-C to the prediction of intracellular concentrations of ara-CTP. As we show later, curve B is much more realistic than the hypothetical curve A: We are able to verify the measurements of Chou by using phosphatase levels corresponding to that shown in curve B.

In other studies of pharmacokinetic simulation, we have considered a slightly different question.

If the Bischoff-Dedrick⁽¹⁻³⁾ model of drug distribution to tissues can predict differences in tissue concentrations, how can such differences be utilized? As an illustration, we have focused on the ara-C tissue concentration that can be predicted in the testis and on how these concentrations may be altered by the manipulation of testicular blood flow.⁽⁷⁾ Simulations show that tissue differences are greatest when the injection is given rapidly by i.v. bolus. The testis may be protected by cutting off the blood flow until the peak blood concentration has passed. Conversely, by decreasing blood flow at the time of peak testicular concentration, higher levels of drug can be maintained in the testis because less drug is returned to the blood stream. The peak tissue concentration may be found by simulation, and the pharmacokinetics can be verified by sampling the testicular venous blood concentration and comparing it with predicted values. For ara-C, tissue distribution is pharmacologically simple because the principal rate-limiting features are tissue volume, tissue-specific blood flows, and the metabolic breakdown to ara-U.

Briefly, we touch on some of the implications of this work in connection with cell cycle specificity and optimum scheduling, and host resistance. It has been noted that the curves for S-phase labeling using tritiated thymidine, and the curves for cell death due to ara-C exposure, are not the same.⁽⁸⁾ More cells are killed by ara-C than are labeled with tritiated thymidine. It would appear that cells can form sufficient ara-CTP prior to entering S-phase, so that the exposure of cellular metabolism to ara-CTP exceeds the time of exposure to ara-C. Cells that have not entered S-phase during an exposure time, but enter S-phase while sufficient ara-CTP is present, will be affected. This

implies that sufficient deoxycytidine kinase is available prior to S-phase to form ara-CTP, and that the half-life of ara-CTP is at least 1 or 2 hr (as indicated by present measurements).⁽⁹⁾ From these considerations, it is clear that calculations of pulse dose kill based only on the interactions of ara-C on S-phase may be considerably oversimplified.

As we have indicated, low levels of ara-CTP are cytostatic. The rate of DNA synthesis may be slowed by a factor as great as 30 without killing the cells.⁽¹⁰⁾ Thus all the cells that are committed to divide, but have not yet completed their passage through S-phase, are nearly totally blocked. In this arrested state, they are subject to any other cytotoxic effect that may be present in their surroundings. As with the use of bacteriostatic agents, slowing the process of cell division magnifies host defenses even when they are relatively weak. Any immunological resistance to tumor cells can thus be increased by cytostatic levels of ara-CTP. This may account for the clinical effectiveness of continuous i.v. infusions where blood levels ($\sim .07 \mu\text{g/ml}$)⁽¹¹⁾ are not sufficient for direct cell death. The cytostatic activity of ara-C may also enhance the effectiveness of other therapeutic agents that, for one reason or another, tend to kill more tumor cells than normal cells. Simulation of this approach is under vigorous investigation.

II. THE PHARMACOKINETIC MODEL

In order to describe the distribution of ara-C and its pharmacologic action, we have extended the Bischoff-Dedrick⁽²⁾ multicompartment flow model by adding another compartment and more metabolic detail. A schematic of the flows appears in Fig. 2. We have used the same organ compartments as employed by Dedrick et al. in their analysis of ara-C, namely the blood, liver, gut, heart, kidney, lean, and marrow, but have added the spleen to the portal circulation because this organ compartment has been shown to be metabolically very active with a high phosphorylating ability.⁽¹²⁾ For each compartment we have constructed mass balances that describe the organ concentrations of ara-C and its metabolites in terms of drug flow in and out of each compartment, coupled with the important reactions within a compartment. The solution of these balances allows one to compare our simulated time-dependent

Fig. 2—The pharmacokinetic flow scheme

nucleotide concentrations with those obtained from experiment. The model is calibrated to the DBA mouse experiments, but is designed to be scaled to other animals and to man.

Because reaction terms describing the metabolism of ara-C are a part of each mass balance equation, we first discuss this metabolism and derive the appropriate enzyme kinetics. We return in the next section to the mass balance expressions themselves.

ARA-C METABOLISM

The active form of ara-C is the triphosphate, ara-CTP. Thus we have selected as the pertinent ara-C biochemistry the minimal set of enzymatic steps that define the time-dependent concentration of ara-CTP. This approach has minimized the work required to solve the many simultaneous equations that arise in the model. It also reduces the problem of missing data posed by other enzyme systems that have only a marginal effect on ara-CTP levels and have not been fully characterized kinetically.

Using this approach, the key steps in the metabolism of ara-C are presented in Fig. 3. Steps 1 through 3 phosphorylate ara-C to ara-CTP.

(Note: Dotted lines with arrows indicate inhibition)

Fig. 3—The metabolism of ara-C

The initial phosphorylation is enzymatically controlled by deoxycytidine kinase (a "salvage" enzyme);^(13,14) this initial step is rate determining. The second phosphorylation^(15,16) by cytosine monophosphokinase and the third by nonspecific nucleoside diphosphokinase⁽¹⁷⁾ exhibit activities much in excess of those found for deoxycytidine kinase. Several nucleotides may act as phosphate donors for these enzymes, but the primary donor is ATP, by virtue of its large steady-state level.

The enzymes catalyzing dephosphorylation, steps 4 through 6 in Fig. 3, are less well studied. It is assumed that there are nucleoside tri- and diphosphatases high in activity compared with deoxycytidine kinase, so that the rate-determining dephosphorylation step (step 4) is the ara-CMP to ara-C conversion. Schrecker⁽¹⁶⁾ has indicated that in L1210 extracts this step is controlled in great part by a 5'-nucleotidase, whereas Tseng and Gurpide⁽¹⁸⁾ have shown that this enzyme dephosphorylates 5'-UMP in rat liver slices. However, it is possible that other phosphomonoesterases present in the cytosol (5'-nucleotidase is bound to the plasma membrane) may also contribute to the dephosphorylation of ara-CMP nucleotide.

Ara-C is deaminated by pyrimidine nucleoside deaminase⁽¹⁹⁾ (step 7), whereas ara-CMP may be deaminated to ara-UMP by deoxycytidylate aminohydrolase⁽²⁰⁾ (step 8). Steps 9 and 10 are controlled by deoxythymidine kinase⁽²¹⁾ and presumably 5'-nucleotidase, respectively.

Because it is also necessary to include the effects of enzyme inhibitors in deriving the kinetics of ara-C metabolism, we briefly mention the principal inhibitors. Deoxycytidine kinase is inhibited by both dCTP and, to a lesser extent, ara-CTP.^(14,16) ATP is the normal phosphate donor in the reaction, and these two inhibitors compete with it. The normal substrate, deoxycytidine, when present in large enough quantities, is also an inhibitor of the enzyme with respect to ara-C phosphorylation. Of the monophosphokinases, inhibition is observed among dCMP, CMP, UMP, and dUMP acting as competing substrates for dCMP kinase,⁽¹⁵⁾ and a similar situation also occurs with nucleoside diphosphokinase because of its lack of specificity. Naturally occurring inhibitors or activators for pyrimidine nucleoside deaminase are not known except the natural substrates deoxycytidine and cytidine.⁽²²⁾

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The metabolic scheme actually employed in the present model includes the kinetics of steps 1 through 7 but generally ignores the deamination of ara-CMP and the phosphorylation steps of ara-U. Schrecker and Urshel⁽²¹⁾ have shown that such steps are small contributors to the overall kinetics in the L1210 system. Chou⁽⁴⁾ and his associates have shown that the ara-U mono- and diphosphokinase steps are of minor importance in other murine tissues. The ara-UMP kinetics have only recently been shown to be of possible importance.⁽⁴⁾ The effects of including these kinetics (steps 8 through 10) in the present model have been partially explored and are discussed separately below.

Not shown in Fig. 3 is the ribonucleotide reductase enzyme system that is largely responsible for controlling the concentration level of the inhibitor dCTP (as well as other deoxyribonucleoside triphosphate levels). It has been omitted principally because Graham and Whitmore,⁽¹⁰⁾ and Chou⁽⁴⁾ have found that dCTP levels change only slightly in cells that have been treated with ara-C. Hence, for any cell type, it is supposed that the reductase system holds dCTP concentrations at their (cell cycle-averaged) normal value and that detailed reductase kinetics are not required to describe the formation of ara-CTP.

We now present the actual kinetic equations employed in our model by enzyme type, as well as the numerical values selected for the principal inhibitor concentrations.

Deoxycytidine Kinase

The kinetic equation for this enzyme has been derived from the data of Schrecker⁽¹⁶⁾ and Momparler et al.^(14,23) Inhibition terms are particularly important here. We have employed as our kinetic scheme the mixed inhibition formalism of Dixon and Webb,⁽²⁴⁾ since the reciprocal plots of ara-C phosphorylation in the presence of varying inhibitor (dCTP) concentrations do not intersect at either the ordinate or the abscissa. An allosteric mechanism need not be invoked because these reciprocal plots, using ara-C as substrate, are linear over the physiological concentration range. The mixed kinetics appear in Fig. 4, where the second substrate, ATP, is indicated. Inhibition by dCTP is equivalent to the direct replacement of ATP. The overall kinetic scheme of

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Fig. 4—The monosubstrate kinetics of deoxycytidine kinase and ara-C at saturating levels of ATP. The equilibrium constants are given here in the mixed inhibition notation used commonly in enzyme kinetics (Ref. 24).

Fig. 4 has monosubstrate character because ATP in intact cells is maintained at a constant saturating level over time.

From Fig. 4, and the additional condition that deoxycytidine may also bind to deoxycytidine kinase, the overall kinetic equation for phosphorylation rate, $\dot{r}_p$, using mixed inhibition, may be derived as

$$\begin{aligned}
 \dot{r}_p &\equiv \left[\frac{d(ap_1 + ap_2 + ap_3)}{dt} \right]_p \\
 &= \frac{V_k}{1 + \frac{K_m}{a} \left(1 + \frac{c}{K_i} + \frac{dCR}{K_i} + \frac{ap_3}{K'_i} \right) + K_m \left(\frac{c}{K_i K'_m} + \frac{ap_3}{K'_i K''_m} \right)}, \quad (1a)
 \end{aligned}$$

where a , ap_1 , ap_2 , and ap_3 are ara-C and its three nucleotides; c is dCTP; dCR is deoxycytidine; K_i is the deoxycytidine inhibitor constant;

K_m corresponds to the usual competitive Michaelis constant; K_1 , K'_1 , K''_m , K'''_m are the mixed inhibition constants of Fig. 3; and the p subscript denotes changes in total nucleotide level due to phosphorylation only. V_k is the kinase activity and depends on the individual tissues being considered.

Note that the left-hand side of Eq. (1a) represents the time rate of change over the entire sum of ara-C nucleotides and not just ara-CMP. This results because the higher phosphorylations and dephosphorylations of ara-C are rapid compared with the first, ⁽¹⁵⁻¹⁷⁾ thus causing the initial kinase product, ara-CMP, to immediately reappportion itself over all the higher nucleotide forms. This rapid reapportionment means that the various ara-C nucleotides will maintain constant steady-state ratios among themselves. Defining the ara-CTP/ara-CDP ratio as α_1 and the ara-CDP/ara-CMP ratio as α_2 , the ara-C nucleotide sum ($ap_1 + ap_2 + ap_3$) may be written only in terms of ara-CTP, and Eq. (1a) may be reexpressed as

$$\dot{r}_p = \left(1 + \frac{1}{\alpha_1} + \frac{1}{\alpha_1 \alpha_2}\right) \left[\frac{d ap_3}{dt}\right]_p. \quad (1b)$$

The nucleotide ratios are calculable from the experimental nucleotide concentrations that have been determined for intact L1210 cells. ⁽²¹⁾ Relative constancy of the coefficient in parentheses in Eq. (1b) is in fact confirmed by the ratio values obtained over a tenfold range of initial ara-C concentrations, i.e., $\alpha_1 = 7.6, 7.8, 4.2$, and $\alpha_2 = 4.7, 5.7, 3.7$ for initial drug concentrations of 10, 51, and 100 μM , respectively, which leads to values of $1 + 1/\alpha_1 + 1/\alpha_1 \alpha_2$ between 1.16 and 1.30.

Values for the Michaelis and inhibition constants in Eq. (1a) have been obtained by applying the mixed intercept values of Dixon and Webb (Table VIII.1, Ref. 24) first to the data on ara-C phosphorylation with dCTP present as inhibitor (Fig. 5 of Ref. 14, Chart 2 of Ref. 16), then to the data with ara-CTP present as inhibitor (Fig. 1 of Ref. 23), and finally to the data on ara-C phosphorylation with deoxycytidine present as inhibitor (Fig. 9 of Ref. 14). Depending on the data source, K_m was found equal to 40, 27, or 25 μM . Because it is only slightly below the

mean of these three numbers, 27 μ M was chosen for further use in our model. Values for the other deoxycytidine kinase constants appear in Table 1.

Table 1
DEOXYCYTIDINE KINASE CONSTANTS^{a,b}

Constant	K _m	K' _m	K'' _m	K _i	K' _i	K _I
Value	27.0	309.0	101.9	1.00	17.9	.84 ^c

^aUnits are μ M.

^bFor this meaning of constants, see Fig. 4 and the text.

^cThis differs slightly from Momparler's value of 1.3 μ M because it was recalculated for a K_m equal to 27 μ M.

Phosphatase

The enzyme 5'-nucleotidase is thought to be the primary phosphatase involved; hence the kinetics of phosphate hydrolysis are based on this enzyme. No inhibitors other than normal competing substrates are known. Therefore a single Michaelis-Menten expression for dephosphorylation rate, $\dot{r}_{dp}$, has been used:

$$\begin{aligned} \dot{r}_{dp} &\equiv \left[\frac{d (ap_1 + ap_2 + ap_3)}{dt} \right]_{dp} = \left(1 + \frac{1}{\alpha_1 \alpha_2} + \frac{1}{\alpha_1} \right) \left[\frac{d ap_3}{dt} \right]_{dp} \\ &= \frac{-V_{dp}}{1 + \frac{\alpha_1 \alpha_2 K_{dp}}{ap_3}}, \end{aligned} \quad (2)$$

where K_{dp} is the Michaelis constant (equal to 900 μ M) as determined by Schrecker for 5'-nucleotidase,⁽²⁵⁾ V_{dp} is the nucleotidase activity, the dp subscript denotes changes in total nucleotide level due to dephosphorylation only, and the rest of the symbols have the same meaning as above. This expression assumes that ara-CMP is the only form that

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reacts with the enzyme and that, as with dCR kinase, instantaneous re-apportionment of the remaining nucleotides occurs in order to maintain time independent nucleotide ratios. The competition due to other mononucleotides, principally AMP, has not been included in Eq. (2). It is estimated that the effect of this exclusion for the spleen would be equivalent to reducing the phosphatase activity by 50 percent. Currently, the activity itself is not known to within this degree of error.

The overall phosphorylation of ara-C in a cell is thus the sum of the phosphorylation and dephosphorylation equations, i.e.,

$$\dot{r}_x = \dot{r}_p + \dot{r}_{dp} \quad (3)$$

Deaminase

The kinetic equation employed for murine pyrimidine nucleoside deaminase rate, $\dot{r}_a$, is the same as that used by Dedrick et al.⁽²⁾ and is a single uninhibited Michaelis-Menten expression, i.e.,

$$\dot{r}_a = \frac{da}{dt} = \frac{V_{da}}{1 + \frac{K_{da}}{a}} \quad (4)$$

where K_{da} and V_{da} have their usual meanings. From Dedrick et al.,⁽³⁾ $K_{da} = 1011.7 \mu M$ (283 $\mu g/ml$). Inhibitions by the competing substrates dCR and CR have been omitted in Eq. (4) because of their estimated small effect. Using simply inhibited Michaelis-Menten kinetics and the Michaelis constants of Creasy,⁽²⁶⁾ we estimate that dCR concentrations in the normal murine 1 to 5 μM range are completely negligible and that CR concentrations in the 50 μM range reduce the deaminase activity by only about 25 percent. In human systems, however, the K_{da} is reported to be lower (139 μM), and so CR inhibition will then have to be included.

The effects of deoxycytidylate deaminase have not been included in the present model because Schrecker has shown that this enzyme is not significantly active in the L1210 system against ara-CMP at concentrations that exceed any attained in our modeling, even though it is present and active against the normal substrate.⁽²¹⁾ However, there is

the possibility that some tissues encountered in our work may have much higher dCMP deaminase activities than the L1210 system, and this may eventually require introduction of the kinetics of this enzyme into the present pharmacokinetic scheme.

Deoxycytidine Triphosphate

This species has been shown to be a relatively strong inhibitor of dCR kinase and thus its normal steady-state level in murine tissues must be estimated. A survey of the literature^(10,13,27-29) shows that this concentration usually lies in the range of 3 to 30 μ M. Accordingly, a mid-range estimate for all murine tissues has been chosen at 10 μ M.

Deoxycytidine

As deoxycytidine is the normal more strongly bound substrate of deoxycytidine kinase, it is necessary to account for its presence in the kinase kinetics. The concentration has not been reported for a very large variety of species or tissue types but is about 40 μ M^(13,30) in rat blood. The data of Rotherham and Schneider⁽³⁰⁾ indicate that levels in murine tissues are nearly an order of magnitude lower, and a recent study⁽³¹⁾ has indicated that 1 to 4 μ M levels are common in murine blood. Thus, for the present calculations, a value of 4 μ M has been used for all tissues that contain significant levels of kinase.

PHARMACOKINETIC EQUATIONS AND PARAMETERS

The method for constructing the mass balances has been described elsewhere,⁽²⁾ and so we present only the results for the compartments of kidney and blood. As in earlier works, the model presumes complete perfusion of the individual compartments and an organ blood/tissue partition ratio of one.

The mass balances for kidney (K) are

$$V_K \frac{da_K}{dt} = q_K (a_B - a_K) - k a_B + V_K \dot{r}_{a_K} - V_K \dot{r}_{x_K}, \quad (5)$$

$$V_K \frac{du_K}{dt} = q_K (u_B - u_K) - k u_B - V_K \dot{r}_{a_K}, \quad (6)$$

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$$\frac{dx_K}{dt} = \dot{r}_{x_K}, \quad (7)$$

where V_K is the total volume of the kidney, a_K is ara-C concentration, u_K is ara-U concentration, x_K is total nucleotide concentration (i.e., $ap_1 + ap_2 + ap_3$), k is clearance (assumed equal for ara-C and ara-U); q is the kidney blood flow; and $\dot{r}_{a_K}$, $\dot{r}_{x_K}$ are the kinetic expressions of Eqs. (1) through (4) as applied to the kidney. The mass balances for blood (B) are

$$V_B \frac{da_B}{dt} = q_H a_H + q_{Li} a_{Li} + q_M a_M + q_K a_K + q_{Le} a_{Le} - q_B a_B + M_1 g(t), \quad (8)$$

$$V_B \frac{du_B}{dt} = q_H u_H + q_{Li} u_{Li} + q_M u_M + q_K u_K + q_{Le} u_{Le} - q_B u_B, \quad (9)$$

where H, Li, M, and Le denote heart, liver, marrow, and lean; M_1 is total dose of drug; and $g(t)$ is either the Bellman injection function⁽²⁾ to simulate i.v. dosing, or the exponential decay function of Dedrick et al.⁽³⁾ used here to simulate subcutaneous injection. No nucleotide expressions appear for the blood because deoxycytidine kinase is, for all practical purposes, absent in murine blood, and nucleotides are not free to pass through cell membranes without first being dephosphorylated. Similar expressions may be derived for the other six compartments, giving a total of 22 equations, including 2 representing ara-C and ara-U accumulation in the urine.

The parameters used in this model appear in Table 2. The volumes and flows of each organ except the spleen have been taken from Dedrick et al.,⁽³⁾ whereas splenic values were scaled from rat data.⁽³²⁾ Enzyme activities for pyrimidine nucleoside deaminase have also been obtained from Dedrick. These values compare well with those obtained by Ho⁽¹²⁾ and may be considered representative of the DBA mouse (for liver, gut, and kidney, Dedrick's values are 16.45, 29.67, 327.11, whereas Ho's values are 12.1, 70.83, 317.33). The kinase activities, taken from Ho,⁽¹²⁾ are extract activities uncorrected for any intact cell differences (see Sec. III). The kidney clearance rate was estimated from the

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Table 2

THE PHARMACOKINETIC PARAMETERS

Item	Organ							
	Blood	Heart	Liver	Marrow	Kidney	Lean	Gut	Spleen
Volume (ml)	1.67	0.10	1.30	0.60	0.34	10.00	1.50	0.08
Flow (ml/min)	4.38	0.28	1.80	0.17	1.30	0.83	1.50	0.07
Deaminase activity ($\mu\text{M}/\text{min}$)	0	0	$\left(\begin{smallmatrix} 16.45^a \\ 400.00^b \end{smallmatrix} \right)$	0	327.11	0	29.67	0
Kinase activity ($\mu\text{M}/\text{min}$) ^c	0	0	0.65	1.28	.02	0	0.10	4.00
Phosphatase activity ($\mu\text{M}/\text{min}$) ^c	---	---	300.00	300.00	300.00	---	300.00	300.00
Clearance (ml/min)	0	0	0	0	0.66	0	0	0

Note: Activities and Michaelis constants have been expressed throughout this model in units of $\mu\text{M}/\text{min}$ or μM (primarily to facilitate the calculation of nucleotide levels). The volume basis for the activities is total organ volume (rather than tissue volume) to be consistent with Eqs. (5) through (9). The molecular weight of ara-C $\cdot$ HCl has been used to convert Dedrick's $\mu\text{g}/\text{ml}$ units to μM .

^aValue for Swiss mouse; (3) similar to DBA/2 value of 12.1 $\mu\text{M}/\text{min}$ obtained by Ho. (12)

^bValue for C3H mouse obtained by Ho. (12)

^cNucleotide ratios used in conjunction with these activities are $\alpha_1 = 7.83$, $\alpha_2 = 5.67$.

data of Chou, so that the total drug nucleoside concentration in blood agreed with the measured value after a 60-min exposure. The phosphatase activity has been derived from the L1210 extract data of Schrecker,⁽¹⁶⁾ using a phosphatase Michaelis constant (K_{dp}) of 900 μ M and a value of 0.7 ml actual cell volume/ml packed cells. Lacking accurate information for other tissue types, this value has currently been assumed to apply to all tissues. That the phosphatase level is in fact nearly constant over diverse tissue types is indicated by the data of Dixon and Webb,⁽²⁴⁾ which show that 5'-nucleotidase activity is nearly constant in the liver, kidney, and small intestinal tissues of the rat. The various Michaelis and inhibitor constants discussed above have been assumed not to vary by tissue type.

For most of the 6- to 12-hr pharmacokinetic simulations, we have assumed that the time scale is too short to require corrections due to cell kinetic phenomena. For example, recruitment, cell lysis, and progression delay, which may result from ara-C exposure and which may, in turn, change enzyme concentrations, have been taken to be negligible over this time period.

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III. RESULTS

The pharmacokinetic model presented above was developed before the drug-distribution data of Chou et al.⁽⁴⁾ became available. To assess the accuracy of the model, we wanted to see how well this extensive experimental data could be simulated. Thus Chou's data became a consistency check on our previous formulation.

Certain requirements of the model immediately became evident when this simulation was attempted. The first was that the kidney clearance had to be increased from Dedrick's⁽³⁾ value of 0.22 ml/min to 0.66 ml/min. This was necessary to fit the total blood nucleoside concentration and to better account for the total amount of ara-C and metabolites in the whole mouse as measured by Chou. The selection of this parameter is nearly independent of the deaminase and kinase activity values. To a first approximation, changing the deaminase activity causes a change in the relative proportions of ara-C and ara-U in the blood but not the total nucleoside concentration, which is the actual quantity fitted in this calculation. Kinase activities, even boosted to levels tenfold higher than those used in our model, are too low to make significant changes in blood nucleoside concentrations after only 60 min, principally because the volume of tissues containing kinase is relatively small. The method of selecting a clearance rate so that blood nucleoside levels are reproduced is the approach employed by Dedrick; it apparently remains a reasonable approach even if deoxycytidine kinase is present. There is no indication of the binding of ara-C to any proteins. Hence, it is plausible to neglect the possibility of any hidden "sink" due to tissue extraction or the long-term binding and storage of ara-C.

The second requirement is that enzyme activities close to the experimentally determined extract values be employed. Dedrick, Forrester, and Ho⁽²⁾ have previously shown that *in vitro* deaminase activities may be used successfully in *in vivo* simulations. In the case of kinase, the Ho extract values closely reproduce the ara-C nucleotide levels at 1-hr exposure in our pharmacokinetic simulation, whereas values sixteenfold higher (a multiplier that is required to convert Ho's⁽¹²⁾ or

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Schrecker's⁽¹⁶⁾ kinase extract activity to a value capable of reproducing Schrecker and Urshel's⁽²¹⁾ intact cell nucleotide levels) give splenic *in vivo* levels that are too high by a factor of 13.8 at dose levels of 2.5 mg/kg.

Furthermore, examination of the DNA synthesis curves obtained by Chou⁽⁴⁾ and by Bhuyan et al.⁽³³⁾ indicated that extract values can relate these curves. If Eqs. (1) through (3) are used to predict ara-CTP concentrations, a value of V_K close to the H_0 extract value will transform the ara-C/percent inhibition curve of Bhuyan to the ara-CTP/percent inhibition curve of Chou. It is possible, of course, that phosphatase activity may be altered to allow use of nonextract values, but the important observation at the present time is that extract activities for all three enzymes--deaminase, kinase, and phosphatase--form a consistent set for pharmacokinetic simulation.

The pharmacokinetic equations were solved by a Runge-Kutta integration procedure, using step sizes of 0.1 min for the first 20 minutes of simulation and 0.25 min for the remainder. Empirically, this corresponds to the minimum step size consistent with stability and accuracy.

After the clearance value and enzyme activities were chosen, the model was solved for the ara-C serum level corresponding to a dose of 5 mg/mouse. This is the dose used by Borsa et al.⁽⁵⁾ in their pharmacokinetic paper. Our simulation is in excellent agreement with this work, since our calculated serum levels nearly perfectly reproduce the line drawn through their data.

For an ara-C dose of 2.5 mg/kg, the simulation results seen in Table 3 and Figs. 5 through 10 were obtained by using the DBA parameters of Table 2. It can be seen in Table 3 that the experimental (E) and simulated (S) liver and spleen ara-CTP values at 60 min agree to within 6 percent. The small intestine estimate is low by an order of magnitude, but it is possible that this may be accounted for by use of a kinase level determined for a tissue sample that does not exactly correspond histologically to that obtained in the pharmacologic study. The exact agreement between simulated and experimental blood nucleoside levels is not a prediction but comes about because the kidney clearance has been adjusted to do so. On the other hand, nucleoside concentrations in other organs are predicted and, except for the kidney, are in close

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Table 3

SIMULATION RESULTS FOR DBA MOUSE AT 60 MIN, 2.5 MG/KG
(S = simulation; E = experimental; ⁽⁴⁾ units are μM)

Organ		Ara-C	Ara-C + Ara-U	Ara-CTP
Blood	(S)	1.10	1.44	0.0
	(E)	---	1.44	0.02
Liver	(S)	1.12	1.52	0.21
	(E)	1.36	1.45	0.22
Spleen	(S)	1.14	1.50	1.34
	(E)	---	2.05	1.26
Kidney	(S)	0.50	0.72	0.003
	(E)	---	1.50	0.05
Intestine	(S)	1.11	1.50	0.03
	(E)	---	1.65	0.46

agreement, especially when the published error bands of $\pm 0.2\mu\text{M}$ are taken into account. The kidney values differ by a factor of two, but this comparison is the weakest case because the experimental observation consisted of the analysis of only one mouse.

Figure 5 shows the computed ara-C and ara-U concentrations in blood, marrow, and lean compartments over a 5-hr period. As noted previously by Dedrick et al., ⁽²⁾ the maximum of the ara-C concentration in the lean compartment occurs later than in blood or marrow compartments. The ara-C curves for all other compartments generally group around the three compartments shown here, except the kidney. The kidney values are consistently found at a level 30 percent lower than that shown in Fig. 5, due to the organ's high deaminase activity.

Figure 6 shows both the ara-C and ara-CTP concentrations in the spleen as a function of time for DBA mice. Results are shown for three levels of phosphatase activity (VDP), one set belonging to the extract value (VDP = $300 \mu\text{M}/\text{min}$) in Table 2 and the others belonging to phosphatase values that are fourfold higher and lower.

The most outstanding characteristic of these curves is that ara-C follows a much different time course than ara-CTP, the nucleotide persisting at relatively high concentrations for long periods of time. For the extract phosphatase value of $300 \mu\text{M}/\text{min}$ (which we take to be

Fig. 5—Ara-C and ara-U contraction in blood, marrow, and lean tissue, for DBA mice

Fig. 6—Ara-C and ara-CTP concentration in spleen, for DBA mice

our best fit), ara-C has dropped three orders of magnitude from its peak concentration in 6 hr, whereas ara-CTP has fallen to only one-sixth of its peak level.

For low values of phosphatase activity, the ara-C curves flatten out after 3 hr instead of continuing their descent. This flattening occurs because nucleotide pools are built up during the first hour of exposure and continue to provide a source of ara-C by dephosphorylation, thus maintaining the serum concentrations at steady levels for a significant period of time. This effect does not appear in the higher 1200 $\mu\text{M}/\text{min}$ case because, at this rate, the nucleoside source, ara-CMP, falls to such a low level after 3 hr that it cannot significantly affect ara-C concentrations in the serum.

Ara-CTP concentrations are plotted for the spleen, liver, and marrow compartments in Fig. 7, using the parameters of Table 2. All three curves are parallel beyond 1 hr because we have assumed equal phosphatase activity in all the organ regions of this model. (The major exception would occur if the model included the testis, which is known to have an extremely high 5'-nucleotidase activity.⁽²⁴⁾) The curve for the liver shows that the ara-CTP concentration decreases to about 0.02 μM 8 hr after a pulse dose of 2.5 mg/kg. According to the ara-CTP/percent DNA inhibition curve determined by Chou, this computed 0.02 μM concentration corresponds to a DNA synthesis rate that is about 43 percent of normal. Chou experimentally measured the synthetic rate in liver at 8 hr and found a rate 20 percent of normal. These percentages are in relatively close agreement but could be brought into exact agreement by adjusting the dephosphorylation rate upwards by about a factor of two. We note that even if this adjustment were made, the time course of ara-C and ara-CTP would still remain quite different.

Figures 8 and 9 show the differences in ara-C and ara-CTP kinetics that occur when the DBA and C3H mouse strains are modeled. Results are shown for the blood and spleen compartments. Ho⁽¹²⁾ measured dCR kinase and pyrimidine deaminase activities only in the liver of the C3H mouse, and hence our model of the C3H mouse has been limited to the assumption that all enzyme parameters are identical except the liver. Even so, the very large deaminase difference between DBA and C3H livers can be

Fig. 7—Ara-CTP concentration in spleen, liver, and marrow, for DBA mice

Fig. 8—Ara-C concentration in blood and spleen, for DBA and C3H mice

Fig. 9—Ara-CTP concentration in spleen, for DBA and C3H mice

seen to make a noticeable difference in the kinetics of ara-C. The ara-C nucleotide kinetics also differ but to a much lesser degree. The C3H half-life of ara-C (based on the first 3 hours of exposure) is about five-sixths the DBA value, and this leads to a fourfold difference in serum ara-C concentration after only 2 hr. Ara-CTP concentrations differ by about 30 percent after 2 hr. The ara-CTP results are relatively insensitive to this deaminase variation because once the bulk of the triphosphate is formed (by the 1-hr point in Fig. 9), the ara-CTP kinetics lose their dependence on ara-C, including most of the interstrain variations shown in Fig. 8, and depend only on phosphatase activity.

In building cell-kinetic models of tumorous bone marrows, it is desirable for ease of computation to separate the marrow cell and enzyme kinetics from the whole body pharmacokinetic scheme. To do this, ara-CTP concentration pertaining to different cell types in the marrow and to various rates of kill may be calculated in a separate marrow model from estimates of the time-dependent ara-C concentrations in the blood being delivered to the marrow. This input ara-C concentration can be determined from the whole-body pharmacokinetic model developed here provided the growth of high kinase activity tumor cells (such as L1210 cells) does not greatly change the blood levels of ara-C as presently calculated from the assumption of normal marrow kinase activity.

To investigate the effects of variable kinase activities, we have calculated both blood and marrow ara-C concentrations for three levels of kinase activity: one corresponding to a normal marrow, one corresponding to a marrow half filled with L1210 cells, and one corresponding to a marrow completely filled with L1210 cells. The results are shown in Fig. 10. The important result is that blood ara-C levels are practically identical for all three cases up to 3 hr. As was discussed above, most of the phosphorylation of ara-C occurs within the first 3 hr, and changes in ara-C concentrations beyond this time have little effect on the time course of the active drug form, ara-CTP. Hence, a whole-body pharmacokinetic model, *uncorrected* for tumor (or even

Fig. 10—Ara-C concentration in blood and marrow, for DBA mice

progression delay induced*) kinase variations, does provide a good approximation for the ara-C supplied to a murine marrow whose cellular makeup changes in time.

*An activity increase due to progression delay is envisioned as resulting from a drug-induced buildup of cells in the early S-phase region where dCR kinase activity is increased over the non-S-phase value by a factor of 1.5 to 2.2.⁽²³⁾

IV. DISCUSSION

The results obtained with the available data are accurate enough to provide us with an increased degree of confidence in our ability to compute ara-CTP levels for particular tissues *in vivo*.

The difference in the time course of ara-C and that of ara-CTP is important. Skipper et al.⁽⁶⁾ estimated the cytotoxic impact of ara-C in terms of an exposure time to at least minimum cytotoxic concentrations in the blood. Concentrations above this threshold were considered lethal to cells carrying out DNA synthesis. These exposure times were used to interpret the effectiveness of particular dose schedules. The active compound, however, is ara-CTP, which is subject to different determinants of production, distribution, and decay than ara-C. Thus the exposure time of a given cell or tissue to ara-CTP is generally different and not directly comparable, and would be so even if a new ara-CTP threshold were defined.

This difference in exposure time is particularly evident in cell culture viability studies that provide a constant level of extracellular exposure for a fixed time period. These conditions focus sharply on the differences between the intracellular kinetics of the two drug forms. After exposure, cells are washed free of ara-C, and viability is determined by plating or bioassay. Ara-C nucleotides are impermeable to cell membrane^(21,34) and may dephosphorylate slowly, leading to ara-CTP exposures that are considerably larger than ara-C exposure. This extended toxicity partially explains a phenomenon noted by Steel:⁽⁸⁾ that an exposure to ara-C is capable of killing more cells than are labeled by a similar exposure to tritiated thymidine, although the thymidine label should identify all drug-sensitive cells passing through S phase. This may be demonstrated by combining intracellular pharmacokinetics with cell-kinetic simulations of an appropriate cell population. Such simulations are in progress.

There are cytotoxic levels for ara-CTP that kill cells by altering DNA synthesis. There are lower levels in which DNA synthesis is inhibited, so that progression through cell cycle is prolonged leading

to the phenomenon of progression delay. Chou et al.⁽⁴⁾ have measured an ara-CTP/percent inhibition curve which shows that as the ara-CTP concentration falls to 0.01 μ M, DNA synthesis returns to 70 percent of its normal rate. We take this as a minimal threshold for inhibition. One may see from Fig. 7 that well over 8 hr are required at 2.5 mg ara-C/kg before ara-CTP levels fall below this value. We note that this long-term inhibition due to ara-CTP is consistent with the lengths of inhibitions measured by Chou in ascites cells, liver, and small intestine.

By virtue of its inclusion of ara-CTP nucleotide kinetics, the pharmacokinetic model that we have developed is thus capable of providing the time-dependent function from which both cytotoxicity and progression delay may be estimated. Furthermore, the real anatomical and physiological nature of each compartment in the Dedrick-type model allows one to use ara-C and ara-CTP concentrations in the actual organ region of concern, instead of having to estimate drug concentrations for all compartments from blood serum levels. Finally, unlike earlier models, the present pharmacokinetic model allows one to more accurately investigate the case where tumors may be present in an organ region. The cells comprising this tumor generally have different dCR kinase and deaminase activities than the cells normally present in the tumor region, and these differences will lead to much different ara-C exposures and, particularly, ara-CTP exposures. This can be included to first order by simply changing the enzyme activity parameters of the model.

Development of the nucleotide-dependent pharmacokinetic model has shown us where our kinetic parameters are not well known, how they interact, and what experiments might be undertaken to improve the situation. An area of principal importance is the more accurate determination of the kinetics of dephosphorylation of ara-CTP, since this directly governs the length of exposure. From Eq. (2) one may derive an expression for the half life of ara-CTP, $t_{1/2} \cong K_{dp} (1 + \alpha_2 + \alpha_1 \alpha_2) (\ln 2) / V_{dp}$. It is apparent that this half-life depends on both the phosphatase activity, V_{dp} , and the ara-C nucleotide ratios, α_1 and α_2 . Errors in these parameters are thus additive in their effects on the actual value of $t_{1/2}$. There is evidence from Chou,⁽⁴⁾ and some from Schrecker and Urshel,⁽²¹⁾ that the nucleotide ratios employed in this modeling, particularly α_2 ,

may be high. However, if the half-life is to remain relatively unchanged in order to keep the DNA synthesis rate depressed for as long as Chou has measured in liver (see Fig. 7 and Sec. III), the phosphatase activity would also have to be lowered. Hence an experiment that measures *both* ara-C nucleotide levels and phosphatase activities in different tissue types should provide us with the best kind of data to improve our half-life calculation.

Chou⁽⁴⁾ has stated that ara-UMP is a significant fraction of the ara-C mononucleotides in the blood, liver, and intestinal tissues of the mouse. If the presence of ara-UMP is confirmed at these high levels (in the range of 0.5 μ M) in future experiments, reactions describing the production of this nucleotide will have to be added to the present model. Unfortunately, the route(s) by which ara-UMP is formed has not been fully clarified, and further experiments are needed. Two possible routes are (1) by deamination of ara-CMP by deoxycytidylate (dCMP) deaminase and (2) by phosphorylation of ara-U by deoxythymidine (TdR) kinase, but neither route is completely consistent with available data. In order for dCMP deaminase to be the active enzyme, significant activity of deoxycytidine kinase must be present in a tissue to form the ara-CMP substrate. Yet Chou found ara-UMP in all murine blood fractions and, presumably, muscle--sites where Ho⁽¹²⁾ or Durham and Ives⁽¹³⁾ found little or no kinase activity in previous studies. Relatively high kinase levels have been found in human muscle,⁽¹²⁾ however, and reinvestigation of murine muscle could possibly reveal dCR kinase activities that, when combined with dCMP deaminase activities, are high enough to produce Chou's ara-UMP levels.

The possibility of producing ara-UMP by TdR kinase phosphorylation of ara-U requires that ara-U first be formed by pyrimidine deaminase. If, however, the lean (muscle) compartment produces the levels of ara-UMP indicated by Chou, the total mass of ara-U derivatives in an anatomical region this large (nearly two-thirds of the entire pharmacokinetic volume) would require that pyrimidine deaminase activities be increased greatly over present model values in order to produce this mass of ara-U in only 60 min. A very approximate simulation has shown that if only liver deaminase activity were changed, the activity would have to be increased from the DBA value of 16.45 μ M/min to about 260 μ M/min

(clearance rates would drop slightly to 0.46). The $V_{dp} = 300 \mu\text{M}/\text{min}$ -curves of Fig. 6 can be reproduced closely with these values.

The present results have shown that noticeable differences may arise in ara-C and ara-CTP concentrations in the organ regions of mice of different strains. For the 2.5 mg/kg dose in Fig. 9, the C3H ara-CTP concentration remains above $1 \mu\text{M}$ (taken here as an approximate lower cytotoxic level⁽⁴⁾) for nearly 2 hr, whereas the DBA concentration never exceeds this value. These kinds of differences suggest that the prevalence of interstrain enzyme variations should be more fully investigated. For instance, the parameters of Table 2 very accurately reproduce the serum ara-C curves of Borsa et al.⁽⁵⁾ obtained on AKR/J mice. However, if AKR/J mice have a liver deaminase level similar to that of the C3H mouse in Table 2 and not to the DBA mouse value, a reasonably good reproduction of the serum curve may also be made by reducing the clearance value to about 0.2 ml/min. Until pyrimidine deaminase activities are determined in AKR/J livers, which of these two possibilities is correct will remain unresolved.

Ultimately the murine pharmacokinetic model will be scaled for human application. Certain differences between the murine and human models have already become apparent. First, the dCR kinase, and especially the pyrimidine deaminase, activities are higher in human marrow relative to other organ sites than in the murine system.⁽¹²⁾ The marrow and variations in marrow activities will thus become a more important determinant of ara-C metabolism in the whole body model. Secondly, the pyrimidine deaminase Michaelis constant is considerably lower in humans,⁽³⁾ greatly increasing the importance of deoxycytidine and cytidine as inhibitors and requiring more accurate treatment of their inhibition than in the mouse. Particularly since deoxycytidine is also an inhibitor of dCR kinase, human application of this model will require that these two nucleoside concentrations be experimentally determined by tissue type.

The pharmacokinetic model described in this report is currently being extended to include a peritoneal cavity thereby allowing simulation of an L1210 tumor in that compartment. Perfusion of a tumor-laden peritoneum is not high, however, and thus an accurate kinetic description of the drug transport involved must be developed. Once present,

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the peritoneal cavity can be used to investigate the ara-CTP time history in both ara-C-sensitive and ara-C-resistant L1210 cells. Some work is also proceeding on more detailed modeling of ara-UMP production, assuming that high ara-UMP levels will continue to be found in murine tissues by other investigators, and assuming that ara-UMP is produced by one of the two biochemical pathways discussed above.

In a preliminary study of toxicity, we have modeled Skipper's LD10 single dose of 3000 mg/kg. The increase in the exposure to ara-CTP above that achieved by a dose of 200 mg/kg is only 2 hr (see Fig. 1). The formation of ara-CTP is limited by the saturability of the kinase. The rapid clearance of ara-C, together with the rate limitation imposed by kinase, appears to protect the animal from a single dose unless that dose becomes so large that extreme physiological effects not treated in this work come into play.

In summary: We have shown that the kinetics of ara-C and ara-CTP *in vivo* are quite different, and thus that the use of ara-C concentrations to predict states of the ara-CTP-dependent properties of toxicity or DNA inhibition can lead to large errors. The parameters of an ara-C nucleotide pharmacokinetic model have been described, as well as the need for various experimental data to improve the accuracy of the model.

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