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PHARMACOKINETICS OF 1-β-D-ARABINOFURANOSYLCYTOSINE (ARA-C) DEAMINATION IN SEVERAL SPECIES

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Abstract—The pharmacokinetics of 1-β-D-arabinofuranosylcytosine (Ara-C) and 1-β-D-arabinofuranosyluracil (Ara-U) are related to enzyme kinetics *in vitro* in mice, monkeys, dogs and humans by means of a mathematical model. The model is physiologic and permits the incorporation of chemical reactions in appropriate anatomic sites with kinetic characteristics of their local environment. A lean tissue compartment plays an important role as a reservoir. The kidney clearance of Ara-C exhibits essentially the same variation with body weight as inulin.

THE DEAMINATION of 1-β-D-arabinofuranosylcytosine (Ara-C) provides an excellent opportunity to study certain quantitative aspects of metabolic differences among species. The kinetics of deamination have been investigated extensively *in vitro*.[1–4] Ara-C is converted to 1-β-D-arabinofuranosyluracil (Ara-U) by pyrimidine nucleoside deaminase (cytidine aminohydrolase). These detailed studies with human liver preparations showed that the deaminase is specific in its substrate requirement. Further, no direct regulatory mechanism or cofactor requirements were demonstrated.

Additional enzymatic reactions are involved in a complete discussion of the metabolism of Ara-C. In particular, the drug must be phosphorylated to nucleotide, which is believed to act as an inhibitor of DNA polymerase.[5] These reactions have not been completely characterized *in vitro* or *in vivo*.

Recently we described a pharmacokinetic model[6] for the detailed distribution and disposition of Ara-C and Ara-U in man. It was designed to be consistent with what had been reported about the behavior of total radioactivity from labeled drug. In addition, it incorporated Michaelis–Menten kinetics for the deamination step (but did not include the phosphorylation of Ara-C to nucleotide or its subsequent metabolic fate). Apparently the formation of small amounts of nucleotide does not play an important role in altering systemic Ara-C concentrations at short times; however, if the nucleotide is resistant to deamination, it can serve as a source which returns Ara-C to the circulation upon hydrolysis, and this may affect systemic levels at longer times.

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Our pharmacokinetic model was used to predict plasma concentrations of Ara-C and Ara-U in man after intravenous injections of 1·2 and 86 mg/kg of the parent compound. The enzyme kinetics were based entirely on work *in vitro*, and we felt that a completely *a priori* prediction of plasma concentrations was a rigorous test of the model. Some of the postulates and conclusions were not verified experimentally. A particularly important aspect was the role of lean tissue in serving as a reservoir of Ara-C and releasing it to the blood, thus maintaining plasma concentrations at longer times. Related to this reservoir effect was our postulate concerning flow limitation of drug and metabolite. In addition, deamination is sufficiently rapid in the human liver that a large fraction of the drug may be reacted on a single pass so that the observed kinetics become strongly dependent on blood flow rate and relatively less sensitive to the precise nature of the local enzyme kinetics.

Large differences exist among species in the location and kinetic characteristics of deaminase.[2,3,7,8] Mellett[9] reported plasma concentrations of Ara-C after its administration to several mammalian species. He observed that the plasma concentrations related qualitatively to enzyme activities *in vitro*.[9] It is the object of this report to present plasma and lean tissue concentrations of Ara-C and Ara-U in mice, monkeys and dogs, and to interpret these quantitatively in the context of the pharmacokinetic model described previously for man.

It is shown that the rates of deamination are consistent with those predicted from measurements *in vitro*, despite a wide range in both enzyme activities and Michaelis constants. The sensitivity of the model predictions to changes in either kinetic parameters or lean tissue flow rate is illustrated. Finally, the variation of kidney clearance of Ara-C with body weight is discussed.

PHARMACOKINETIC MODEL

The pharmacokinetic model for Ara-C, model assumptions and the observations leading to its development have been discussed previously.[6] Principles of pharmacokinetic modeling on physiologic bases have been discussed in general or with reference to a number of other drugs or endogenous substances.[10–15] Only an outline of the mathematical model and its key assumptions are presented here.

Figure 1 is a flow diagram of the compartmental model. It contains the major tissues which are thought to be involved in the metabolism, storage, elimination and toxicity of the drug. Development of the mathematical model is based on the concept of a mass balance which may be written for any of the compartments. In a word equation:

Rate of accumulation of drug in compartment	=	Rate of injection or absorption into compartment	+	Rate of inflow with blood	−	Rate of outflow with blood	−	Rate of conversion by chemical reaction
		+ Rate of formation by chemical reaction		− Rate of elimination by other processes				

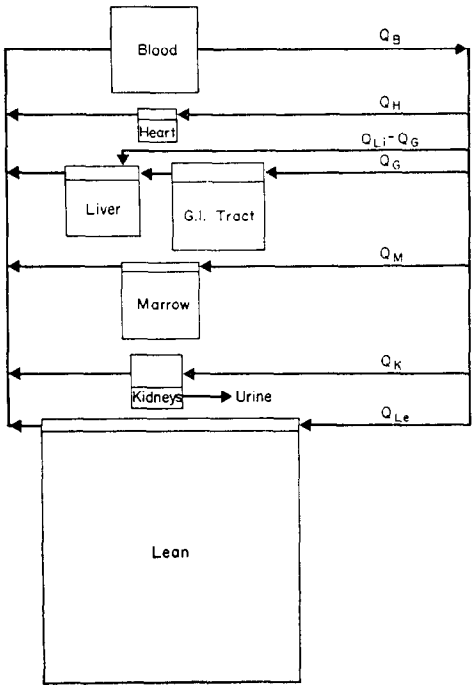

FIG. 1. Compartmental model for Ara-C pharmacokinetics. The compartment volumes are approximately proportional to areas in the figure.

The differential equation for the concentration of Ara-C in the kidney, for example, is:

$$V_K \frac{dC_K}{dt} = Q_K C_B - Q_K C_K - k_K C_B - \left[\frac{v_{\max,K} C_K}{K_{m,K} + C_K}\right] V_K \tag{1}$$

where: V = compartment size (ml or g); C = concentration (μg/ml); Q = blood flow rate to compartment (ml/min); k = clearance (ml/min); $v_{\max}$ = enzyme activity at saturation [μg/(g) (min)]; K_m = Michaelis constant (μg/ml); and the subscript identifies the compartment.

The balance equation on the blood is:

$$V_B \frac{dC_B}{dt} = Q_H C_H + Q_{Li} C_{Li} + Q_M C_M + Q_K C_K + Q_{Le} C_{Le} - Q_B C_B - \left[\frac{v_{\max,B} C_B}{K_{m,B} + C_B}\right] V_B + Mg(t). \tag{2}$$

In equation 2, the subscripts indicate the compartment, M is the total dose of drug and $g(t)$ is an injection function which may be a short pulse to simulate an intravenous injection or an exponential function of time to simulate an intraperitoneal injection. Other functions or locations can be used, for example, to represent intravenous or intraarterial infusion or oral administration.

The key assumptions implicit in the balance equations are: (1) The concentration of drug or metabolite in the blood leaving any compartment is the same as that in the compartment. This assumption involves two others: flow limitation on transport and uniform equilibrium distribution between blood and tissue. A distribution coefficient other than unity can be incorporated if needed. (2) The deamination occurs within a compartment according to a simple Michaelis–Menten process with kinetic parameters appropriate for the site. Additional chemical reactions, e.g. formation of nucleotide, could be included in the balance equation in the same way if the kinetic characteristics were available.

Similar equations are written for Ara-C in each of the other compartments (heart, liver, gut, marrow and lean). Then balance equations are written for Ara-U in each compartment. After selection of the parameters (flows, volumes, kinetic constants etc.), the set of 14 ordinary nonlinear differential equations is solved on a digital computer.* The results are the predicted concentrations of Ara-C and Ara-U in each of the seven compartments as functions of time.

The rate of drug or metabolite accumulation in the urine is $k_K C_B$, and the cumulative amount is:

$$U = \int_0^t k_K C_B \, dt'. \tag{3}$$

Estimation of model parameters

A very large number of parameters is required to solve the model equations. Those used in this study are shown in Table 1. The parameters which are anatomic (volumes) or physiologic (blood flow rates) are not functions of the particular drug. These were obtained from a variety of sources or estimated as documented in the Appendix.

The volume of distribution had to be reduced slightly from that derived on anatomic bases in the three animal species, as was previously found necessary for man. Mathematically, this was accomplished by reducing the volume of the lean tissue compartments. Lean tissue blood flow rates were reduced proportionately. The consistently greater anatomic than physiologic volume of distribution suggests that an alternate basis of calculation could be tissue water rather than tissue volume. Such a basis would differ only slightly from the adjusted anatomic basis used in our calculations.

Most of the kinetic parameters were obtained from data *in vitro* reported in the literature: K_m for mouse; v_{max} and K_m for monkey; v_{max} for dog; v_{max} and K_m for man. The value of v_{max} in the mouse was estimated from our data *in vivo;* it is about 20 per cent greater than the lower limit reported *in vitro*. The value of K_m for the dog was obtained from our data *in vivo*, thus permitting a comparison with the other species. The Michaelis constants listed in Table 1 are based on an aqueous solution of deaminase; they were corrected to a tissue volume basis for consistency with the model equations as described in the Appendix.

The kidney clearances were obtained by trial in order to fit the data for total radioactivity (Ara-C plus Ara-U) in blood or serum. It is assumed that the same clearance

* Our solution was based on IBM's Scientific Subroutine Package HPCG. The subroutine uses Hamming's modified predictor–corrector method. Since the method is not self-starting, starting values were obtained by a special Runge–Kutta procedure, and one iteration step was added to the predictor–corrector method.

TABLE 1. MODEL PARAMETERS FOR CYTOSINE ARABINOSIDE IN SEVERAL SPECIES

Parameter	Mouse	Monkey	Dog	Man
Body wt (g)	22	5000	10,000	70,000
Volume (ml)				
Blood	1·67	367	670	2670
Liver	1·30	135	250	1700
Gut	1·50	230	400	3180
Heart	0·095	17	54	450
Kidney	0·34	30	50	1060
Lean	10·0	2000	4500	27,000
Marrow	0·60	135	270	2000
Blood flow (ml/min)				
Blood	4·38	431	805	4040
Liver	1·80	153	270	1450
Gut	1·50	125	216	1100
Heart	0·28	65	54	240
Kidney	1·30	123	216	1240
Lean	0·83	67	225	930
Marrow	0·17	23	40	180
Michaelis constant (μg/ml H_2O)	283	39	115	39
Deaminase activity (μg/g-min)				
Blood		1·6		
Liver	4·6	80·2	7	119
Gut	8·3			
Heart		57·0		6
Kidney	91·5	71·8		20
Lean		34·3		
Marrow				
Kidney clearance (ml/min)	0·18	14	32	90

is appropriate for Ara-C and Ara-U individually. Predictions of urinary excretion have verified this assumption in man.[6]

The injection function (equation 2) was a short pulse for all intravenous doses (monkey, dog, man). For the intraperitoneal injections (mouse), it had the form $g(t) = 1/\tau \exp(-t/\tau)$. The value of the time constant, τ, for the mouse was chosen equal to 10 min, and the function was truncated at a value of $t = 5\tau$.

EXPERIMENTAL

Swiss mice (No. MAC-1), in groups of five, were treated with 30 mg/kg of ^{3}H-Ara-C intraperitoneally. The ^{3}H-Ara-C was purchased from Schwarz/Mann and, after preliminary treatment according to the procedure of Mulligan and Mellett,[7] had a specific activity of 20·94 μCi/mg. The labeled stock material was diluted with unlabeled Ara-C in a 1:9 ratio for administration to the animals. The groups of animals were sacrificed at 5, 15, 30, 60, 120 and 180 min after drug administration. Pooled

blood from the animals was collected in tubes containing 1×10^{-4} M tetrahydrouridine (H_4U), an inhibitor of the deaminase, and the serum was separated after clotting. Tissues from the animals were removed and the organs pooled and immediately frozen on solid CO_2 and kept frozen until analysis.

At the time of analysis, the tissues were thawed and a 1·0-g sample was homogenized in 9·0 ml of physiological saline containing 1×10^{-4} M H_4U. The tissue homogenates were deproteinized and analyzed according to the method of Mulligan and Mellett.[7] The results are expressed in terms of total radioactivity per sample and micrograms of Ara-C or Ara-U per ml of fluid or per g of tissue. (This system also estimates phosphorylated products of Ara-C, but does not distinguish between the mono-, di- or triphosphates.)

Two rhesus monkeys (*Macaca mulatta*) received 50 mg/kg of ^{3}H-labeled Ara-C intravenously. Blood samples were collected periodically in tubes containing H_4U. One animal was sacrificed at 60 min after drug administration and tissues were collected and frozen. The other animal was sacrificed at 120 min and tissues were collected and frozen. Serum and tissues were analyzed in a fashion identical to that described for the mouse.

Five beagle dogs received 50 mg/kg of ^{3}H-labeled Ara-C intravenously. Blood samples were collected periodically through 24 hr, and the individual animals were sacrificed at 1, 2, 4, 8 and 24 hr. Tissues were collected at these times and processed in the manner described above for the mouse and monkey.

RESULTS AND DISCUSSION

Mice. The predicted kinetic events accompanying the distribution of Ara-C and Ara-U between two major compartments in the mouse after an intraperitoneal injection are shown in Fig. 2. The concentration of Ara-C in the blood peaks at about 10 min and then falls exponentially with time. The early phase to about 25 min is a result of several simultaneous processes: absorption of Ara-C from the peritoneal cavity; redistribution into other compartments; metabolism in the kidney, liver and gastrointestinal tract; and clearance by the kidney. Well-perfused regions (not shown) follow the blood concentration quite closely, except as influenced by rapid metabolism. The relatively poorly perfused lean compartment cannot follow the blood concentration closely and thus lags, reaching a peak at about 25 min and, subsequently, serving as a reservoir from which Ara-C exhibits net transfer back into the blood.

Ara-U is formed in the kidney, liver and gastrointestinal tract from which it rapidly enters the blood. Again, the concentrations in blood and well perfused regions follow each other quite closely, except as influenced by enzymatic formation. The blood concentration of Ara-U peaks at 1 hr. It becomes less than the lean tissue concentration at about 75 min and, subsequently, the lean tissue serves as a reservoir from which Ara-U also shows a net transfer from lean to blood.

Figure 3a and b shows comparisons between model simulations and experimental data for mice given 30 mg/kg of Ara-C i.p. The simulations agree fairly well with the data for Ara-C, Ara-U and their total in both lean tissue and serum. The data point for muscle at 30 min is about 40 per cent less than the model prediction. This discrepancy could reflect differences among the individual mice, or it could suggest

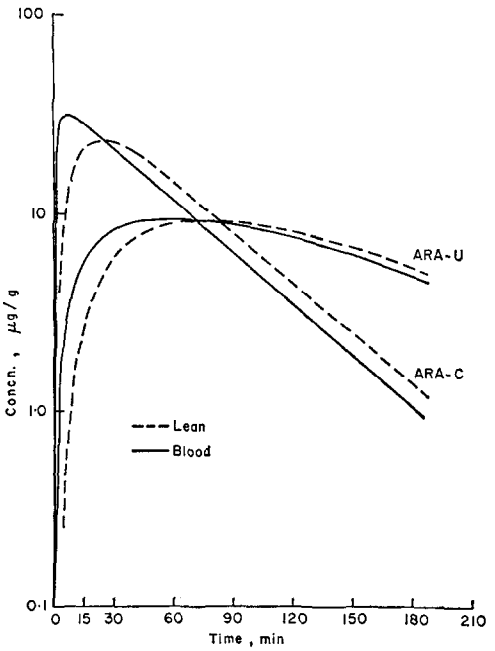

FIG. 2. Model simulations of the concentrations of Ara-C and Ara-U in the blood and lean compartments after an i.p. injection of 30 mg/kg of Ara-C in mice.

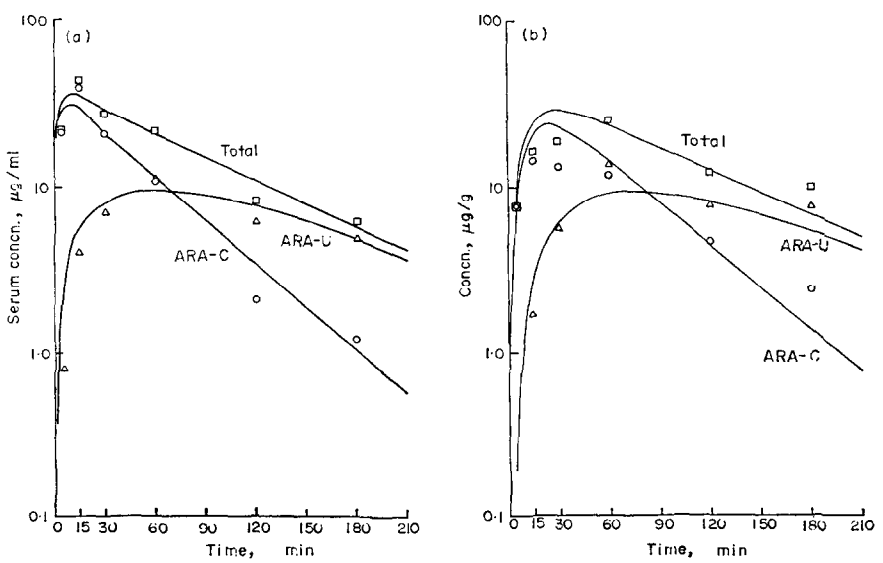

FIG. 3. Model simulations (solid lines) compared with experimental data for Ara-C and Ara-U concentrations after 30 mg/kg i.p. of Ara-C in mice: (a) blood or serum; (b) muscle.

that transfer from blood into muscle is slower than would be predicted on the basis of average blood flow.

Cytosine arabinoside was never detected in mouse kidneys. This reflects the high deaminase levels in this tissue. The model predicts that the concentration of Ara-C in the blood leaving the kidney (and the kidney tissue) would be reduced by 23 per cent from the arterial concentration. When blood flow stops, the kidney becomes a batch rather than flow reactor. At concentrations well below the Michaelis constant, the process would be expected to obey first-order kinetics with a rate constant equal to

$$\frac{91{\cdot}5\ \mu g/(g)\ (min)}{(283\ \mu g/ml\ water)\ (0{\cdot}83\ ml\ water/g)} = 0{\cdot}39\ min^{-1}$$

or a half-life of 1·8 min. We conclude that significant deamination may have occurred in mouse kidney after cessation of blood flow, despite the precautions that were taken.

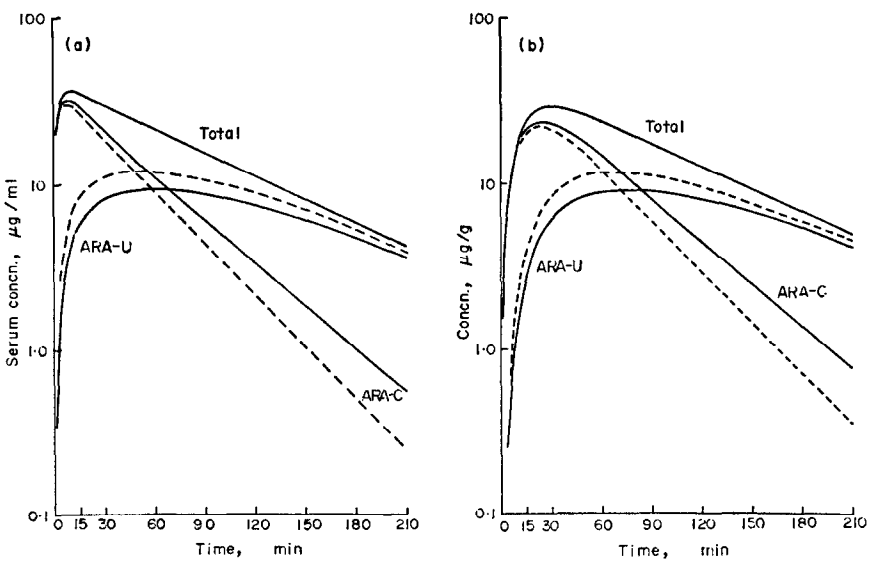

FIG. 4. Effect on model simulations of increasing enzyme activity in mouse by 50 per cent (dashed line) compared with simulations shown in Fig. 3 (solid line): (a) blood or serum; (b) muscle.

We have not undertaken a detailed analysis of the sensitivity of the model to the individual parameters; however, certain of them have been perturbed to illustrate their influence. Figure 4a and b illustrates the effect of increasing enzyme activities in the mouse tissues by 50 per cent. Figure 5a and b shows the effect of reducing the lean tissue perfusion rate by 50 per cent. This could represent a real physiologic flow if our estimate were not close for these mice or if there were significant maldistribution (shunt) in flow. The lower flow could also approximately represent some resistance to diffusion between the blood in the lean tissue and its intracellular space. A rigorous criterion for flow limitation is available;[6,11] however, it is not possible to apply it without data on membrane permeability.

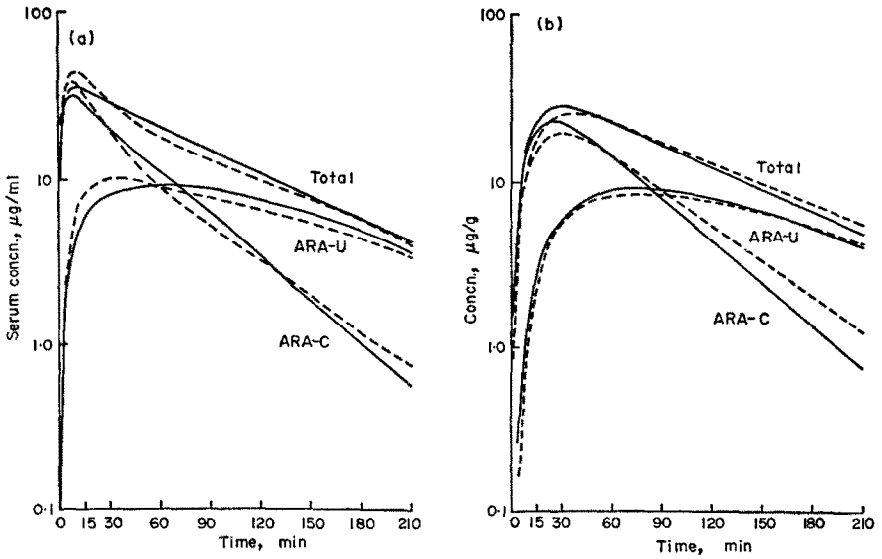

FIG. 5. Effect on model simulations of reducing lean tissue perfusion in the mouse by 50 per cent (dashed line) compared with simulations shown in Fig. 3 (solid line): (a) blood or serum; (b) muscle.

Monkeys. Very high and quite variable deaminase activities have been reported in tissue homogenates of liver, kidney, heart and muscle from the rhesus monkey.[2] Deaminase activity also occurs in serum.[7] It would be anticipated that Ara-C concentrations in individual monkeys could not be quantitatively predicted by a model incorporating average parameters. This problem is indicated in Fig. 6, where con-

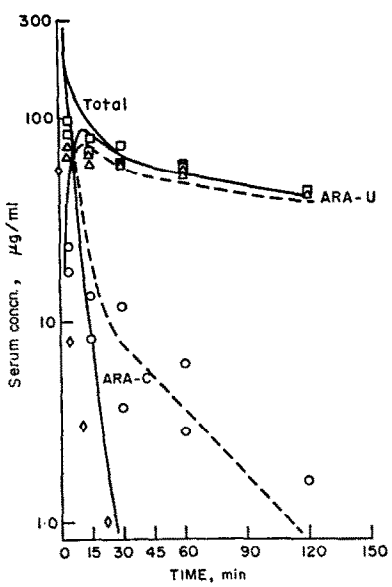

FIG. 6. Model simulations (solid lines) compared with experimental data for Ara-C and Ara-U after 50 mg/kg i.v. in the monkey. The dashed line shows the effect of assuming neglible deaminase activity in the lean compartment. The diamonds are Ara-C data from Mulligan.[16]

centrations of Ara-C, Ara-U and their total are shown for two monkeys in this study. Also shown are Ara-C data from Mulligan.[16] The model predictions shown by the lines are in general agreement with the data, confirming very rapid redistribution and metabolism along with elimination from the body; however, there are large individual differences in the serum concentrations of Ara-C, particularly at longer times. This latter observation illustrates intraspecies differences that occur.

We note that even in the monkey which exhibited the slowest decrease in serum Ara-C, the concentration was only about 5–10 per cent of that of Ara-U after 30 min. This amount could be accounted for by a poorly perfused compartment, which does not metabolize the parent compound and which can serve as a reservoir for release into the blood. This appears to be at least part of the explanation for the prolonged presence of Ara-C in human plasma.[6] To obtain the effect in monkeys, we simulated

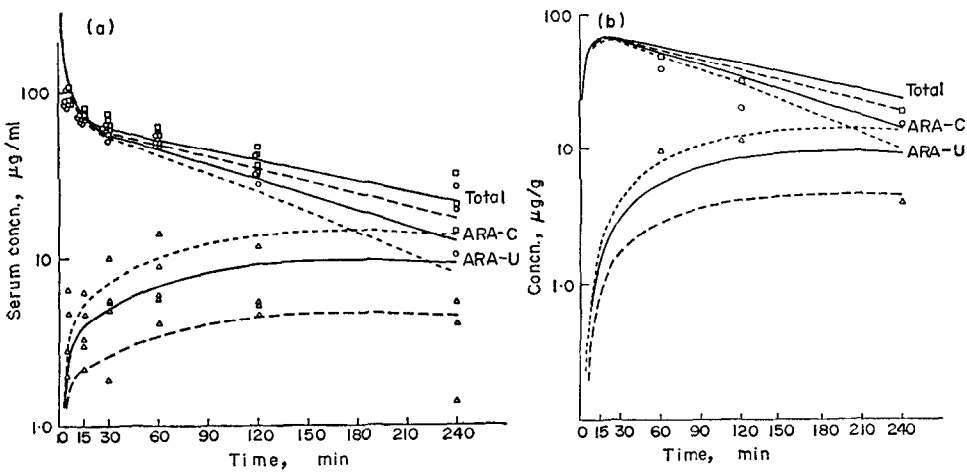

FIG. 7. Model simulations compared with experimental data for Ara-C and Ara-U after 50 mg/kg i.v. in the dog. Dotted line, K_m for monkeys and humans; dashed line, K_m for mice; solid line, $K_m = 115$ μg/ml of tissue water; (a) blood or serum; (b) muscle.

plasma concentrations of Ara-C and Ara-U in the monkey with no deaminase activity in the lean compartment. This simulation is shown in Fig. 6 as dashed lines; the total is unchanged. The simulation is much better for the two monkeys in this study. The possible role of the lean tissue in storing Ara-C is suggested but not proven by the presence of significant amounts (relative to serum) at the termination of the experiments: at 1 hr, 1·5 μg/g; at 2 hr, 1·7 μg/g. The Ara-U concentrations at these same times are 52 and 32 μg/g.

Dogs. Dogs have been reported to have the least relative amount of deaminase activity of the several species we have studied. Camiener and Smith[2] obtained significant but limited activity only in liver. The Michaelis constant for dog deaminase appears not to have been measured; so, an operational K_m *in vivo* within the context of the model is 'reported here. Figure 7a and b presents serum and muscle concentrations of Ara-C, Ara-U and their total. The slow deamination of Ara-C and the kinetics of distribution between blood (or well perfused inactive tissue) and lean

tissue are illustrated. Two different simulations were conducted. In one, the Michaelis constant was chosen equal to $1{\cdot}4 \times 10^{-4}$ M (39 μg/ml on a water basis), which is that for human liver homogenates *in vitro*[4] and monkey serum.[7] In the other, the constant was chosen equal to $1{\cdot}0 \times 10^{-3}$ M (283 μg/ml), or equal to that for mouse kidney *in vitro*.[8] Based on these two simulations, the effective Michaelis constant in dog liver *in vivo* is approximately 115 μg/ml or three times that for humans and monkeys.

Body-weight relationships. Adolph[17] observed that a variety of physiologic functions could be correlated with body weight if they were expressed as:

$$\text{Function} = K\,(\text{body wt})^n$$

where the coefficient, K, and the exponent, n, are characteristic of the function, e.g. inulin clearance. We have used similar reasoning previously in explaining certain aspects of species differences in the disposition of methotrexate in several species.[15,18] Methotrexate is not metabolized significantly by these species.

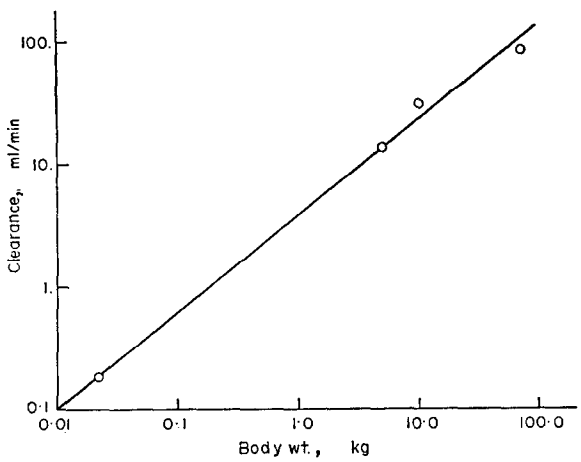

FIG. 8. Kidney clearance of Ara-C and Ara-U vs body weight for mice, monkeys, dogs and humans.

Ara-C is metabolized, and important species differences occur in the location and kinetic characteristics of the deaminase. A rather complex pharmacokinetic model has thus been required to account for these differences in a quantitative way and permit valid *in vitro–in vivo* correlations of kinetic data. Inspection of plasma or serum Ara-C concentrations from mice, monkeys, dogs and man shows that no simple body-weight correlation exists. There is, however, an orderly progression of kidney clearances ranging from 0·18 ml/min in a 22 g mouse to 90 ml/min in a 70 kg man.

The kidney clearances we have obtained from mice, monkeys, dogs and humans are plotted vs body weight on log–log paper in Fig. 8. A straight line correlation is obtained. It is interesting to note that the slope of the curve is 0·80, which is very similar to the value of 0·77 for inulin obtained by Adolph.[17] The value of 90 ml/min for Ara-C clearance in the human is somewhat less than the generally accepted inulin clearance of 125 ml/min for man;[19] however, it may be a reasonable average

for glomerular filtration rate in the limited number of patients (2 men and 2 women) for whom simulation was attempted.

General. The pharmacokinetics of Ara-C and Ara-U are related to enzyme kinetics in mice, monkeys, dogs and humans by means of a physiologic model. The model has permitted the use of enzyme activities and Michaelis constants determined *in vitro* to be applied *in vivo* at the actual sites of chemical reaction. In those cases where enzyme kinetics have not been determined *in vitro* or are not available in a form that allows complete *a priori* prediction of deamination *in vivo*, the model defines an operational basis for estimation of the kinetic parameters.

While general agreement has been obtained between theory and experiment, the predictive validity for any individual animal or patient should not be overemphasized. Very large intraspecies variations occur in most of the relevant parameters, so that no model based on averages can yield highly precise results in all cases. The model can, of course, be adapted to any individual if the parameters appropriate to that individual can be obtained. Even "chronobiologic" effects[20] may need to be included in some applications. A model containing averages can organize and use many diverse observations to explain major aspects of interspecies variability, provide better predictive bases, and illustrate the dominant features of pharmacokinetic behavior.

In addition to its usefulness in relating data obtained *in vitro* to events *in vivo* and to elucidating interspecies differences, pharmacokinetics can play an important role in cancer chemotherapy.[21] Werkheiser[22] has discussed mathematical modeling in chemotherapy. Two simulations were included: one for the action of methotrexate *in vivo*; the other, DNA biosynthesis by *de novo* pathways. He pointed out that explanation of the action of a drug *in vivo* requires the consideration of many dynamic processes. Pharmacokinetics provides a formal mechanism for considering the joint effects of drug absorption, blood flow, physicochemical interactions, chemical reactions, and elimination in determining drug concentration as a function of time at any site of action. Cytokinetic effects can be included directly[23] or through more fundamental, intracellular reactions. We have not been able to incorporate the biologically very important phosphorylation of Ara-C or its subsequent interactions and metabolic fate; however, a conceptual framework is provided by the model.

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APPENDIX

This appendix gives the references and assumptions that we used in the derivation of the model parameters. We obtained all human values from Dedrick *et al.*[6] The values for the 10-kg dog were interpolated on a log–log graph of the values for 5 and 17-kg dogs[15] vs body wt. We converted plasma values to blood values based on a hematocrit of 40.

Volumes. Liver, kidney, gut, blood.[15] Marrow[6]: human value linearly scaled in proportion to body wt. Lean: muscle[15] added to skin (mouse,[24] monkey,[25] dog[25]). The values obtained were: 13·9 ml, mouse; 3·3 l. monkey; 6·6 l. dog. Heart: mouse,[26] monkey,[27] dog.[17]

Blood flow rates. Liver, kidney, gut.[15] Marrow: mouse,[28] with the assumption that the perfusion of marrow in mice equals that in rats; monkey and dog, interpolation on log–log plot of flow in man[29] and rats[28] vs body wt. Lean: mouse,[15] with the assumption that skin and muscle have equal perfusion rates; monkey and dog, muscle flow[15] added to skin flow.[30, 31] Heart: mouse,[32] monkey,[30] dog.[31] The blood flow to the blood compartment is the sum of the appropriate flows (Fig. 1).

Michaelis constants. Kidney, mouse;[8] serum, monkey.[7] The reported values had units of moles/l. of water. To convert moles to grams, we used the molecular weight of Ara-C-(HCl). To convert the Michaelis constants to a basis of tissue volume, we multiplied by the fraction of water in the appropriate tissue.[19]

Deaminase activity. Kidney: mouse,[2] for lower limit. Liver and gut: mouse.[16] These values were not obtained at saturation. We assumed that the ratio of liver to kidney and gut to kidney deaminase activities reported by Mulligan[16] remained constant. We then used the kidney deaminase activity to calculate liver and gut values. Liver, heart, kidney and lean: monkey.[2] The value used for the lean compartment was adjusted from the value for muscle[2] so that the total deaminase was the same. Blood, monkey[16]; liver, dog,[2] mean value of reported range.