

Some Pharmacokinetic Aspects of 5-(Dimethyltriazeno)-imidazole-4-carboxamide in the Dog

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The renal clearance of 5-(dimethyltriazeno)-imidazole-4-carboxamide (DIC, NSC-45388) has been determined in the dog using radioactive inulin as the reference compound. The clearance ratio of DIC to inulin was greater than one, suggesting tubular secretion of DIC. The volume of distribution of DIC in the dog was determined from plasma clearance curves. In experiments lasting 1 hr., this volume was greater than total body water, indicating the localization of the drug in some tissues. However, when the experimental period was extended to 2 hr., the localized drug was eliminated from the body by both biotransformation and excretion, and the result was a reduction in the volume of distribution. A new mathematical treatment is described for analysis of the plasma clearance curves from 2-hr. experiments.

A POTENT CANCER chemotherapeutic agent of recent development, 5-(dimethyltriazeno)-imidazole-4-carboxamide (DIC, NSC-45388) (1), compares quite favorably with existing clinically useful agents such as cyclophosphamide and 6-mercaptopurine against the solid form of mouse Ehrlich carcinoma (2). In addition, it has shown activity against certain human malignant melanomas during clinical trials (3). Studies of its pharmacology have been reported (4). In this paper, certain pharmacokinetic aspects of DIC in the dog are described.

The renal clearance of DIC has been determined using radioactive inulin as the reference compound (5). The drugs were administered either by a single, rapid intravenous injection (6), or by constant infusion (7). Radioactive inulin was given either in trace dosage or diluted with unlabeled inulin.

The volume of distribution of the drug is estimated by a single, rapid intravenous injection technique (8-11). Frequent blood samples are taken at intervals. The plasma levels of DIC are plotted against time on a semilog scale. In experiments lasting 1 hr., the data are fitted by two exponential functions of time. This is interpreted to mean that the body behaves like a two-compartment open system in which the drug equilibrates between an immediately permeable compartment and a second compartment not immediately permeable to DIC. In addition, the drug is eliminated from the body by biotransformation and excretion following first-order kinetics (10).

An alternative interpretation similar to that above is that the drug leaves the first compartment both by elimination and by passive diffusion into a second compartment in accordance with Fick's law (8). In either case a second-order linear homogeneous differential equation with constant coefficients is derived. The volumes of the two compartments and the two clearance constants are computed from the graphic data. By either model, exactly the same numerical values are obtained in each experiment.

However, in volume of distribution experiments lasting over 1 hr., the data can best be fitted by three instead of two exponential functions of time. In other words, the differential equation relating plasma levels of DIC to time is now a third-order linear homogeneous one with constant coefficients. Again, two models can be constructed to arrive at such an equation. Either the body can be thought of as a three-compartment open system (9) or the previous two-compartment concept may be retained but the intercompartmental clearance written as a linear function of time. The volumes of distribution of the reference compounds, inulin and mannitol, are invariant with time in the dog, and their values agree with published data (8).

EXPERIMENTAL

Materials—Both DIC and DIC-²¹⁴C were supplied by the Drug Development Branch of the Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda, Md. For intravenous administration to the dog, DIC was dissolved in 0.1 *N* hydrochloric acid containing 0.88% of sodium chloride. No significant pH change in plasma after such an injection was ever observed in the experiments. Inulin carboxyl-¹⁴C, inulin methoxy-³H, and mannitol-¹⁴C were supplied by New

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England Nuclear Corp., Boston, Mass., and dissolved in normal saline for administration.

Colorimetric Determination—DIC was determined by a colorimetric method developed by the authors (12). The principle involves the photodecomposition by ultraviolet light irradiation of a solution of DIC in dilute acid in the presence of *N*-(1-naphthyl)-ethylenediamine (Bratton-Marshall reagent), followed by measurement of the color intensity of the azo compound thus formed. The method is specific for dialkyltriazeno compounds, and metabolite interference has not been encountered. Plasma and urine standards were prepared from samples collected just before the experiment.

Radioactivity Determination—A liquid scintillation spectrometer (Packard Tri-Carb, model 3375) was used. All plasma and urine samples (0.2 ml.) were counted directly as a suspension in 11 ml. of counting solution described previously (13). Quenching was determined by channel ratios of an automatic external standard. Solutions containing precisely measured amounts of radioactive drugs were counted together with the samples.

Dogs—Mongrel dogs, 13–30 kg., of both sexes were lightly anesthetized with phenobarbital. Drug administration and blood sampling were performed in different veins. Heparin was used as an anticoagulant. Urine was collected by an indwelling Foley catheter.

Renal Clearance—The renal clearance of DIC in comparison with inulin was determined by both constant infusion and single i.v. techniques. Prior to all experiments the bladder was emptied and preinjection blood samples obtained.

Blood samples were taken at the midpoint of urine collection intervals over a 2-hr. period. A Sigmamotor 18 SH pump (Sigmamotor Inc., Middleport, N.Y.) was used in constant infusion experiments. The priming dose consisted of 20 mg./kg. DIC in all experiments while inulin ranged from 0.25 mg./kg. (radioactive inulin only) to 60 mg./kg. (a mixture of radioactive inulin and unlabeled inulin). The sustaining dose for DIC was 10–15 mg./kg./hr. and for inulin was 0.49 (radioactive only) to 131 mg./kg./hr.

In single i.v. experiments the dose of DIC was 20

mg./kg. and inulin ranged from 0.65 mg./kg. (radioactive only) to 100 mg./kg.

Volume of Distribution—Intravenous infusion of normal saline (about 2 ml./min.) was started 30 min. prior to each experiment. The bladder was emptied and a blood sample taken immediately before the injection. DIC was administered at a dose range of 10–20 mg./kg. The dose of the reference substance used in combination with DIC (mannitol and inulin) was 100 mg./kg. Blood samples were taken every 5 min. over a 2-hr. period.

RESULTS AND DISCUSSION

Renal clearance of DIC and that of inulin are compared in Table I. The sustaining dose of DIC used to maintain a fairly constant plasma level of the drug in the dog is based on previous experience (4). The sustaining dose of inulin follows a recommendation in the literature (14). There appears to be no significant difference in renal clearance whether the single intravenous injection technique or the constant-infusion technique is adopted. Also, the amount of inulin used does not seem to be critical.

The authors inulin clearance value is lower than that reported in the literature (14) because the urine flow in the anesthetized dog is much lower than the published average. However, since the clearances of both DIC and inulin are equally affected by the low urine flow, clearance ratios of the two substances must be substantially correct. The average clearance ratio of DIC to inulin is 1.8, indicating tubular secretion of DIC. Compounds with dipolar structure, such as *p*-aminohippuric acid (I) are known to be secreted by renal tubules; DIC (II) also has a dipolar structure as shown below. Renal tubular secretion of DIC is therefore not surprising.

A typical 1-hr. plasma clearance curve plotted on a semilog scale is illustrated in Fig. 1. It is evident that the data can be adequately described by two exponential functions of time, thus:

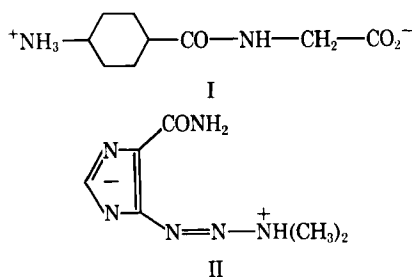
$$C_1 = Ae^{-r_1t} + Be^{-r_2t} \quad (\text{Eq. 1})$$

In other words, the plasma clearance curve is resolvable into two exponential (straight lines on a semilog

TABLE I—RENAL CLEARANCE OF DIC IN COMPARISON WITH INULIN

Dog No.	Wt., kg.	Priming Dose, mg./kg.		Sustaining Dose, mg./kg./hr.		Clearance ml./min./kg.		Clearance Ratio, DIC/Inulin
		DIC	Inulin	DIC	Inulin	DIC	Inulin	
Single i.v.								
53	14.6	20	100			2.6	1.4	1.9
85	21.6	20	100			5.1	2.6	2.0
87	19.3	20	100			4.3	2.6	1.7
277	20.3	20	0.65 ^a			1.7	1.6	1.1
					Average	3.4	2.1	1.7
Infusion								
16	14.8	20	50	10	131	3.0	2.0	1.5
18	19.9	20	50	15	98	3.9	3.9	1.0
37	19.6	20	0.25 ^a	15	0.49 ^a	4.4	2.2	2.0
41	16.0	20	50	15	121	9.4	3.0	3.1
279	26.0	20	60	10	75	3.6	2.2	1.6
					Average	4.9	2.7	1.8

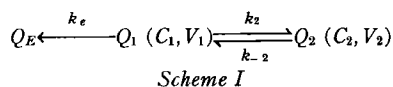
^a Radioactive material only.



scale) curves. The differential equation of which Eq. 1 is the general solution is

$$D^2C_1 + (r_1 + r_2)DC_1 + r_1r_2C_1 = 0 \quad (\text{Eq. 2})$$

Consider now the distribution of the drug in a two-compartment open system according to Scheme I.



Eq. 2 may be rewritten in terms of the rate constants of Scheme I. Thus,

$$D^2C_1 + (k_2 + k_{-2} + k_e)DC_1 + k_{-2}k_eC_1 = 0 \quad (\text{Eq. 3})$$

From the graphically obtained values of A , B , r_1 , and r_2 , the rate constants are readily computed (11):

$$k_2 = \frac{AB(r_2 - r_1)^2}{(A + B)(Ar_2 + Br_1)} \quad (\text{Eq. 4})$$

$$k_{-2} = \frac{Ar_2 + Br_1}{A + B} \quad (\text{Eq. 5})$$

$$k_e = \frac{(A + B)r_1r_2}{Ar_2 + Br_1} \quad (\text{Eq. 6})$$

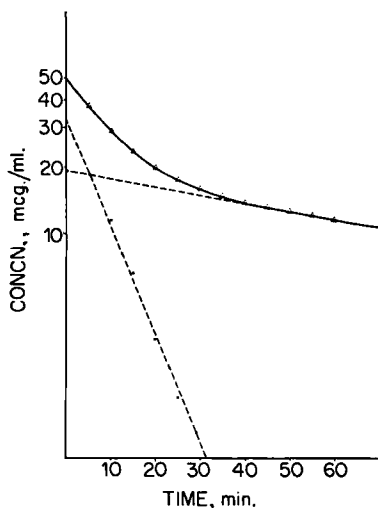


Fig. 1—Plasma level of DIC in dog No. 57 after a single i.v. injection (20 mg./kg.). A 1-hr. experiment. $C_p = 19.2 e^{-0.008t} + 32.7 e^{-0.111t}$.

V_1 is simply

$$V_1 = \frac{Q_1}{A + B} \quad (\text{Eq. 7})$$

The volume of the second compartment which is not immediately permeable to DIC, and the volume of distribution are also explicit in A , B , r_1 , and r_2 .

$$V_2 = \frac{AB(r_1 - r_2)^2 Q_1}{(A + B)(Ar_2 + Br_1)^2} \quad (\text{Eq. 8})$$

$$V_d = \frac{(Ar_2^2 + Br_1^2) Q_1}{(Ar_2 + Br_1)^2} \quad (\text{Eq. 9})$$

The derivation of Eqs. 8 and 9 will be discussed later.

Results of eight experiments are shown in Table II. In all cases the volumes of distribution of DIC in the dog are in excess of total body water content of the animal, suggesting local concentration of DIC in some tissues of the dog.

An alternative model for the fitting of the experimental data by two exponential terms has been offered by Sapirstein *et al.* (8). These authors considered the mass transfer from the first compartment by both elimination and passive diffusion into the second compartment. If the elimination follows first-order kinetics and the diffusion obeys Fick's law, the following equation is readily derived;

$$D^2C_1 + \left(\frac{\alpha + G}{V_1} + \frac{\alpha}{V_2} \right) DC_1 + \frac{\alpha G}{V_1 V_2} C_1 = 0 \quad (\text{Eq. 10})$$

If the drug suffers biotransformation, and if the method of drug determination is specific for the drug alone, the rate constant for biotransformation can be absorbed into G . Equation 10 can be rewritten

$$D^2C_1 + \left(\frac{\alpha}{V_1} + \frac{\alpha}{V_2} + \frac{G}{V_1} \right) DC_1 + \frac{\alpha G}{V_1 V_2} C_1 = 0 \quad (\text{Eq. 10a})$$

A comparison of Eq. 10a with Eq. 3 reveals

$$\frac{\alpha}{V_1} = k_2, \quad \frac{\alpha}{V_2} = k_{-2}, \quad \text{and} \quad \frac{G}{V_1} = k_e$$

or

$$\alpha = k_2 V_1 = k_{-2} V_2 \quad (\text{Eq. 11})$$

and

$$G = k_e V_1 \quad (\text{Eq. 12})$$

Since

$$\alpha = \frac{AB(r_1 - r_2)^2 Q_1}{(A + B)^2 (Ar_2 + Br_1)} \quad (\text{Eq. 13})$$

Equations 8 and 9 are readily derived from Eqs. 11, 12, and 13.

When the volume of distribution experiments were extended to 2 hr., the plasma clearance curve could best be resolved into three instead of two exponential terms (Fig. 2), thus:

$$C_1 = Ae^{-r_1 t} + Be^{-r_2 t} + Fe^{-r_3 t} \quad (\text{Eq. 14})$$

Apparently under these circumstances the body behaves like a three-compartment open system. This situation is frequently encountered in tracer problems in which the body is treated not as a three-compartment system but as a multiple-compartment open system (15-17). At steady state, the number

TABLE II—VOLUME OF DISTRIBUTION OF DIC (1 hr.)

Dog No.	Wt., kg.	Dose, mg./kg.	G, ml./min.	α , ml./min.	V_1 , l.	V_2 , l.	Volume of Distribution, % Body wt.	$k_1 \times 10^3$, min. ⁻¹	$k_{-1} \times 10^3$, min. ⁻¹	$k_e \times 10^3$, min. ⁻¹
57	30.3	10	162	578.1	9.3	25.7	115.5	6.3	2.2	1.8
57	30.3	20	225	628	11.7	13.6	83.5	5.4	4.6	1.9
63	13.5	10	104	255.2	4.1	7.0	82.2	6.3	3.6	2.6
63	13.5	20	174	256.9	7.3	5.9	97.8	3.5	4.3	2.4
72	22.3	20	512	444	10.2	9.8	89.7	4.4	4.5	5.0
76	23.6	20	146	481.2	9.5	7.3	71.2	5.1	6.9	1.5
87 ^a	19.3	20	146.1	548	5.7	6.0	60.6	9.6	9.2	2.6
91 ^b	18.5	20	170.6	203.5	8.0	5.4	72.4	2.6	3.7	2.1

^a DIC-2-¹⁴C used in combination with unlabeled DIC and inulin-methoxy-³H. The volume of distribution of inulin was 32% of body weight. ^b Unlabeled DIC used in combination with mannitol-1-¹⁴C. The volume of distribution of mannitol was 18% of body weight.

of postulated compartments and the rate constants of intercompartmental transport (namely, intercompartmental clearances) are estimated by nonlinear statistical regression analysis. Such a treatment requires a certain amount of mathematical sophistication and skill, and computer programs have been written to aid the analysis. Even in the present three-compartment problem, when the rate equations are written down, the solutions, though by no means difficult, are extremely cumbersome (see *Appendix*). Finally, on pharmacokinetic grounds alone, one finds it hard to explain why DIC becomes distributed in three instead of two functional compartments as the period of observation is extended.

However, the simpler two-compartment treatment can still be made applicable to the present problem if all functional body compartments except the one immediately permeable to the drug (plasma water in most cases) are conceptually combined and considered as one composite compartment. Intuitively one may write V_c , the volume of the composite compartment, as a weighted mean of all the compartments

$$V_c = \frac{\alpha_1 V_1 + \alpha_2 V_2 + \alpha_3 V_3 + \dots + \alpha_n V_n}{\alpha_1 + \alpha_2 + \alpha_3 + \dots + \alpha_n} \quad (\text{Eq. 15})$$

The intercompartmental clearance between the central compartment and the composite peripheral compartment is the mean of all the intercompartmental clearances.

$$\alpha = \frac{\alpha_1 + \alpha_2 + \dots + \alpha_n}{n} \quad (\text{Eq. 16})$$

To derive a third-order linear homogeneous differential equation with constant coefficients for the description of the above two-compartment open system, one writes α' as a linear function of t , thus:

$$\alpha' = \alpha + \beta t, \beta \ll \alpha \quad (\text{Eq. 17})$$

Consider the rate of decrease of a quantity of the drug from the central compartment both by elimination (and biotransformation) and by passive diffusion into the composite peripheral compartment (8):

$$DQ_1 = V_1 DC_1 = -GC_1 - \alpha'(C_1 - C_2) \quad (\text{Eq. 18})$$

$$Q_1 = C_1 V_1 + C_2 V_2 + \int_0^t GC_1 dt$$

or

$$C_2 = \frac{1}{V_1} \left(Q_1 - C_1 V_1 - \int_0^t GC_1 dt \right) \quad (\text{Eq. 19})$$

Substitution of Eqs. 17 and 19 into Eq. 18 followed by rearrangement gives

$$DC_1 + \left(\frac{\alpha + \beta t + G}{V_1} + \frac{\alpha + \beta t}{V_2} \right) C_1 + \frac{(\alpha + \beta t)G}{V_1 V_2} \int_0^t C_1 dt - \frac{(\alpha + \beta t)Q_1}{V_1 V_2} = 0 \quad (\text{Eq. 20})$$

The integral is removed by differentiating Eq. 20 twice with respect to t

$$D^3 C_1 + \left(\frac{\alpha + \beta t + G}{V_1} + \frac{\alpha + \beta t}{V_2} \right) D^2 C_1 + \left(\frac{2\beta V_1 + 2\beta V_2 + G(\alpha + \beta t)}{V_1 V_2} \right) DC_1 + \frac{2\beta G}{V_1 V_2} C_1 = 0 \quad (\text{Eq. 21})$$

The general solution of Eq. 21 is complex. How-

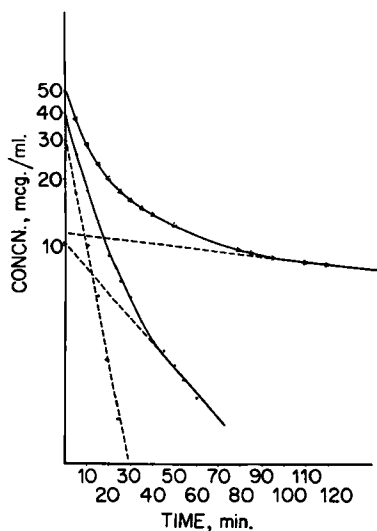


Fig. 2—Plasma level of DIC in dog No. 57 after a single i.v. injection (20 mg./kg.). A 2-hr. experiment. $C_p = 11.4 e^{-0.003t} + 10.3 e^{-0.028t} + 30.5 e^{-0.117t}$.

TABLE III—VOLUME OF DISTRIBUTION OF DIC (2 hr.)

Dog No.	Wt., kg.	Volume of Distribution, % Body wt.	G, ml./min.	α' , ml./min.	β , ml./min. ²
57	30.3	50.8	585	279	0.32
72	22.3	49.3	862	100	0.39
76	23.6	52.5	354	136	0.28
85 ^a	21.6	35.2	322	68	0.09
87 ^a	19.3	47.6	309	259	0.12

^a DIC-2-¹⁴C used.

ever, remembering $\beta \ll \alpha$, provided t is not too large, $\alpha + \beta t \rightarrow \alpha'$. Under these conditions Eq. 21 is therefore reduced to

$$D^3C_1 + \left(\frac{\alpha' + G}{V_1} + \frac{\alpha'}{V_2} \right) D^2C_1 + \frac{\alpha'G}{V_1V_2} DC_1 + \frac{2\beta G}{V_1V_2} C_1 = 0 \quad (\text{Eq. 22})$$

This is precisely the equation desired because the general solution of Eq. 22 is Eq. 14, (i.e.,

$$C_1 = A_e^{-r_1t} + B_e^{-r_2t} + F_e^{-r_3t}.$$

The usual boundary conditions are: at $t = 0$,

$$C_1 = C_0 = A + B + F \quad (\text{Eq. 23})$$

and also

$$DC_1 = -(Ar_1 + Br_2 + Fr_3) = -(\alpha + G)C_0/V_1 \quad (\text{Eq. 24})$$

By simple algebraic manipulations, V_1 , V_2 , G , and α are now expressed in A , B , F , r_1 , r_2 , and r_3 .

Therefore:

$$V_1 = Q_I/(A + B + F) \quad (\text{Eq. 25})$$

$$V_2 = \alpha'G/(r_1r_2 + r_2r_3 + r_1r_3)V_1 \quad (\text{Eq. 26})$$

$$G = \frac{(r_1r_2 + r_2r_3 + r_1r_3)Q_I}{A(r_2 + r_3) + B(r_1 + r_3) + F(r_1 + r_2)} \quad (\text{Eq. 27})$$

$$\alpha' = \frac{(Ar_1 + Br_2 + Fr_3)V_1}{A + B + F} - G \quad (\text{Eq. 28})$$

$$\beta = \frac{r_1r_2r_3}{2G} V_1 V_2 \quad (\text{Eq. 29})$$

and, finally, the volume of distribution, V_d , is

$$V_d = \frac{V_1 + V_2}{W} \times 100 \quad (\text{Eq. 30})$$

Table III shows the numerical values of the volume of distribution and G , α' , and β . In the 2-hr. experiments, the volume of distribution of DIC averages 47% of body weight of the dog, or 80% of the total body water content. It may be recalled that, in all experiments of 1-hr. duration, the volumes of distribution exceed total body water content, suggesting the localization of the drug in some tissues. This contention receives support from recent preliminary studies. Using DIC-2-¹⁴C, it has been shown that 15 min. after an i.p. injection in the mouse radioactivity is indeed localized in the liver and the small intestine.¹ When the volume of

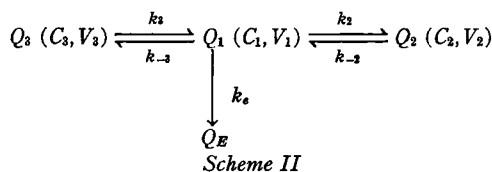
distribution experiment is carried on to 2 hr., the localized drug is eliminated from the body by both biotransformation and excretion, and consequently a reduction in the volume of distribution is observed.

NOTATIONS

- C = Concentration of DIC. The subscripts denote individual compartments.
 A, B, F = Intercepts of plasma clearance curve (plotted on a semilog scale) on the y -axis (concentration).
 r_1, r_2, r_3 = Slopes of the individual exponential lines.
 t = Time.
 D = The differential operator d/dt .
 k_2 = First-order rate constant of drug transport from the central, immediately permeable compartment (plasma) to Compartment 2, with the dimension of t^{-1} .
 k_3 = Similar constant for Compartment 3.
 k_{-2} = First-order rate constant of reverse drug transport from Compartment 2 back to the central compartment.
 k_{-3} = Similar constant for Compartment 3.
 Q = Quantity of DIC. The subscripts refer to individual compartments.
 Q_E = Quantity of DIC eliminated from the body.
 k_e = First-order rate constant of drug elimination, with the dimension of t^{-1} .
 G = Glomerular filtration rate, with dimension L^3t^{-1} . (L stands for length.)
 V = Volume (dimension L^3) of individual functional compartment. The subscripts have the usual meaning.
 α, α' = Intercompartmental clearance with a dimension L^3t^{-1} .
 Q_I = Quantity of DIC injected.
 β = A constant of dimension L^3t^{-2} .
 V_d = Volume of distribution.
 V_c = Volume of the composite compartment.
 W = Body weight.

APPENDIX

Distribution of a Drug in a Three-Compartment Open System—Consider Scheme II.



$$DC_1 = -(k_2 + k_3 + k_e)C_1 + k_{-2}C_2 + k_{-3}C_3 \quad (\text{Eq. 31})$$

$$DC_2 = k_2C_1 - k_{-2}C_2 \quad (\text{Eq. 32})$$

$$DC_3 = k_3C_1 - k_{-3}C_3 \quad (\text{Eq. 33})$$

Substitute Eqs. 32 and 33 into Eq. 31 and rearrange

$$D^3C_1 + (k_2 + k_3 + k_{-2} + k_{-3} + k_e)D^2C_1 + (k_2k_3 + k_3k_{-2} + k_e k_{-2} + k_e k_{-3} + k_{-2}k_{-3})DC_1 + (k_{-2}k_{-3}k_e)C_1 = 0 \quad (\text{Eq. 34})$$

¹ Housholder, G. E., unpublished observation.

TABLE IV—FIRST-ORDER RATE CONSTANT ($\times 10^3$ IN MIN.⁻¹) OF TRANSPORT OF DIC^a IN THE DOG IN 2-HR. EXPERIMENTS

Dog No.	k_2	k_3	k_e	k_{-2}	k_{-3}
57	3.8	2.5	1.2	6.0	1.4
72	2.7	1.9	4.3	5.3	2.2
76	3.1	0.2	1.3	5.3	1.5
85	2.3	2.5	1.1	6.0	1.7
87	4.5	2.6	0.1	14.0	1.0

^a Dose of DIC injected: 20 mg./kg.

The general solution of Eq. 34 is Eq. 14. Therefore,

$$r_1 + r_2 + r_3 = k_2 + k_3 + k_{-2} + k_{-3} + k_e \quad (\text{Eq. 35})$$

$$r_1 r_2 + r_1 r_3 + r_2 r_3 = k_2 k_{-3} + k_3 k_{-2} + k_e k_{-2} + k_e k_{-3} + k_{-2} k_{-3} \quad (\text{Eq. 36})$$

$$r_1 r_2 r_3 = k_{-2} k_{-3} k_e \quad (\text{Eq. 37})$$

The boundary conditions are, at $t = 0$: $C_1 = C_0 = A + B + F$, which is Eq. 23, and

$$DC_1 = -(k_2 + k_3 + k_e)C_0 = -(Ar_1 + Br_2 + Fr_3) \quad (\text{Eq. 24a})$$

It follows that

$$k_2 + k_3 + k_e = \frac{Ar_1 + Br_2 + Fr_3}{A + B + F} \quad (\text{Eq. 38})$$

From Eqs. 35 and 38,

$$k_{-2} + k_{-3} = \frac{B + Fr_1 + (A + F)r_2 + (A + B)r_3}{A + B + F} \quad (\text{Eq. 39})$$

Since

$$C_0 = k_e \int_0^\infty C_1 dt = k_e \int_0^\infty (Ae^{-r_1 t} + Be^{-r_2 t} + Fe^{-r_3 t}) dt = k_e \left(\frac{A}{r_1} + \frac{B}{r_2} + \frac{F}{r_3} \right) \quad (\text{Eq. 40})$$

therefore,

$$k_e = \frac{(A + B + F)r_1 r_2 r_3}{Ar_2 r_3 + Br_1 r_3 + Fr_1 r_2} \quad (\text{Eq. 41})$$

and from Eq. 36,

$$k_{-2} k_{-3} = \frac{Ar_2 r_3 + Br_1 r_3 + Fr_1 r_2}{A + B + F} \quad (\text{Eq. 42})$$

From Eqs. 39 and 42, k_{-2} and k_{-3} are computed; and from Eqs. 36 and 38, k_2 and k_3 are computed. Table IV presents the numerical values of these rate constants.

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Keyphrases

5-(Dimethyltriazeno)-imidazole-4-carboxamide(DIC)—pharmacokinetics
 Renal clearance—DIC
 Volume of distribution—DIC, DIC-2¹⁴C
 Pharmacokinetic equations—plasma clearance, DIC
 Colorimetric analysis—spectrophotometer
 Liquid scintillation counting—radioactivity determination