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Measurement of Sulfamethizole Clearance Rate by Nonthrombogenic Constant Blood-Withdrawal System

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Abstract □ A method for the measurement of the total body clearance rate (*CR*) of drugs is described. It involves a single intravenous injection of a known quantity of the drug (*D*) and automatic integration of the plasma concentration curve, using a portable, nonthrombogenic, constant blood-withdrawal system. When blood withdrawal is carried out until the concentration of the drug in the plasma approaches zero, the concentration of the drug in the collected pool, the integrated concentration (IC_T) multiplied by the time of collection (*T*) yields the integral of the concentration curve: $\int_0^\infty X'\,dt = IC_T \times T$ and $CR = (D/\int_0^\infty X'\,dt)$. The method was tested by measuring the clearance rate of sulfamethizole in five dogs by the established constant infusion method. At three plasma levels (25, 75, and 200 mg/liter), the plasma concentration had no significant effect on the clearance rate. The clearance rate of sulfamethizole was subsequently measured in the same dogs by the new single-injection constant withdrawal method. Multiple blood samples were collected at 15-min intervals simultaneously with the constant withdrawal of blood. There was no significant difference between the clearance rate of sulfamethizole measured by the two methods. The initial peak mean concentration of the drug from the time of injection ($t = 0$) to the time of the first blood sampling ($t = 15$ min) was calculated from the difference between $\int_0^\infty X'\,dt$ obtained by the constant withdrawal method and that obtained from the results of the multiple blood withdrawals by the trapezoidal rule. The integrated concentration IC_{15} was significantly higher than its estimation by the semilogarithmic linear regression method.

Keyphrases □ Sulfamethizole—measurement of total body clearance rate, nonthrombogenic constant blood-withdrawal system, dogs □ Clearance rate, total body—sulfamethizole, measured using nonthrombogenic constant blood-withdrawal system, dogs □ Blood-withdrawal system—nonthrombogenic, use in measurement of total body clearance rate of sulfamethizole, dogs

The total body clearance rate of drugs (*CR*) is defined as the volume of blood containing the amount of the drug under study that is irreversibly removed in a unit of time. Two established methods for measuring the clearance rate are currently in use (1–5): (*a*) a steady-state constant infusion method, in which the clearance rate is derived from the infusion rate at a situation where both reach equilibrium; and (*b*) a single-pulse injection method, in which the value $\int_0^\infty X'\,dt$ of the disappearance curve is calculated from multiple determinations of X'.

A new method for the measurement of $\int_0^\infty X'\,dt$ is proposed. The new method became possible by the development of a nonthrombogenic, constant blood-withdrawal system (6). A single determination of the integrated concentration of the drug in the pool obtained by constant withdrawal yields the $\int_0^\infty X'\,dt$. This method is not dependent on an assumed knowledge of the equation of the disappearance curve. The purposes of this work were to demonstrate the use of this new method and to test its accuracy in comparison with the established method.

EXPERIMENTAL

Apparatus—A constant infusion pump[1] was used for the constant infusion method. Nonthrombogenic, presterilized, disposable Kowarski sets and withdrawal pump[2] were used for the withdrawal method.

The method of Bratton and Marshall (7) was used to assay sulfamethizole[3] spectrophotometrically[4] in plasma and in saline solutions.

Clearance Rate Measurement by Constant Infusion Method—An intravenous catheter was placed in a jugular vein and another in a hindleg cephalic vein. The jugular catheter was then connected to a 100-ml syringe containing a sterile solution of sulfamethizole in saline. Three experiments were carried out on each dog. In each experiment, the concentration of sulfamethizole was maintained at 15, 35, or 100 mg/ml.

The syringe was then placed in a constant infusion pump. The pump was set to infuse at a rate of 4 ml/hr and continued without interruption for at least 5.5 hr. While the sulfamethizole solution was infused through the jugular vein, blood samples were drawn every 15 min from the cephalic vein into a heparinized syringe. Each blood sample was centrifuged and the plasma was stored at 4°.

At the end of 5.5 hr of infusion, the syringe was disconnected from the catheter in the jugular vein, without stopping the pump. The pump effluent was delivered into a measuring cylinder for an additional hour, and the rate of delivery was noted. The concentration of sulfamethizole in both the plasma sample and in the infusion solution was measured.

Since there was no detectable fluctuation in the plasma level of sulfamethizole during the final 2 hr of infusion, the mean of these results was considered the concentration at equilibrium (X^c). The infusion rate (*IR*) was calculated from the measured concentration in the infusion solution and the volume actually delivered into the measuring cylinder during the final hour. The total body clearance rate was then calculated:

$$CR = \frac{IR}{X^c} \qquad \text{(Eq. 1)}$$

[1] Model 352, Sage Co., Oriane Research, Cambridge, Mass.
[2] Sigmamotor Inc., Middleport, N.Y.
[3] Ayerst Laboratories, New York, N.Y.
[4] Beckman DU.

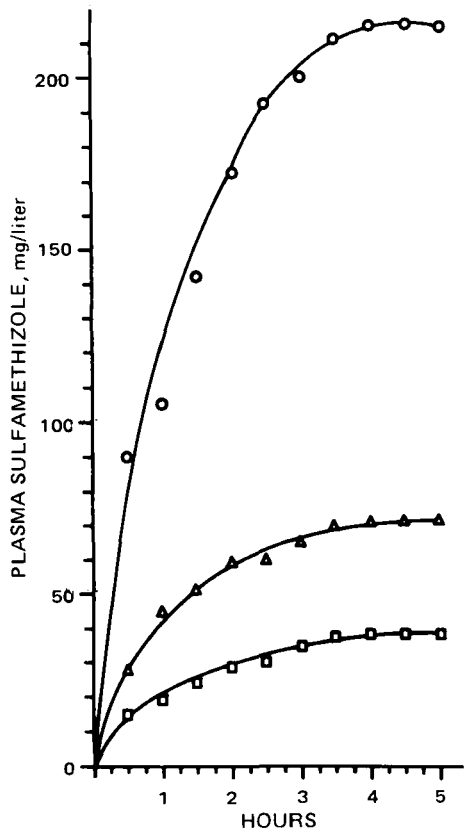

Figure 1—*Plasma concentrations of sulfamethizole in Dog 3 during 5 hr of constant intravenous infusion at 68 (□), 141 (△), and 385 (○) mg/hr.*

The experiment on each dog was repeated at three different infusion rates to demonstrate that the clearance rate of sulfamethizole remained constant over a wide range of blood concentrations.

Clearance Rate Measurement by New Constant Withdrawal Method—The same five dogs used in the constant infusion method were restudied. Five milliliters of a 40-mg/ml sterile solution of sulfamethizole was injected as a single pulse through an intravenous catheter. The catheter was left in place and flushed by a slow drip of saline. A constant withdrawal of blood from a vein in another limb was initiated at the moment of the pulse injection, using a nonthrombogenic, presterilized, disposable Kowarski set and withdrawal pump. The use and function of this system were described previously (6). The rate of withdrawal was set at 6 ml/hr and was continued for 5 hr.

In addition to the constant withdrawal, 2-ml blood samples were withdrawn from another vein every 15 min. The concentration of sulfamethizole in the plasma samples obtained every 15 min, as well as in the plasma from the pool of blood collected by constant withdrawal, was measured (6). The concentration of sulfamethizole in the constant withdrawal pool was the integrated concentration (IC_{5hr}).

Calculations—The integral of the concentration curve was calculated as follows: $\int_0^\infty X'\,dt = IC_{5hr} \times 5$; the clearance rate was calculated from this integral and the amount of injected sulfamethizole (D) using:

$$CR = \frac{D}{\int_0^\infty X'\,dt} \qquad \text{(Eq. 2)}$$

The integral of the concentration curve from the time the first blood sample was taken (t = 15 min) was calculated from the results of the multiple blood withdrawals by the trapezoidal rule:

$$\int_0^\infty X'\,dt = 7.5\,(X_{15} + 2X_{30} + 2X_{45} + \cdots + 2X_{285} + X_{300}) \qquad \text{(Eq. 3)}$$

Table I—Sulfamethizole Clearance Rate at Various Plasma Levels

Dog	Infusion Rate, mg/hr	Plasma Concentration at Equilibrium, mg/liter	Clearance Rate, liters/hr
1	56.8	28.2	2.0
	137.6	64.8	2.1
	420.0	196.0	2.1
2	70.0	25.6	2.8
	143.0	49.3	2.9
	415.0	143.0	2.9
3	68.0	36.2	1.9
	140.8	72.8	1.9
	385.0	214.0	1.8
4	65.6	45.0	1.5
	138.0	92.0	1.5
	375.0	251.0	1.5
5	60.8	31.5	1.9
	163.0	76.0	2.1
	343.0	156.0	2.2

The initial peak, IC_{15}, was then calculated using:

$$IC_{15} = \frac{\left(\int_0^\infty X'\,dt\right) - \left(\int_{15}^\infty X'\,dt\right)}{15} \qquad \text{(Eq. 4)}$$

The result was compared to an estimated peak concentration of sulfamethizole obtained by the extrapolation to t = 0 of the semilogarithmic linear regression of $X_{15}\cdots C_{300}$.

RESULTS AND DISCUSSION

In the present study, the clearance rate of sulfamethizole was first measured by the constant infusion method. The study was repeated three times on each dog. A different infusion rate was used each time, leading to a different level at which the equilibrium concentration was reached.

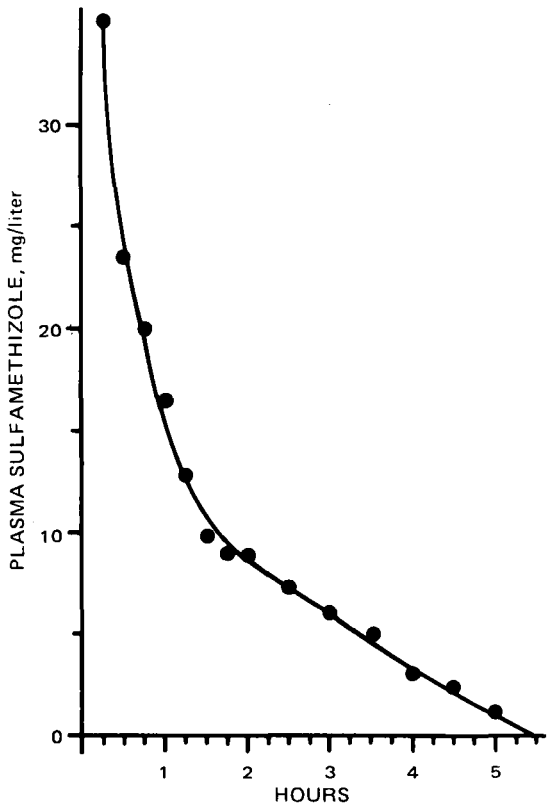

Figure 2—*Disappearance curve of plasma sulfamethizole in a dog after a single intravenous injection.*

Table II—Sulfamethizole Clearance Rate (liters/24 hr)

Dog	Constant Infusion Method	Constant Withdrawal Method
1	2.0	2.1
2	2.9	2.9
3	1.9	1.8
4	1.5	1.5
5	2.1	2.5

The plasma concentrations of sulfamethizole that were registered during these three tests in one dog are shown in Fig. 1. Although the plateau level of sulfamethizole was different in each experiment, the calculated clearance rates were essentially equal. The results in all of the dogs are given in Table I. As can be seen, the clearance rate remained unaffected by changes in the sulfamethizole concentration.

After the clearance rate of sulfamethizole was measured by the constant infusion method, each dog was restudied by the single intravenous injection method. After a pulse injection of sulfamethizole, the level of sulfamethizole in the blood was followed by multiple blood withdrawals from one limb and a continuous constant withdrawal from another limb. A representation of the disappearance curve for one dog is given in Fig. 2.

The results obtained by the two methods are given in Table II. There was no significant difference between the clearance rates obtained ($p < 0.001$ by the paired t test).

The initial peak concentration of the drug, which occurs a short time after the pulse intravenous injection, has a major effect on the final integral, but there is no way to predict the initial integral from the instant of injection to the first blood sampling. This problem is solved by the continuous constant withdrawal, since $\Delta t \rightarrow 0$ in this method. The integral of the concentration curve from the first moment of sampling (t = 15 min) was calculated by the trapezoidal rule. The difference between the two integrals allows the calculation of the initial integral ($\int_0^{15} X' dt$) and the initial IC_{15}.

In the five described experiments, IC_{15} was always higher than the extrapolated concentration at $t = 0$ calculated by the semilogarithmic linear regression (Table III). The accuracy of the constant blood-withdrawal method was demonstrated by the similarity between the clearance rate obtained by the two methods.

Table III—Calculated Initial Concentration of Sulfamethizole

Dog	Extrapolation to $t = 0$, mg/liter	IC_{15}, mg/liter
1	37.8	278.6
2	31.2	161.9
3	56.7	250
4	60.3	365.1
5	37.0	232.6

REFERENCES

(1) J. F. Tait and S. Burstein, in "The Hormones," vol. 5, G. Pincus, K. V. Thimann, and E. B. Astwood, Eds., Academic, New York, N.Y., 1964, pp. 41, 550.

(2) J. F. Tait, *J. Clin. Endocrinol. Metab.*, **23**, 1285(1963).

(3) J. C. K. Loo and S. Riegelman, *J. Pharm. Sci.*, **57**, 918(1968).

(4) M. Rowland, S. Riegelman, and W. L. Epstein, *ibid.*, **57**, 984(1968).

(5) S. Riegelman and W. Sadee, in "Clinical Pharmacokinetics," G. Levy, Ed., American Pharmaceutical Association, Washington, D.C., 1974, p. 169.

(6) A. Kowarski, R. G. Thompson, C. J. Migeon, and R. M. Blizzard, *J. Clin. Endocrinol. Metab.*, **32**, 356(1971).

(7) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537(1939).

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Volume of Distribution as a Function of Time

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Abstract □ A single definition for all volume terms in pharmacokinetic modeling is proposed; this parameter is considered as a function of time. This definition will represent the kinetic nature of pharmacokinetic models and will provide a highly sensitive parameter for correlation with pharmacological responses.

Keyphrases □ Volume—terms in pharmacokinetic modeling, single definition proposed as a function of time □ Pharmacokinetic modeling—single definition for all volume terms proposed as a function of time □ Distribution volume—volume terms in pharmacokinetic modeling, single definition proposed as a function of time

In the pharmacokinetic modeling of the absorption, distribution, metabolism, and excretion of drugs, various volume terms have been defined, such as the volumes of central (V_c) and tissue (V_T) compartments, the steady-state volume of distribution ($V_{d_{ss}}$), and the volume of distribution following pseudo-distribution equilibrium (V_{d_β} or $V_{d_{area}}$) (1–9).

The purpose of this paper is to propose a single definition for volume terms that can be used regardless of the complexity of the pharmacokinetic model. If this term is considered as a variable rather than a constant, this parameter will be more meaningful and add a new dimension to the characterization of drug response through pharmacokinetic studies.

THEORETICAL

When a drug is introduced in the body such as by intravenous injection, it starts to eliminate and to distribute to other parts of the body instantaneously (Scheme I). The measured concentra-