isolated from beef pineal gland and purified according to the method of Axelrod and Weissbach (4).

The stock solutions of inhibitors II, III, IV, and VII were prepared in propylene glycol, and those of V, VI, VIII, and IX were prepared in dimethylsulfoxide. The previous findings showed that the same magnitude of inhibitory activity was obtained regardless of the use of these two solvents (1).

Incubation was carried out with *N*-acetylserotonin and *S*-adenosyl-L-methionine-methyl-^{14}C according to the previously described procedure (1).

REFERENCES

(1) Ho, B. T., McIsaac, W. M., and Tansey, L. W., *J. Pharm. Sci.*, in press.

(2) Potts, K. T., and Saxton, J. E., *J. Chem. Soc.*, **1954**, 2641.

(3) Katritzky, A. R., *ibid.*, **1955**, 2586.

(4) Axelrod, J., and Weissbach, H., *J. Biol. Chem.*, **236**, 211(1961).

Keyphrases

Enzyme inhibitors
N-Acetyltryptamines—synthesis
Hydroxyindole-*O*-methyltransferase inhibition—*N*-acyltryptamines

Effect of Flow Rate on the Distribution Kinetics of a Drug From Perfusate to a Perfused Organ

By RENPEI NAGASHIMA and GERHARD LEVY*

A pharmacokinetic analysis of the distribution of a drug into a perfused organ or hypothetical compartment of the body yields mathematical expressions which may be useful for ascertaining if the distribution of a drug is rate limited by the flow of the perfusate, by the diffusion of the drug from the perfusate into the organ, or if it is a function of both of these processes. These expressions have been used to analyze the distribution of a drug from a perfusing fluid into the isolated perfused rat liver, and of several drugs from a hypothetical central compartment to a hypothetical peripheral compartment in man.

THE DISTRIBUTION of a drug from a perfusion fluid such as the blood to a perfused organ such as the liver is likely to be rate limited by the flow of the perfusate, by the diffusion of drug from perfusate to the organ, or it may be a function of both of these processes. It is useful therefore to undertake a kinetic analysis of such a system so that the rate-limiting step in the distribution of a given drug under defined conditions may be determined.

For the purpose of this analysis, the liver is viewed as a tissue with numerous parallel channels through which perfusate flows. The concentration of the drug in the perfusate leaving the liver is lower than the drug concentration in the fluid entering the liver, due to the diffusion of drug from perfusate into the liver. Elimination of the drug by the liver and back-diffusion of the drug from the liver to the perfusate are considered to be negligible during the early distribution phase. These assumptions lead to the same model as has been presented in an earlier report from this laboratory dealing with drug elimination kinetics in a perfused organ (1). Equation 8 of that report then takes the form:

$$k_{\text{dist.}} = \frac{u}{V_R}[1 - \exp(-k_{\text{trans.}}V_L/u)] \quad \text{(Eq. 1)}$$

where $k_{\text{dist.}}$ is the apparent first-order rate constant for the distribution of drug from perfusate to the perfused organ or tissue, u is the flow rate of perfusate, V_R is the real or apparent volume of the extrahepatic perfusate (which in practice usually is equivalent to the real or apparent volume of total perfusate), V_L is the volume of the channels in the organ or tissue through which the perfusate flows, and $k_{\text{trans.}}$ is a rate constant for the transfer of the drug from perfusate to the organ or tissue.[1] When drug distribution is rate limited by the flow of the perfusate, *i.e.*, $u \ll k_{\text{trans.}}V_L$, the bracketed term in Eq. 1 approaches unity, and

$$k_{\text{dist.}} = \frac{u}{V_R} \quad \text{(Eq. 2)}$$

If, on the other hand, the distribution of the drug is rate limited by the transfer of the drug from perfusate to the tissue, $k_{\text{trans.}}V_L/u < 0.1$ and it can be shown (1) that

$$k_{\text{dist.}} = \frac{V_L}{V_R} \cdot k_{\text{trans.}} \quad \text{(Eq. 3)}$$

It should be noted that Eq. 3 does not contain the flow-rate term.

One may now turn to a consideration of the more general case, namely, the distribution and elimination of the drug during the entire experimental

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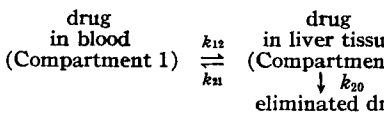
[1] This transfer rate constant may be the rate constant for diffusion of the drug through the blood, through a barrier between blood and tissue, or through tissue. It may also be a complex function of more than one of these, and it may include a factor for plasma protein binding of the drug.

period. When sampling intervals are sufficiently short, it is possible to observe a bi-exponential decline in the drug concentration in the perfusate of an isolated liver perfusion system as a function of time (2). Then,

$$C = A \exp(-\alpha t) + B \exp(-\beta t) \quad \text{(Eq. 4)}$$

where C is the drug concentration in the perfusate at any time t, B is the extrapolated zero-time concentration and $-\beta/2.3$ is the slope of the terminal exponential phase in a plot of log C *versus* t, A is the extrapolated zero-time intercept and $-\alpha/2.3$ the slope obtained when the differences between the early experimentally determined drug concentrations and the values obtained by back-extrapolation of the terminal exponential phase of the concentration *versus* time curve are plotted semilogarithmically. The transfer of a drug between liver and blood and the elimination of drug from the system *via* the liver can be represented by the following model,



where the k's are apparent first-order rate constants defined in terms of the rate of the changes in the amount of drug in the respective compartments. It is evident that $k_{dist.}$ in Eqs. 1–3 is essentially equivalent to k_{12}. Considering the usual case where $V_R \gg V_L$, V_R approximates the total blood volume ($V_R + V_L$). The value for k_{12} can be determined from the equation:

$$k_{12} = (\alpha A + \beta B)/(A + B) \quad \text{(Eq. 5)}$$

which is derived from the relationship described by Rescigno and Segre for a more general model (3).

In a recent study of the pharmacokinetics of bishydroxycoumarin elimination in isolated perfused rat liver systems (4), the following average values were obtained: V_R = 100 ml., k_{12} = 0.063 min.$^{-1}$ (see *Footnote 2*), and u = 19 ml./min. These data yield a $k_{trans.}V_L$ value of 7.6 ml./min. Since this value is not much smaller than the flow rate, u, it can be concluded that the distribution of bishydroxycoumarin from the perfusate to the liver tissue is not rate limited solely by the diffusion of drug from the perfusate to the liver. Also, since $k_{12}V_R$ = 6.3 ml./hr. and $< u$, the distribution is not rate limited solely by the flow rate of perfusate. Rather, on the basis of the kinetic model used, the kinetics of the distribution of bishydroxycoumarin is a function of both the flow rate of the perfusate and the process described by the transfer rate constant, $k_{trans.}$, for the diffusion of drug from perfusate to liver tissue.

A similar kinetic analysis can be used to get some indication of the rate-limiting process (or the relative importance of blood flow and diffusion) in the distribution of a given drug from blood to other tissues, both *in vitro* and *in vivo*. Considering, for example, the distribution of a drug in man, $k_{dist.}V_R$ would equal the plasma flow rate if the latter is the rate-limiting step in the distribution of the drug (Eq. 2). A test of the mathematical model presented here is that $k_{dist.}V_R$ should never exceed a value equivalent to the known plasma flow rate through the compartment into which the drug is diffusing. Riegelman and his associates have pointed out recently that the distribution and elimination of certain drugs can be described adequately by a two-compartmental open-system model consisting of a central and a peripheral compartment (5). Using their reported values for α, β, A, and B, k_{12} values were calculated (6). Their V_p and k_{12} are equivalent to V_R and $k_{dist.}$ as defined here, and thus $k_{dist.}V_R$ can be determined. The results of these calculations for the five drugs reported on by Riegelman *et al.* (5) are listed in Table I. The highest $k_{dist.}V_R$ value, 0.80 l./min., was obtained with thiopental. If the peripheral compartment is considered to be equivalent to the poorly perfused tissues as classified by Price (7), the plasma flow rate through this compartment is 0.9 l./min. (see *Footnote 3*). This is in good agreement with the flow rate calculated from the thiopental data and it appears therefore that the distribution of thiopental into the peripheral compartment is essentially blood flow rate limited. On the other hand, the $k_{dist.}V_R$ values for the other drugs are such that blood flow rate does not appear to be the rate-limiting factor in their distribution from the hypothetical central compartment to the hypothetical peripheral compartment. Neither is their distribution rate limited by the transfer process represented by the rate constant, $k_{trans.}$, since their $k_{trans.}V_L$ values are not much smaller than u (Table I). It appears rather that their distribution is a function of both blood flow rate and transfer rate.

The significant result of this analysis is that the pharmacokinetic data for one drug yielded a calculated plasma flow rate essentially equal to the actual flow rate reported in the literature and, just as important, that none of the data yielded theoretical plasma flow rates ($k_{dist.}V_R$) that were higher than the actual value. One complicating factor in the pharmacokinetic analysis of drug distribution in intact animals or man (as opposed to isolated perfused organ systems) is the possible contribution to the $k_{dist.}$ values due to direct diffusion of drug between adjacent tissues (9, 10). This can lead to $k_{dist.}V_R$ values which are higher than the flow rate, or it can

TABLE I—DISTRIBUTION KINETICS OF SEVERAL DRUGS IN MAN[a]

Drug	k_{12}, min.$^{-1}$	V_p, l.	$k_{12}V_p$ or $k_{dist.}V_R$, l./min.	$(k_{trans.}V_L)/u$
Acetylsalicylic acid	0.085	6.3	0.54	0.92[c]
Salicylic acid	0.070	5.6	0.39	0.57
Griseofulvin	0.0038	59.3	0.23	0.29
Thiopental[b]	0.0132	60.5	0.80	2.21
Pentobarbital[b]	0.0118	62.0	0.73	1.67

[a] k_{12} and V_p values from *Reference 5* except where indicated otherwise. [b] Calculated using data from *Reference 5* and Eqs. 16 and 17 from *Reference 6*. [c] The value of u used in the calculations in this column is 0.9 l./min. (see *Footnote 3* in text).

[2] The perfusate was sampled initially every 10 min. In most cases, there was very little difference between the drug concentration in the perfusate at 10 min. and the theoretical 10-min. value obtained by back extrapolating the linear segment of the log concentration *versus* time plot. Therefore, the estimated value of α is only a minimum value, and thus, the calculated k_{12} is only a minimum value.

[3] Price lists a blood flow rate of 1.65 l./min. Considering an average hematocrit value of 45% (8), this equals a plasma flow rate of 0.9 l./min.

yield values equivalent to the flow rate although the latter is not distribution rate limiting.

REFERENCES

(1) Nagashima, R., and Levy, G., *J. Pharm. Sci.*, **57**, 1991 (1968).
(2) Nagashima, R., and Levy, G., unpublished data.
(3) Rescigno, A., and Segre, G., "Drug and Tracer Kinetics," Blaisdell, Waltham, Mass., 1966, p. 84.
(4) Nagashima, R., Levy, G., and Sarcione, E. J., *J. Pharm. Sci.*, **57**, 1881(1968).
(5) Riegelman, S., Loo, J., and Rowland, M., *ibid.*, **57**, 128 (1968).
(6) *Ibid.*, **57**, 117(1968).
(7) Price, H. L., in "Uptake and Distribution of Anesthetic Agents," Papper, E. M., and Kitz, R. J., Eds., McGraw-Hill, New York, N. Y., 1963, p. 123.
(8) "Blood and Other Body Fluids," Altman, P. L., and Dittmer, D. S., Eds., Fed. Am. Soc. Exptl. Biol., Washington, D. C., 1961, p. 1.
(9) Rackow, H., Salanitre, E., Epstein, R. M., Wolf, G. L., and Perl, W., *J. Appl. Physiol.*, **20**, 611(1965).
(10) Perl, W., Rackow, H., Salanitre, E., Wolf, G. L., and Epstein, R. M., *ibid.*, **20**, 621(1965).

Keyphrases

Drug distribution—perfusate to rat liver
Flow rate, perfusate—drug-distribution effect
Pharmacokinetics—flow rate-drug distribution

Rapid Assay Method for the Determination of Methotrexate

By M. K. BALAZS, C. A. ANDERSON, and P. LIM

A rapid procedure for the assay of methotrexate samples (bulk or formulated) has been developed in these laboratories. This procedure, which employs quantitative paper chromatography, makes use of relatively inexpensive equipment and is simple to execute.

METHOTREXATE (MTX),[1] a clinically useful anticancer agent, is without exception an impure preparation consisting of MTX, contaminants related to MTX, and water. Efforts to assay MTX preparations have led to the development of column chromatographic procedures[2] that utilize adsorbents such as DEAE cellulose (1, 2) and diatomaceous earth.[3] These elegant procedures are adequate, but they require elaborate equipment and considerable time. The paper chromatographic procedure, which constitutes the basis of this note, requires relatively inexpensive equipment, is rapid and simple to execute, and affords a better separation of MTX from its contaminants.

The sensitivity of the method is limited only to the amount of MTX needed to give a useful UV spectrum. In this laboratory, a sample size of 1 mg. has been found to be convenient. The results of this procedure are comparable to those obtained by column methods.

Procedure—One milligram of methotrexate is dissolved in 0.2–0.3 ml. of 0.1 *N* sodium hydroxide and applied as a narrow band on Whatman No. 1 chromatographic paper 22.8 × 48.2 cm. (9 × 19 in.). The chromatogram is developed by descending chromatography with 0.5% aqueous sodium carbonate solution for 2.5 hr. The solvent front travels approximately 43 cm. (17 in.) during this time. The chromatogram is dried (in a fume hood) and the major band (MTX) is located ($R_f \simeq 0.6$–0.8) by illumination of the paper with shortwave UV light. This band is cut from the chromatogram and divided into 6 to 8 pieces, which are placed in a glass-stoppered, 125-ml. conical flask. Fifty milliliters of 0.1 *N* sodium hydroxide solution is added to the flask, which is then shaken vigorously until the paper is reduced to a pulp. A portion of the pulpy mixture is centrifuged at high speed for 3 min. or until a clear supernatant is obtained. The UV absorption of the supernatant solution is measured at 303 mμ, and the amount of methotrexate in the sample is calculated, using as a standard the molar absorptivity, 2.24×10^4.[4] A blank is prepared by the procedure cited above, and the slight increase in the base line is subtracted from the absorption of each sample. Due to the photo-instability of MTX, the entire analysis is carried out under minimal illumination.

Results and Discussion—The examples presented in Table I illustrate the precision of this method. The authors have observed that this procedure generally yields values as much as 2% lower than those obtained when the column chromatographic assay is used. The higher values obtained by column method are due to the inclusion of materials which contribute to the absorbance of the MTX but are in reality impurities. These impurities, which are absent in purified[5] samples of MTX, travel similarly to it but fluoresce brightly under UV light in contrast to the MTX, which absorbs. This visible difference permits excellent mechanical separation

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[1] *N*-{*p*-[(2,4-Diamino-6-pterdinyl)methyl]methylamino}-benzoylglutamic acid, Lederle Laboratories.
[2] Unpublished procedure from Lederle Laboratories.
[3] Celite, Johns-Manville, New York, N. Y.
[4] The average ε value of four recent MTX bulk samples. Since the chromophores in the impurities are indistinguishable from those in MTX, the percent of MTX is calculated from this value.
[5] Purified by the Procedure described by Noble, E. P., *Biochem. Prep.*, **8**, 20(1961).