cold acetone–water. The yield was 1.2 g. (70%), m.p. 106–107°.

1,3 - Bis(piperidinomethyl) - 2 - phenylindolizine (VIII)—Six milliliters of 37% aqueous formaldehyde (0.075 mole) and 8.4 g. piperidine (0.10 mole) were combined with 75 ml. dioxane. One-tenth milliliter of 50% sodium hydroxide was added and the mixture was allowed to stand for 15 min. at room temperature. Two grams 2-phenylindolizine (0.01 mole) was dissolved in the mixture which was then allowed to stand at room temperature for 48 hr. The reaction mixture was transferred to an evaporating dish and cold air was blown across the surface. Evaporation was accompanied by vigorous scratching with a glass rod. The crystalline product which was obtained during the evaporation process was removed by filtration and washed with 50% ethanol. The yield was 3.3 g. (85%). On recrystallization from hot ethanol the compound gave m.p. 98.5–99.5°.

1,3 - Bis(morpholinomethyl) - 2 - phenylindolizine (IX)—Six milliliters of 37% aqueous formaldehyde (0.075 mole) and 8.7 g. morpholine (0.10 mole) were combined with 75 ml. dioxane. One-tenth milliliter of 50% sodium hydroxide solution was added and the mixture was allowed to stand for 15 min. at room temperature. Two grams 2-phenylindolizine (0.01 mole) was added and the flask swirled to achieve solution. The reaction mixture was allowed to stand at room temperature for 48 hr. and then transferred to an evaporating dish. Evaporation was carried out by blowing cold air across the surface accompanied by vigorous scratching with a glass rod. The crystalline product obtained was removed by filtration and washed with 50% ethanol. The yield was 3.2 g. (81%). The product was recrystallized from ethanol and gave m.p. 156–157°.

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Keyphrases

Mannich bases—synthesis
2-Phenylindolizine derivatives—Mannich bases
3-Diethylaminomethyl-1,2-diphenylindolizine—pharmacological screening

Effect of Perfusion Rate and Distribution Factors on Drug Elimination Kinetics in a Perfused Organ System

By RENPEI NAGASHIMA and GERHARD LEVY*

The effects of flow rate and volume of perfusate on the elimination of a drug from a perfusion fluid by an organ or tissue such as the liver, kidney, or intestine are examined. It is shown that the volume of perfusate (or the apparent volume of distribution of a drug) has a pronounced effect on the rate constant for the decline of drug concentration in the perfusate, but that perfusion rate has an effect only if the concentration of drug in the perfusate leaving the organ is appreciably lower than the concentration in the fluid entering the organ.

MANY KINETIC studies of drug absorption or metabolism are carried out by perfusing a segment of intestine, or an organ such as the liver, and determining the change in the concentration of the drug in the perfusate as a function of time. Two important variables in such experiments are the volume of perfusate and its rate of flow through the organ. These variables are encountered also *in vivo* since blood flow rate can be affected by shock, in certain disease conditions, and by some drugs, and since the apparent volume of distribution of drugs (which is analogous in certain respects to the perfusate volume in *in vitro* studies) differs as a function of plasma protein concentration, type and concentration of drug, and other factors. The pharmacokinetic analysis to be presented here will deal specifically with the isolated perfused liver system, but the principles which will be outlined apply equally to the other systems mentioned above.

The liver is viewed as a tissue with numerous parallel channels through which perfusate flows. The concentration of drug in the perfusate leaving the liver is lower than the drug concentration in the fluid entering the liver, due to biotransformation of the drug in that tissue. This decrease in the drug concentration is a function of the activity of the biotransformation process (assuming that drug transfer from perfusate to liver is not rate limiting), and the contact time of a given increment of perfusate with liver tissue (1). If the elimination is a zero-order process, the drug concentration in the

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outflow is given by:

$$C_o = C_i - k^\circ\tau \qquad \text{(Eq. 1)}$$

where C_i and C_o refer to the drug concentrations in the inflowing and outflowing perfusate, respectively, k° is the zero-order rate constant for drug elimination (concentration change per unit time), and τ is the contact (or transit) time. If drug elimination is a first-order process, the appropriate equation is given by:

$$C_o = C_i \exp(-k\tau) \qquad \text{(Eq. 2)}$$

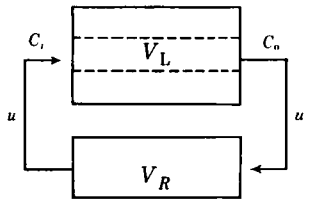
where k is the first-order rate constant for drug elimination, in reciprocal time units.

The mean contact time for perfusate in all the channels in the liver is given by:

$$\tau = V_L/u \qquad \text{(Eq. 3)}$$

where V_L is the total volume of the channels, and u is the flow rate of perfusate (2).

Consideration must now be given to the extrahepatic space, *i.e.*, the real or apparent (in the case of an *in vivo* system) volume of perfusate in the reservoir and tubings. This volume is designated as V_R. The described model is illustrated in Scheme I.



Scheme I

Immediately upon leaving the liver, the perfusate is incorporated in and mixed with V_R. The drug concentration in V_R is identical to C_i. The net rate of decrease in the amount of drug in V_R is given by:

$$-V_R \cdot \frac{dC_i}{dt} = u(C_i - C_o)$$

where dC_i/dt is the rate of drug concentration change in V_R.[1] Upon rearrangement,

$$-\frac{dC_i}{dt} = \frac{u}{V_R} \cdot (C_i - C_o) \qquad \text{(Eq. 4)}$$

Substituting for C_o in Eq. 4 from Eqs. 1 or 2, and for τ from Eq. 3 yields:

$$-\frac{dC_i}{dt} = \frac{V_L}{V_R} \cdot k^\circ \qquad \text{(Eq. 5)}$$

and

$$-\frac{dC_i}{dt} = \frac{u}{V_R} \cdot [1 - \exp(-kV_L/u)]C_i \qquad \text{(Eq. 6)}$$

respectively. The apparent rate constants for the decline of drug concentration are then:

$$k^\circ_{app.} = \frac{V_L}{V_R} \cdot k^\circ \qquad \text{(Eq. 7)}$$

and

$$k_{app.} = \frac{u}{V_R} \cdot [1 - \exp(-kV_L/u)] \qquad \text{(Eq. 8)}$$

If kV_L/u is smaller than 0.1, Eq. 8 may be reduced to:

$$k_{app.} = \frac{V_L}{V_R} \cdot k \qquad \text{(Eq. 9)}$$

The error introduced by this simplification is less than 5 %.[2] Note that Eqs. 7 and 9 do not contain a flow rate term.

Regardless of whether drug elimination in the perfused organ proceeds by zero- or first-order kinetics, the apparent rate constants are inversely proportional to the volume of perfusate (Eqs. 7 to 9). Therefore, when serial sampling of perfusate reduces the volume of that fluid as a function of time, appropriate corrections must be made to account for the volume change. This is shown in another report from this laboratory (4).

A change in the flow rate of perfusate has absolutely no effect on drug elimination rate constants when the kinetics are zero-order,[3] and essentially no effect when the kinetics are first-order and kV_L/u is small. Taking for example the results of an isolated rat liver perfusion study of bishydroxycoumarin elimination described in another report (4), u = 1,140 ml./hr., V_R = 100 ml., and the largest $k_{app.}$ = 0.565 hr.$^{-1}$. Thus, from Eq. 8, kV_L/u = 0.05, which shows that flow rate should have no effect on the elimination kinetics of bishydroxycoumarin.

A similar analysis can be carried out with respect to intact animals, assuming rapid distribution of drug between plasma and tissues. In this case,

$$V_R = V_d - W(C_L/C_P) \qquad \text{(Eq. 10)}$$

where V_d is the apparent volume of distribution of the drug, W is the weight of the liver, and C_L/C_P is the liver:plasma concentration ratio of the drug. Using data for bishydroxycoumarin elimination by intact rats presented in other reports (8, 9), where V_d = 100 ml./kg. of body weight, C_L/C_P = 0.44 ml./g., and W = 33.5 g./kg. of body weight (5), it was calculated that V_R = 85 ml./kg. of body weight. Considering a hepatic blood flow rate of about 50 ml./min./kg. of body weight (6) and a hematocrit value of 45% (4), the hepatic plasma flow rate in rats is 1,350 ml./hr./kg. of body weight. The value of kV_L/u may then be calculated from Eq. 8 and from the *in vivo* $k_{app.}$ value of 0.14 hr.$^{-1}$ (4). By these calculations, kV_L/u = 0.009, which shows that the elimination kinetics of bishydroxycoumarin in the intact rat are apparently not affected by hepatic blood flow. The same probably applies to man, considering the similar magnitude of hepatic blood flow (6), the lower $k_{app.}$ value (7), and the similar relative apparent volume

[1] See *Reference 1* for a similar derivation for chemical reactions in flow systems.

[2] Using the Mclaurin series (3):

$$\exp(-x) = 1 - x + \frac{x^2}{2!} - \frac{x^3}{3!} + \ldots$$

When $x \ll 1$: $\exp(-x) \simeq 1 - x$. Specifically when x = 0.1, the difference in $k_{app.}$ calculated by Eqs. 8 and 9, respectively, is 5%.

[3] Provided that the rate of supply of drug to the perfused organ is not elimination rate limiting.

of distribution of bishydroxycoumarin in rats and humans (8). On the other hand, it is possible that hepatic blood flow rate will affect the elimination kinetics of certain drugs with very short biologic half-lives which are metabolized in the liver.

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Keyphrases

Perfused organ system—drug elimination
Elimination kinetics—perfusion rate effect
Distribution factors, effect—elimination kinetics
Kinetic equations—perfused organ system

Effect of Deuterium Oxide on the Culturing of *Penicillium janczewskii* III. Antifungal Activity of Fully Deuterated Griseofulvin

By D. A. NONA, M. I. BLAKE, H. L. CRESPI*, and J. J. KATZ*

The *in vitro* antifungal activity of fully deuterated, partially deuterated, and protiogriseofulvin was compared. The antibiotics were evaluated by plate assay with *Microsporum gypseum* (ATCC 14683) as the test organism. A statistically significant enhancement in antifungal activity was observed in zones of inhibition when fully deuterated griseofulvin was compared with partially deuterated and protiogriseofulvin. No significant difference was apparent in a comparison of biological activity of partially deuterated and ordinary griseofulvin. The enhanced antifungal activity demonstrated by fully deuterated griseofulvin may be due to an increased efficiency of action. The antifungal target site may involve the metabolism of a C—D bond directly or indirectly. Since C—D bonds are generally more stable than C—H bonds, the increased stability of the molecule may alter the rate of griseofulvin metabolism by the fungus.

In the first paper (1) of this series the nutritional requirements for optimal growth of *Penicillium janczewskii* in heavy water were reported. The effects on antibiotic production were noted. The second study (2) described in detail the isolation, purification, and characterization of the deuterated griseofulvin biosynthesized by the organism in a replacement culture. In the present report comparison is made of the *in vitro* antifungal activity of ordinary griseofulvin and the antibiotics obtained from the organism grown in a deuterated environment.

EXPERIMENTAL

Antifungal activity of the antibiotic was determined by plate assay using *Microsporum gypseum* as the test organism. A modification of the USP (3) procedure for microbiological assay of griseofulvin was employed.

Medium I—The medium was composed of dextrose, 40.0 g.; peptone, 10.0 g.; agar, 15.0 g.; chloramphenicol, USP, 0.05 g.; and sufficient distilled water to make 1,000 ml. The pH was adjusted, if necessary, to 5.65 ± 0.05. The medium was placed into 100-ml. vials, fitted with rubber closures, sealed, and capped. The vials were autoclaved at 15 p.s.i. for 15 min. The medium was used immediately after cooling to 50°, or was stored for future use. After storage at room temperature, the solidified medium was melted by gentle warming on a steam bath.

Medium II—The composition of this medium differed from Medium I in that 2 ml. of a 1% aqueous solution of cycloheximide (Actidione, Upjohn) was added for each 100 ml. of the agar medium. The cycloheximide solution was injected through the rubber closure of the vial using a needle and syringe fitted with a Swinney adapter. The membrane filter had a porosity of 0.22 μ.

Preparation of Inoculum—The test organism, *Microsporum gypseum* (ATCC 14683), was grown

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