Per Cent Absorbed *Versus* Time Plots from Metabolite Level in Blood

Sir:

Equations to calculate per cent of dose absorbed as a function of time from drug blood level or urinary excretion data have been presented previously (1), and the utility of these equations to elucidate the kinetics of the absorption of several drugs has been demonstrated (2). This communication presents a method by which per cent of dose absorbed *versus* time data may be calculated from information on the drug's metabolite level in blood at various times after drug administration.

It will be assumed that drug and drug metabolite instantaneously distribute themselves in their apparent volumes of distribution after absorption and metabolism, respectively, and that they are removed from their apparent volumes of distribution by first-order processes. These assumptions are consistent with those previously made (1).

At any time the following material balance holds

$$A = X'_c + X_c + X_d \qquad \text{(Eq. 1)}$$

where A is the amount of drug absorbed, X'_c is the amount of drug in its volume of distribution, X_c is the amount of the drug's metabolite in its volume of distribution, and X_d is amount of metabolite that has been eliminated either by further metabolic transformations or by elimination as such by excretory pathways. It is implicit in Eq. 1 that all quantities are expressed in terms of either the drug or its metabolite. The derivative of Eq. 1 with respect to time is

$$\frac{dA}{dt} = \frac{dX'_c}{dt} + \frac{dX_c}{dt} + \frac{dX_d}{dt} \qquad \text{(Eq. 2)}$$

where dA/dt is the absorption rate of drug, dX'_c/dt is the rate of change of amount of drug in the body with time, dX_c/dt is the rate of change of amount of metabolite in the body with time, and dX_d/dt is the rate of elimination of the metabolite. Under the assumptions made concerning the nature of the metabolism process, the rate of change of amount of metabolite in the body with time is given by

$$\frac{dX_c}{dt} = k_1 X'_c - k_2 X_c \qquad \text{(Eq. 3)}$$

where k_1 is rate constant for metabolism, and k_2 is rate constant for elimination of the metabolite. Both k_1 and k_2 have the units of reciprocal time.

The derivative of Eq. 3 is

$$\frac{d^2X_c}{dt^2} = k_1 \frac{dX'_c}{dt} - k_2 \frac{dX_c}{dt} \qquad \text{(Eq. 4)}$$

which may be rearranged to yield

$$\frac{dX'_c}{dt} = \frac{1}{k_1}\frac{d^2X_c}{dt^2} + \frac{k_2}{k_1}\frac{dX_c}{dt} \qquad \text{(Eq. 5)}$$

The rate of elimination of the metabolite by further metabolism or excretion is given by

$$\frac{dX_d}{dt} = k_2 X_c \qquad \text{(Eq. 6)}$$

Equations 5 and 6 may be substituted in Eq. 2 to give, after rearrangement

$$\frac{dA}{dt} = \frac{1}{k_1}\frac{d^2X_c}{dt^2} + \left(1 + \frac{k_2}{k_1}\right)\frac{dX_c}{dt} + k_2 X_c \qquad \text{(Eq. 7)}$$

In accordance with the assumptions made previously, $X_c = VC$, where V is the apparent volume of distribution of the metabolite and C is the concentration of the metabolite in the body fluid sampled for assay (*i.e.*, blood, serum, or plasma). Therefore, $d^2X_c/dt^2 = Vd^2C/dt^2$ and $dX_c/dt = VdC/dt$ which allows dA/dt of Eq. 7 to be given by

$$\frac{dA}{dt} = \frac{V}{k_1}\frac{d^2C}{dt^2} + V\left(1 + \frac{k_2}{k_1}\right)\frac{dC}{dt} + Vk_2C \qquad \text{(Eq. 8)}$$

Integration of Eq. 8 between the limits $t = 0$ and $t = T$ yields, on rearrangement

$$\frac{A_T}{V} = \frac{1}{k_1}\frac{dC}{dt} + \left(1 + \frac{k_2}{k_1}\right)C_T + k_2\int_{t=0}^{t=T} C\,dt \qquad \text{(Eq. 9)}$$

where A_T is the amount absorbed in time T, and the quantity under the integral sign is the area under the C *versus* t curve between the indicated limits. If the value of the right-hand side of Eq. 9 is determined at increasing longer times an asymptotic value should be obtained indicating that the maximum amount of the dose of drug administered has been absorbed. Thus, in agreement with a procedure presented earlier (1),

$$\% \text{ absorbed} = \frac{\frac{1}{k_1}\frac{dC}{dt} + \left(1 + \frac{k_2}{k_1}\right) C_T + k_2 \text{ area}\Big|_{t=0}^{t=T}}{A_\infty} \times 100 \quad \text{(Eq. 10)}$$

where A_∞ is the asymptotic value obtained by the procedure described.

It is of interest to point out that when the rate of biotransformation of the absorbed species is very rapid (large k_1), and when the ratio k_1/k_2 is also large, Eq. 9 takes the form of the equation previously presented (1) which was based on the use of blood level *versus* time data on the absorbed species.

(1) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **52**, 610(1963).
(2) *Ibid.*, **53**, 1392(1964).

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Paper Chromatographic Method for Detecting Phenethicillin Contamination in Drugs

Sir:

Recently promulgated Federal regulations (1) dealing with possible cross-contamination of other drugs manufactured on the same premises or with the same equipment as penicillin products have created a multitude of problems for the pharmaceutical industry. Among these problems is the necessity of testing a large backlog of samples representing batches of drugs produced prior to the advent of the new regulations.

Microbiological methods thus far proposed (2) are expensive, and many small laboratories are not equipped to conduct microbiological assays. Chemical methods currently in use for assaying the penicillins lack the sensitivity required for detecting the minute amounts specified by the regulations (not more than 0.5 unit of penicillin contamination per maximum single oral dose, and not more than 0.05 unit per maximum single parenteral dose).

To meet this problem, the authors have developed a quick and inexpensive paper chromatographic method for traces of phenethicillin. Sensitivity is of the order of 5 units, so that the sample spotted on the paper strip must represent approximately 10 maximum single oral doses or 100 maximum single parenteral doses. The method is applicable mainly to oral dosage forms, although the efficiency of the extraction procedure employed makes it feasible for many parenterals. Preliminary experimentation has shown that penicillins G and V are detected equally as well as phenethicillin by this test.

Reagents.—*Phosphate Buffer, pH 2.5, 10% Stock Solution.*—Dissolve 100 Gm. of monobasic potassium phosphate in 800 ml. of distilled water, adjust to pH 2.5 with concentrated hydrochloric acid (about 0.2 ml.), and dilute to 1000 ml. with distilled water.

Phosphate Buffer, pH 2.5, 1% Solution.—Dilute the 10% stock solution 1:10 with distilled water, and readjust to pH 2.5, if necessary.

Mobile Phase for Chromatographic Development.—Equilibrate 2 parts *n*-butanol and 1 part 2% aqueous oxalic acid. Use upper layer.

Spray Reagent.—0.02 *M* iodine, prepared by diluting 0.1 *N* iodine test solution (U. S. P. XVI, p. 1080) 1:5 with distilled water.

Other Solutions and Solvents.—1 *N* hydrochloric acid, ammonium hydroxide, chloroform, and methanol.

All reagents and glassware must be free of penicillin contamination.

Preparation of Test Sample.—Assuming one tablet or capsule per maximum single oral dose pulverize 20 tablets (or combine contents of 20 capsules) and dampen the powder with a small amount of distilled water. Dissolve or suspend in sufficient distilled water to make 18 ml. Filter through a prewetted filter paper, and transfer 9 ml. of the filtrate to a separator. Adjust to pH 2.5 with 1 *N* hydrochloric acid, add 1 ml. of 10% phosphate buffer, pH 2.5, and extract with two 20-ml. portions of chloroform. Combine the chloroform extracts and wash with one 20-ml. portion of 1% phosphate buffer, pH 2.5. Transfer the chloroform layer to a beaker and air dry. Wash the residue into a 15-ml. centrifuge tube with two 1-ml. portions of methanol. Take the methanol solution to dryness with forced air and a warm water jacket. Dissolve the residue in 0.1 ml. of methanol.

Preparation of Control Sample.—Repeat the above procedure with an additional 20 tablets or capsules from a batch of the same formulation known to be penicillin-free, adding 10 μl. of phenethicillin standard (10 units of activity) to the powder prior to dampening with water.

Preparation of Phenethicillin Standard.—Dissolve an accurately weighed amount of