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Theoretical Approach to Sustained-Release Multiple-Dose Therapy: Noncumulative Attainment of Desired Blood Level

JOSEPH R. ROBINSON* and STUART P. ERIKSEN†

Abstract □ Equations are presented to allow calculation of doses and dosing interval for multiple-dose therapy of sustained-release dosage forms. Both zero- and first-order release of drug from the dosage form are considered in developing these equations. Although special problems are associated with multiple dosing of sustained-release dosage forms because of their unique design, application of the appropriate equations yields relatively uniform blood levels of drug.

Keyphrases □ Sustained-release products—multiple-dose therapy □ Equations—doses, dosing intervals, calculation □ Blood levels—noncumulative multiple-dose therapy □ Theoretical approach—noncumulative blood levels, sustained-release therapy

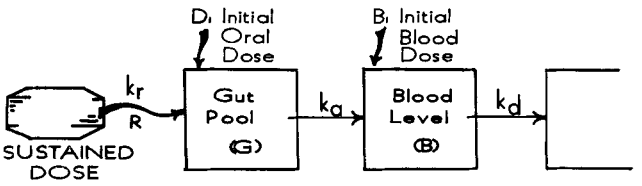
Considerable effort has been expended in the mathematical development of dosage regimens for multiple dosing of nonsustained-release dosage forms (1–7). In recent work (7), the authors developed equations to allow calculation of doses and dosing intervals to produce and maintain a desired blood level (noncumulative approach). Surprisingly, the problem of multiple dosing with sustained-release dosage forms has not been considered. In the present work, equations are presented which will allow a rational (and hopefully pragmatic) approach to such therapy.

MODEL USED

A simple four-compartment model was used throughout this study (Scheme I). All of the customary assumptions of pharmacokinetics with respect to exponential rate processes, constants, *etc.*, as well as the validity of the model are assumed in this study (see *References Ia* and *7* for a discussion of these points).

ELEMENTARY REGIMEN

The elementary regimen design, using two units initially followed by one unit each elimination half-life later, was found to be only very approximately valid for oral nonsustained-release products, as reported in another paper (7). Simple extension of this elementary concept from nonsustained- to sustained-release dosage forms suggests that for a sustained-release dosage form, which is designed to provide flat blood levels over a full treatment period, the dosing frequency, $\tau_{sust.}$, should be



Scheme I—Model used in development and evaluation of sustained-action equations

$$\tau_{sust.} = h + t_{1/2\ elimination} \qquad \text{(Eq. 1)}$$

where h is the number of hours of desired sustained effect for which one dose of the sustained-release dosage form has been designed, and $t_{1/2\ elimination}$ is the elimination half-life of the drug. Similar, but more severe, problems than those encountered with nonsustained medications are encountered here. The solution to these problems depends somewhat upon the mathematics to describe the release of drug from the dosage form, *i.e.*, zero- or first-order release, but in general involves corrections for the same difficulties present in nonsustained multiple-dose therapy, *i.e.*, accumulation of drug in the body.

As in any therapy, the interest of both the patient and the physician is safe, but rapid, and maintained relief. A therapy dependent upon accumulation does not achieve this goal. The desired sustained-action product should rapidly attain and maintain the desired blood or tissue level of drug. All subsequent doses must be designed and taken to reachieve the plateau blood level established by the first dose.

The theoretical concepts behind the design of a single sustained-action dosage form have been reported (8–10). This earlier work was designed to produce the optimum blood picture for one administered dose. While identical concepts are involved when multiple doses are to be administered, slight alterations must be made to eliminate the undesired accumulation effect.

SATISFACTORY SUSTAINED-ACTION PATTERN

The nonsustained-action definition of "satisfactory therapeutic blood level patterns" (7), *i.e.*, a rapid rise to a peak suitable to produce the desired biological action followed by a regular rise and fall between constant values, must be altered slightly for sustained-action dosage forms. Satisfactory blood level patterns, for

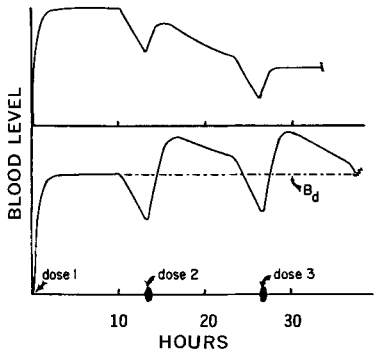


Figure 1—*Simulated blood level patterns produced with an adequately designed "zero - order released" sustained-action dosage form. Curve A (lower curve) is one dosage unit initially followed by one dosage unit every* $\tau_{sust.}$ *hr.; Curve B (upper curve) is two dosage units initially followed by one every* $\tau_{sust.}$ *hr. Both curves utilize* $\tau_{sust.}$ *as calculated from Eq. 1.*

this purpose, will be produced by dosages and regimens yielding a rapid rise to blood levels suitable to produce the therapeutic effect, maintained at that level for a desired length of time, and followed at regular intervals by suitable doses to reestablish and maintain the same blood levels. The equations required will be discussed according to the type of release produced by the dosage form used, *i.e.*, zero- or first-order release.

DRUGS RELEASED BY ZERO-ORDER PROCESSES

Calculation of Doses Required—The second dose of a sustained-release dosage form,[1] administered at time $\tau_{sust.}$ (using Eq. 1) after the start of therapy, produces blood levels similar to those shown in Fig. 1. Because of their unique design, sustained-release dosage forms are unable to maintain any blood level but the one for which they were designed (B_d). The dosage regimen, where a single sustained-release dose is taken after two initial ones, produces a slow drop of the blood level down to that value for which the single dose was designed. Single doses administered one after the other produce accumulation.

The immediately available portion of the second and subsequent sustained-action doses must be corrected to obtain the optimum flat blood level pattern for which these dosage forms are designed. For the initial dose, the maintenance portion (Dm) is prepared according to standard equations (8):

$$Dm = R \times h \qquad \text{(Eq. 2)}$$

where $R = k_d B_d$ and is the zero-order availability rate of drug from the dosage form, h is the selected time of sustained action for one dose, k_d is the elimination constant,[2] and B_d is the desired amount of drug in the blood, all ascertained from the pharmacokinetics of single, nonsustained doses. The immediately available portion (D_1), when both the immediate and maintenance portions begin release of drug from time zero, is normally calculated to be (8):

$$D_1^{corr.} = D_1 - Rt_p \qquad \text{(Eq. 3)}$$

where D_1 is the nonsustained dose required to obtain the desired peak blood level of B_d, and Rt_p is the correction on the initial dose necessary due to supply of drug from the maintenance portion over the time period zero to peak time (8). For any subsequent sustained-action dose, however, the immediately available portion must be decreased to take into account that the desired blood level (B_d) has decreased to NB_d by the time of the next dose [the fraction N is defined as the fraction of the peak height (B_d) to which the blood level is allowed to drop before the next dose is administered]. Thus, the optimum dose for the subsequent doses, $D_2^{corr.}$, can be estimated to be

$$D_2^{corr.} \ldots n \cong (1 - N)D_1 - Rt_p \qquad \text{(Eq. 4)}$$

using the approximations developed in earlier work (7, 8). It is apparent that if the half-life time is used

$$D_2^{corr.} \cong \frac{1}{2} D_1 - Rt_p \qquad \text{(Eq. 4a)}$$

To offer a complete picture, if a dosage form involving a delayed start of maintenance dose is used (8):

$$D_2 \ldots n \cong (1 - N)D_1 \qquad \text{(Eq. 5)}$$

Calculation of Dosing Interval—The calculation of $\tau^0_{sust.}$, the treatment time for the zero-order release dosage form, involves a sum of the time to empty the dosage form (h) and the time for the blood level to drop to one Nth of the plateau value:

$$\tau^0_{sust.} = h + \frac{2.3}{k_d} \log \left(\frac{1}{1 - N} \right) + \Delta t \qquad \text{(Eq. 6)}$$

where the first two terms are the general expression for which Eq. 1 is the example at $N = 1/2$. The Δt term is a correction for drug being absorbed from the gut after the moment (h) where the dosage form has ceased to yield drug (7).

The correctness of Eq. 1 assumes that the dosage form is completely empty (probably true) and that all absorption ceases at the moment h. Under these circumstances, the drop of the blood level to one Nth of the plateau value requires a time equal to $2.3/k_d \log [1/(1-N)]$ time units. The time error involved in making the assumption that all absorption ceases at the moment h for oral dosage forms can be estimated to be Δt, where Δt is the horizontal distance between that "no absorption line" and the "eventual elimination line," as shown in Fig. 2. It can be shown mathematically that the time error is identical to that previously derived for nonsustained forms (7).

At the moment that the dosage form is empty, h, the concentration in the gut is G_h, where $G_h = k_d B_d / k_a$. The equality of the rate of absorption, $k_a G_h$, and the rate of loss from the blood, $k_d B_d$, is a requirement for sustained action. From that time on, the blood level is

$$B = B_d e^{-k_d t} + \frac{G_h k_a}{k_a - k_d} (e^{-k_d t} - e^{-k_a t}) \qquad \text{(Eq. 7)}$$

Substituting $k_d B_d$ for $G_h k_a$,

$$B = B_d \left[e^{-k_d t} + \frac{k_d}{k_a - k_d} (e^{-k_d t} - e^{-k_a t}) \right] \qquad \text{(Eq. 8)}$$

The eventual elimination line for such a dosage (letting $e^{-k_a t} \to 0$) is described by the equation:

$$B = B_d e^{-k_d t} \left(\frac{k_a}{k_a - k_d} \right) \qquad \text{(Eq. 9)}$$

This equation is identical to the similar eventual elimination line described for a nonsustained dose (7). The time error, Δt, is therefore again described by the equation previously reported (7):[3]

$$\Delta t = \frac{2.3}{k_d} \log \left(\frac{k_a}{k_a - k_d} \right) \qquad \text{(Eq. 10)}$$

One precisely designed sustained-action dose, followed at intervals of $\tau^0_{sust.}$ by corrected sustained-action follow-up doses, will produce

[1] Sustained-action dosage forms, as used here, mean a system composed of an immediately available portion plus a sustaining or maintenance portion.

[2] The terms elimination and absorption refer specifically to the mathematical model considered and are so termed only for purposes of comprehension. From the standpoint of dosage form design, the only constants of concern are those describing the rise of drug concentration (or amount) in the tissue measured (blood in these discussions) and the decrease of that concentration (or amount). For a simple model, these may be called absorption and elimination constants, k_a and k_d, but they are only related to the actual constants for the physiologic processes of absorption and excretion or metabolism.

[3] The derivation of this equation involves setting the equations describing the blood level of an intravenous injection and an oral dose (Eq. 9) equal and solving for the time difference between them, *i.e.*,

$$Be^{-k_d t_1} = \frac{Bk_a}{k_d - k_d} e^{-k_d t_2}$$

$$t_2 - t_1 = \Delta t = \frac{2.3}{k_d} \log \left(\frac{k_a}{k_a - k_d} \right)$$

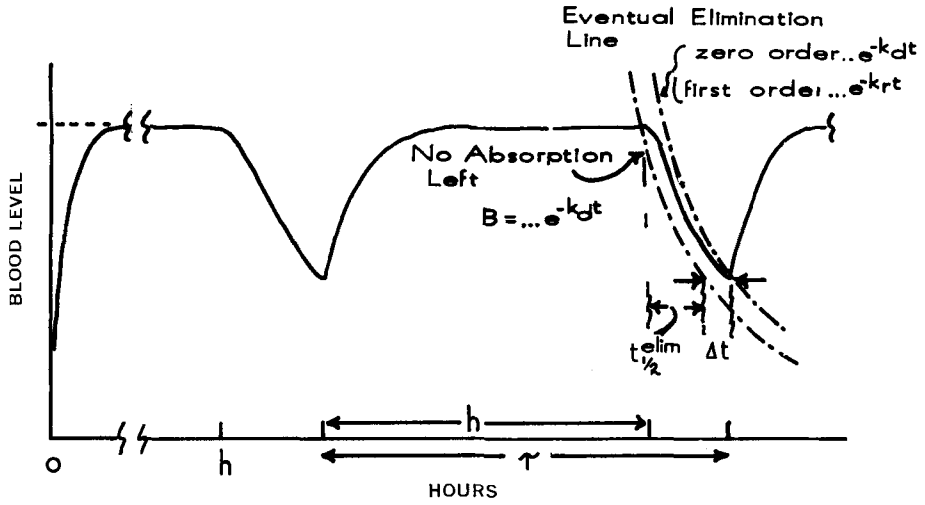


Figure 2—*Computer-drawn construction showing the development of the* Δt *correction for sustained-action dosage forms.*

the blood level patterns shown in Figs. 3 and 4. While the impractical method of administering a different dosage unit initially ($D_1^{corr.}$ and then $D_2^{corr.}$) produces the fastest rise to B_d, the use of the same corrected second dose at each interval of $\tau^0_{sust.}$ hr. achieves essentially the same result. Figure 4 shows the computer-drawn curves for a sustained-release penicillin product using zero-order release procedures and the constants of Juncher and Rasschou (11); quite acceptable sustained action is demonstrated.

DRUGS RELEASED BY FIRST-ORDER PROCESSES

Calculation of Doses Required—For the initial dose, the maintenance portion (Dm) is prepared according to standard procedures (8):

$$Dm = \frac{k_d}{k_r} B_d \qquad \text{(Eq. 11)}$$

where $k_r = k_d e^{-k_d h}$ and is the first-order availability rate of drug from the dosage form. The immediately available portion (D_1), when both the immediate and maintenance portion begin release at time zero, can be calculated using Eq. 12:

$$D_1^{corr.} = D_1 - k_r D_m t_p \qquad \text{(Eq. 12)}$$

As with the zero-order case, a correction is applied for the amount of drug contributed by the maintenance portion from time zero to the peak time (8).

Subsequent doses require correction for the immediately available portion:

$$D_2^{corr.} \ldots n = (1 - N)D_1 - k_r D_m t_p \qquad \text{(Eq. 13)}$$

If the maintenance dose has a delayed start, the equation is

$$D_2 \ldots n = (1 - N)D_1 \qquad \text{(Eq. 14)}$$

Calculation of Dosing Interval—The second and subsequent doses of a dosage form whose maintenance portion releases drug by a first-order process must be corrected in a manner similar to zero-order dosages to prevent excessive peaking. The frequency of therapy, however, plays a larger part in first-order release therapy, because after the designed sustained-release time, h, has passed, considerable drug is still available and is being released from the dosage form. First-order release forms achieve their action through small release constants, k_r, and relatively large maintenance doses, D_m (8). The blood level very rapidly becomes controlled by the dosage form release constant, so that

$$B = \frac{D_m k_r}{(k_d - k_r)} e^{-k_r t} \qquad \text{(Eq. 15)}$$

after the immediately available portion of the dosage form is no longer contributing to the blood picture. This is the equation for the "eventual elimination line" of such a dosage form (Fig. 2).

The time error (Δt_1), resulting from assuming immediate cessation of absorption and that the blood level decreases in a manner based solely on k_d, is then

$$\Delta t_1 = \frac{2.3}{k_r} \log \left(\frac{k_d}{k_d - k_r} \right) \qquad \text{(Eq. 16)}$$

making the dosing interval for first-order release dosage forms

$$\tau^1_{sust.} = h + \frac{2.3}{k_r} \log \left(\frac{1}{1 - N} \right) + \Delta t_1 \qquad \text{(Eq. 17)}$$

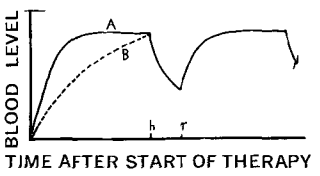


Figure 3—*Simulated blood level patterns for multiple sustained-release therapy. Curve A is initial dose unit of* ($D_1 + D_m$) *and subsequent dose units of* [$(1 - N)D_1 + D_m$] *every* $\tau_{sust.}$ *hr.; Curve B is repeated dosings with* [$(1 - N)D_1 + D_m$] *every* $\tau_{sust.}$ *hr.*

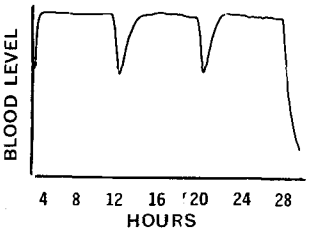


Figure 4—*Computer-drawn blood level patterns for a zero-order sustained-release penicillin product, using the pharmacokinetic constants of Juncher and Rasschou (11), calculated for a blood level of 47.65 units,* $k_a = 4.3\ hr.^{-1}$, $k_d = 0.852\ hr.^{-1}$, $R = 40.5$, $D_1^{corr.} = 51.2$, *and* $D_2^{corr.} = 25.6$.

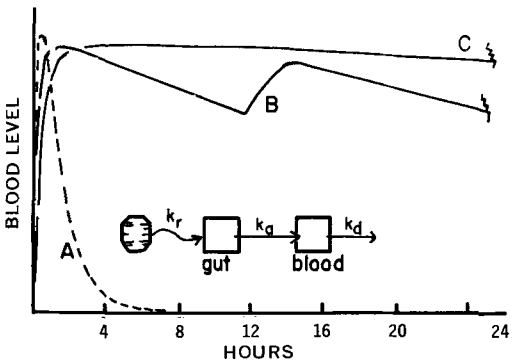


Figure 5—*A "practical" solution to sustained-action for penicillin* ($k_a = 4.3\ hr.^{-1}$, $k_d = 0.852\ hr.^{-1}$) *with a first-order release dosage form. Curve A is a computer-simulated blood level diagram for a 0.024-g. dose. Curve C is the diagram for a single sustained-action dose, the parameters calculated as described in earlier work* ($D_1^{corr.} = 0.024$ g., $D_m = 81$ g., $k_r = 0.00174\ hr.^{-1}$). *Curve B is the "practical, multiple sustained-release dosage method" described here* ($D_1^{corr.} = 0.013$ g., $D_m = 0.488$ g., $k_r = 0.028\ hr.^{-1}$).

Table I—Required Equations for Design of Sustained- and Nonsustained-Release Dosage Forms

Term	Nonsustained	Zero-Order Sustained	First-Order Sustained
D_1	D	D	D
$D_1^{corr.}$	—	$D_1 - Rt_p$	$D_1 - k_r D_m t_p$
$D_{2 \ldots n}$	$(1 - N)D$	$(1 - N)(D_1)$	$(1 - N)D_1$
$D_{2 \ldots n}^{corr.}$	—	$(1 - N)D_1 - Rt_p$	$(1 - N)D_1 - k_r D_m t_p$
D_m	—	$R \cdot h$	$\frac{k_d}{k_r} B_d$
R or k_r	—	$R = k_d B_d$	$k_r = k_d e^{-k_d h}$
τ or $\tau_{sust.}$	$t_p + \Delta t + 2.3 \log\left(\frac{1}{1-N}\right)\left(\frac{1}{k_d} - \frac{1}{k_a}\right)$	$h + \Delta t + \frac{2.3}{k_d} \log\left(\frac{1}{1-N}\right)$	$h + \Delta t_1 + \frac{2.3}{k_r} \log\left(\frac{1}{1-N}\right)$
Δt or Δt_1	$\frac{2.3}{k_d} \log\left(\frac{k_a}{k_a - k_d}\right)$	$\frac{2.3}{k_d} \log\left(\frac{k_a}{k_a - k_d}\right)$	$\frac{2.3}{k_r} \log\left(\frac{k_d}{k_d - k_r}\right)$

For the design of sustained-release dosage forms intended to be administered in more than one dose, a summary of the required equations is shown in Table I and a glossary of terms is given in Table II. For comparison, the equations required for nonsustained-release dosage forms are also shown.

PRACTICAL DESIGN OF DOSAGE FORMS

Because therapy with sustained-action dosage forms is for the benefit of the patient, not every treatment frequency is equally convenient, nor are varying dosage schedules. From a pragmatic point of view, the dosage units must be all the same or, at most, simple fractions of the first dose. In addition, treatment frequency must be constant and, by convention, be held to a very few values.

Nonsustained-Action Forms—Nonsustained-action forms are commonly administered at intervals of one, two, three, or four per day. The dosage multiples represent the only variable available to the formulator for maintaining an even series of blood level peaks and valleys.

The frequency of treatment equation (τ, see Table I) may be solved for N using τ = 6, 8, 12, and 24 hr. (7). Any simple, even fraction approximating N will enable the initial and subsequent dose ratios to be calculated (if N = 0.75 or $^3/_4$, the initial dose represents three units and the subsequent doses one unit).

Sustained-Action Forms—*Zero-Order Release*—The dosing interval contains only the sustained-action time, h, as an adjustable variable. Since $\tau_{sust.}$ for sustained-action forms is normally limited to 12 or 24 hr., h can have but two possible values for any drug. Using a selected value for $\tau_{sust.}$, the resulting value of h may be used to calculate the remaining factors.

The size of the total dose is customarily limited to 0.5 g. for one dosage unit (an administered dose could, of course, be two units).

Table II—Glossary of Terms Used

Symbol	Definition
D	Equals the 100% available oral dose required to produce the desired blood level peak, B_d
R or k_r	Zero- and first-order dosage form availability constants
t_p	Time after administration for peak blood level to occur
N	Fraction of peak or sustained blood level present at the time when the next dose is administered
h	Designated sustained action for one dose, or the time to "empty" the dosage forms
D or $D^{corr.}$	Immediately available dose (D) and corrected dose ($D^{corr.}$), with the subscript referring to the dose number
D_m	Maintenance dose
$\tau_{sust.}$	Dosing interval for sustained-action dosage forms: $\tau^0_{sust.}$ (zero order), $\tau^1_{sust.}$ (first order)
k_a, k_d	Absorption and elimination constants for the drug involved

Thus, because $D_m = R \times h$ where R is fixed by the desired blood level and the elimination constant,

$$D_m = 0.5 - (1 - N)D_1 \quad \text{(Eq. 18)}$$

$$h^* = (\text{desired } h) = D_m/R \quad \text{(Eq. 19)}$$

$\tau^0_{sust.}$ = selected value that produces a calculated value of h closest to h^* (Eq. 20)

subsequent $D_{2 \ldots n} = (1 - N)D_1$ (Eq. 21)

Note that several values of N may be tried; larger values decrease the valleys and decrease the subsequent $D_{2 \ldots n}$, smaller ones increase them.

First-Order Release—Since the total dose is again limited to approximately 0.5 g. and $\tau_{sust.}$ is again constrained to 12 or 24 hr., the required parameters are calculated from these points:

$$D_m = 0.5 - (1 - N)D_1 \quad \text{(Eq. 22)}$$

$$k_r = k_d \frac{B_d}{D_m} \quad \text{(Eq. 23)}$$

$$h^* = (\text{desired } h) = \frac{2.3}{k_d - k_r} \log\left(\frac{k_d}{k_r}\right) \quad \text{(Eq. 24)}$$

$$h = \tau^1_{sust.} - \frac{2.3}{k_r} \log\left(\frac{1}{1-N}\right) - \Delta t \quad \text{(Eq. 25)}$$

and $\tau^1_{sust.}$ is selected as 12 or 24 to produce a calculated value of h as close as possible to h^*. Several values of N should be tried. Then,

$$D_1^{corr.} = D_1 - k_r D_m t_p \quad \text{(Eq. 26)}$$

Calculation of sustained-action dosage forms, based on this pragmatic approach, will not produce the optimum in blood level patterns. Unfortunately, however, to obtain the optimum blood level pattern with these nonideal availability kinetics, very large maintenance doses may be required (especially if k_d is large). Within the limits of practical oral dosages, the "practical approach" produces the only satisfactory dosage form. The difference between the blood level pattern for the optimum and the practical design is shown in Fig. 5 for a drug with a large k_d.

Satisfactory sustained- and nonsustained-release dosage forms can be designed with relatively simple equations. The equations provided in this report are based on simple models and are intuitively correct. They have been derived completely so that the assumptions involved are apparent.

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Metabolism of Flurazepam, a Benzodiazepine, in Man and Dog

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Abstract □ The metabolism of ^{14}C-flurazepam hydrochloride, 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one-5-^{14}C dihydrochloride, was studied in a dog administered oral and intravenous 2-mg./kg. doses and in two human subjects who each received a 28-mg. oral dose. In both species, evidence was obtained for rapid and essentially complete absorption followed by a rapid elimination of plasma flurazepam. Biotransformation of the drug was rapid and virtually complete in man and dog. Pathways of biotransformation were similar in both species. All the metabolites identified either showed some alteration in the N_1-diethylaminoethyl moiety or lacked the N_1-substituent altogether. The major metabolite in the dog was a carboxylic acid, the N_1-acetic acid analog of flurazepam. In man, the analogous alcohol (the N_1-ethanol analog) predominated.

Keyphrases □ ^{14}C-Flurazepam HCl metabolism—humans, dogs □ Metabolites, flurazepam HCl—isolation, identification □ Urinary, fecal excretion—^{14}C-flurazepam □ Plasma levels—^{14}C-flurazepam □ TLC—separation □ UV spectrophotometry—analysis □ Scintillometry—analysis

The synthesis (1) and the pharmacology (2) of flurazepam hydrochloride,[1] 7-chloro-1-(2-diethylaminoethyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one dihydrochloride, have been reported as well as the clinical use of this compound (also designated Ro 5-6901) as a hypnotic (3, 4).

In a previous report (5), the isolation by TLC and the characterization by high-resolution mass spectrometry of five urinary metabolites from the dog and one human urinary metabolite were described. The structures of these metabolites are shown in Table I, together with those of flurazepam, the 3-hydroxy derivative of flurazepam (F-3-OH), and Compound III. While neither of the latter two compounds had been detected as metabolites, F-3-OH was of interest as a reference compound to test for 3-hydroxylation of intact drug, and Compound III was a likely metabolic precursor of metabolites III-OH and IV. All the compounds of Table I, except the incompletely characterized III-OH, have been synthesized (6).

The synthesis of ^{14}C-labeled flurazepam hydrochloride allowed for a study of the disposition of this drug in terms of absorption, distribution, biotransformation, and excretion.

EXPERIMENTAL

Labeled Compound and Counting of ^{14}C—Flurazepam-5-^{14}C hydrochloride[2] was diluted with unlabeled compound to a specific activity of 3.12 μc./mg. flurazepam base for the dog studies and 2.10 μc./mg. base for the human studies. Before administration to the human subjects and the dog, the labeled drug was shown to be radiochemically pure by TLC with System A (described later).

All samples were counted in a Nuclear-Chicago Corp. Mark I liquid scintillation spectrometer equipped with a ^{133}Ba external standard; the external standard-channels ratio technique was used to determine counting efficiency. Aliquots of urine and extracts of both urine and plasma were counted in Phosphor I (7), while plasma (0.2–1.0 ml.) and silica gel segments from chromatoplates were counted as suspensions in Phosphor II (7) which contained a thixotropic gel. The ^{14}C in aliquots of 50% ethanol homogenates of feces was determined by the Schoniger combustion, carbon dioxide trapping, and counting procedures of Kelly *et al.* (8).

Dog Studies—A nonfasted 13-kg. male dog (beagle) was first given an oral dose of 2 mg./kg. of ^{14}C-flurazepam HCl in a gelatin capsule. Two months later, it was given the same dose as a solution in 2.6 ml. of physiological saline by rapid intravenous injection. Urine and feces were collected until the excretion of radioactivity reached the limits of detection. Blood (10 ml., heparinized) was drawn at 0, 1, 2, 3, 4, 7, 12, and 24 hr. after oral dosing and at 0, 5, 15, and 30 min., and 1, 2, 3, 4, 7, 11, and 24 hr. after intravenous dosing of the dog.

For fractionation of the plasma radioactivity, aliquots (0.2–0.5 ml.) of plasma were brought to 1 ml. with water, mixed with 1 ml. of 0.5 *M* borate buffer, pH 9.0, and extracted twice with 5 ml. of ether. This procedure quantitatively removed flurazepam (9). The aqueous phases were adjusted to pH 7.0 and were extracted twice with 5 ml. of ether and then twice with 5 ml. of ethyl acetate. Finally, the aqueous phases were brought to pH 3.0 and again extracted twice with 5 ml. of ethyl acetate. These extracts were concentrated and counted.

The urinary radioactivity was fractionated in a similar manner. Aliquots (10 ml.) of urine were extracted twice with equal volumes of ethyl acetate at various pH's before incubation at pH 5.5 and 37° for 3 hr. with a commercial preparation[3] containing β-glucuronidase

[1] Flurazepam hydrochloride is the active ingredient in the trademarked product Dalmane of Hoffmann-La Roche Inc., Nutley, N. J.

[2] The labeled flurazepam hydrochloride synthesized by H. H. Kaegi and G. Bader, Isotope Synthesis Laboratory, Hoffmann-La Roche Inc., was labeled with ^{14}C at the C-5 position. The synthesis has not been published but is available from these chemists.

[3] Glusulase, Endo Laboratories, Garden City, N. Y.