to study the effects of drugs or procedures on isolated perfused hearts, lungs, and blood vessels. The very small current employed (approximately 0.2 mamp) has no observable effects on either the preparation or the actions of the drugs studied.

In short, this apparatus provides a method for rapidly detecting changes in flow by using the Marriotte bottle to provide graphic information as well as to serve simply as a reservoir for the perfusion solution. Ease of construction and low cost make it particularly useful for teaching purposes, since each group of students can be supplied with one of these devices.

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Controlled Release from Matrix Systems

Keyphrases □ Matrix systems—controlled release of ethynodiol diacetate from silicone rubber devices, comments □ Silicone rubber matrix—release of ethynodiol diacetate, comments on controlled release □ Release, controlled—ethynodiol diacetate from silicone rubber devices, comments

To the Editor:

Two publications by Chien and coworkers (1, 2) described the release of ethynodiol diacetate from silicone rubber devices. These authors treated two cases for the matrix release process: matrix-controlled and partition-controlled drug release mechanisms. The equations describing the diffusional process were previously presented by Higuchi (3) and Roseman and Higuchi (4). With the assumptions that (*a*) the matrix acts as the diffusion medium, (*b*) a pseudo-steady-state condition exists during the release process, and (*c*) the drug particles are uniformly distributed throughout the matrix and are quite small relative to the average distance of diffusion, Higuchi (3) derived the following relationship for the release of drug from a planar homogeneous matrix:

$$Q = [D_s C_s (2A - C_s)t]^{1/2} \quad \text{(Eq. 1)}$$

where Q = amount of drug released per unit area, D_s = diffusion coefficient of drug in the homogeneous matrix phase, C_s = solubility of drug in the matrix phase, t = time, and A = total amount of drug present per unit volume of matrix.

When $A \gg C_s$, Eq. 1 reduces to:

$$Q = (2AD_s C_s t)^{1/2} \quad \text{(Eq. 2)}$$

Chien *et al.* (1), however, incorrectly quoted the Higuchi equation as:

$$Q = [D_s C_s (2A - C_a)t]^{1/2} \quad \text{(Eq. 3)}$$

where C_a is the solubility of drug in the elution medium[1]. Although the $(2A - C_a)$ term does not appear to have any physical significance (except, of course, when $C_a = C_s$), Eq. 3 does yield Eq. 2 when $2A$ is much greater than C_a.

The equations derived by Roseman and Higuchi (4) are an extension of the concepts discussed by Higuchi (3) for the specific case where diffusion from the surface of the device is considered in series with the diffusional step through the matrix. In this instance, the release of drug from a planar matrix is given by the following expressions, when $A \gg C_s$:

$$Q = Al \quad \text{(Eq. 4)}$$

$$l^2 + \frac{2D_e h_a l}{D_a K} = \frac{2D_e C_s t}{A} \quad \text{(Eq. 5)}$$

where[1] l = diffusional distance in the matrix (depleted zone); K = partition coefficient (C_a/C_s); h_a = diffusional distance in the boundary diffusion layer; $D_e = D_s\epsilon/\tau$ where ϵ and τ are the volume fraction and tortuosity of the matrix,respectively; and D_a = diffusion coefficient of drug in the elution medium. The other terms were defined previously.

Except for consideration of the boundary diffusion layer, the basic assumptions in the derivation of these equations are the same as those used to derive Eq. 2. Equations 4 and 5 describe a general case for matrix release, which was termed the matrix-boundary diffusion layer model (4). This nomenclature corresponds to the partition-controlled and matrix-controlled cases subsequently used by Chien and Lambert (2) for the two limiting cases. When $l \gg 2D_e h_a/D_a K$, Eqs. 4 and 5 yield Eq. 2 (matrix-controlled case)[2]. Conversely, when $l \ll 2D_e h_a/D_a K$, Eqs. 4 and 5 yield:

$$Q = \frac{D_a C_a t}{h_a} \quad \text{(Eq. 6)}$$

which is boundary layer controlled release (partition-controlled release).

In the publication by Chien and Lambert (2), the Higuchi equation is again misquoted as the authors derived a series of equations following the theoretical treatment resulting in Eqs. 4 and 5. For the limiting condition that yields Eq. 6, Chien and Lambert (2) presented the following expressions[1]:

$$Q = \frac{KD_a C_s t}{h_a} \quad \text{(Eq. 7)}$$

$$Q = \frac{KD_a C_a t}{h_a} \quad \text{(Eq. 8)}$$

Equation 7 is correct since it reduces to Eq. 6 (note that $K = C_a/C_s$). However, Eq. 8 is only valid for the trivial case when $C_s = C_a$. But the data on ethynodiol diacetate indicate that C_s does not equal C_a; therefore, analysis of the data does not support the contention that Eq. 8 is valid.

I hope this communication clarifies the discrepancies between the original equations reported in Refs.

[1] Symbols used for the various parameters differ among authors. In this communication, each term is defined to avoid confusion. For example, in Ref. 1, D_m, C_p, and C_s are equivalent to D_s, C_s, and C_a, respectively, while in Ref. 2, D_s, δ_D, and δ_m are equal to D_a, h_a, and l, respectively.

[2] Equation 2 was derived for a homogeneous matrix; therefore, ϵ and τ were unity.

3 and 4 and the recent work of Chien and coworkers (1, 2).

(1) Y. W. Chien, H. J. Lambert, and D. E. Grant, *J. Pharm. Sci.*, **63**, 365(1974).
(2) Y. W. Chien and H. J. Lambert, *ibid.*, **63**, 515(1974).
(3) T. Higuchi, *ibid.*, **52**, 1145(1963).
(4) T. J. Roseman and W. I. Higuchi, *ibid.*, **59**, 353(1970).

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Metabolic Fate of Flurazepam II: A New Potent Metabolite Obtained by *In Vitro* Liver Drug-Metabolizing Enzyme System

Keyphrases □ Flurazepam—isolation of new metabolite using *in vitro* liver drug-metabolizing enzyme system □ Metabolism—flurazepam, isolation of potent metabolite, *in vitro* liver drug-metabolizing enzyme system

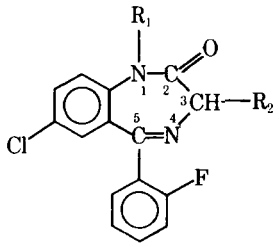
To the Editor:

Although a few investigations on the metabolism of flurazepam (I), 7-chloro-1-[2-(diethylamino)ethyl]-5- (*o*-fluorophenyl) -1,3-dihydro-2*H*- 1,4-benzodiazepin-2-one, in humans (1,2) and experimental animals (1, 3, 4) have been reported, its detailed *in vitro* metabolism is still unknown. This communication describes a new potent metabolite found using an *in vitro* liver drug-metabolizing enzyme system[1].

Reaction mixtures contained the following: 0.5 ml of male DDY mouse or Wister rat liver 9000×*g* supernate or the microsomal fraction (150 mg as fresh liver), NADPH generating system (1 μmole of NADP, 30 μmoles of glucose-6-phosphate, 25 μmoles of nicotinamide, 37.5 μmoles of magnesium chloride, 1.4 units of glucose-6-phosphate dehydrogenase), 5 μmoles of substrate, and 225 μmoles of tromethamine–hydrochloric acid buffer in 2.75 ml of total volume. After incubation at 37° for 90 min, the reaction mixtures were adjusted to pH 9.0 and were extracted once with 10 ml of ethyl acetate. Organic phases were concentrated *in vacuo*, and aliquots of the concentrated organic phases were applied to TLC plates [Kieselgel GF_{254}, benzene–methanol–acetic acid (90:10:10)].

Besides some of the known major metabolites, *e.g.*, II–IV, a minor unknown spot was detected under UV light when I was used as the substrate. Since this metabolite (V) was the predominant metabolite of IV, which is one of the known metabolites of I, 90 mg of authentic IV was then metabolized using mice liver enzyme for the preparation of V.

The metabolite was purified by preparative TLC (Kieselgel GF_{254}) followed by alumina (Woelm, neutral) column chromatography to yield 11.7 mg of oily material. Purified V gave a single spot with several TLC solvent systems, and its chromatographic behavior differed from that of the known metabolites. The IR spectrum of the metabolite showed the increased strength of the OH stretching band at 3400 cm^{-1} and the appearance of a CO stretching band at 1130 cm^{-1}, suggesting that the introduction of the secondary OH to the parent compound (IV) had occurred.



I: $R_1 = CH_2CH_2N(C_2H_5)_2$, $R_2 = H$
II: $R_1 = CH_2CH_2NHC_2H_5$, $R_2 = H$
III: $R_1 = CH_2CH_2NH_2$, $R_2 = H$
IV: $R_1 = CH_2CH_2OH$, $R_2 = H$
V: $R_1 = CH_2CH_2OH$, $R_2 = OH$

The mass spectrum (25 ev) of V showed a weak, but apparent, molecular ion at *m/e* 348 (11%) followed by other prominent fragments at *m/e* 320 (21), 319 (100), 301 (33), 287 (14), 275 (28), and 260 (13). This increment of molecular weight by 16 mass units from that of IV also indicated that one oxygen atom had been introduced to the substrate (IV), but the lack of an M − 16 ion suggested that it might not be any oxide form. The molecular ion of acetylated V (*m/e* 432) and the successive split of two ketenes, giving fragments at *m/e* 390 and 348, were additional supports for the idea.

The proton NMR spectrum of V run in $CDCl_3$,

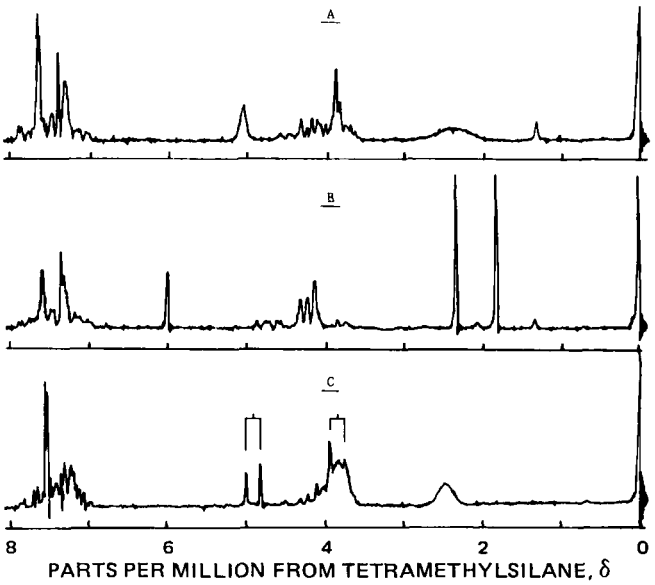


Figure 1—*Proton NMR spectra of V, V-acetate, and authentic parent compound IV in $CDCl_3$: Key: A, V; B, V-acetate; and C, authentic IV (two methylene protons of C_3 at 3.84 and 4.90 ppm are specified).*

[1] For part I, see M. Hasegawa and I. Matsubara, *Chem. Pharm. Bull.*, in press.