Subcutaneous Absorption Kinetics of Benzyl Alcohol

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Benzyl alcohol (BA) was used as a model compound to study subcutaneous (s.c.) drug absorption kinetics in the rat. It has been used extensively as an adjuvant in pharmaceuticals intended for parenteral administration. Benzyl alcohol has a high lipid–water partition coefficient, and is a neutral, relatively nontoxic local anesthetic. The area of s.c. tissue exposed to the BA solution was controlled by a glass absorption cell affixed to the s.c. tissue by a silicone adhesive. The about 1 percent (w/v) BA in normal saline solution was stirred in the cell at an equilibrium temperature. Multiple doses of the drug were used to monitor any volume changes in the cell. Benzyl alcohol was extracted from the fluids at the site into *n*-hexane and assayed spectrophotometrically at 208 mμ. The BA mean absorption half-life in the s.c. cell was 1.6 hr.

THE PURPOSE of this study was to develop general experimental methods for studying the kinetics of drug absorption from the subcutaneous (s.c.) site following the administration of multiple doses of a drug. Schou (1) pointed out that the experimental method of choice where feasible for studying the rate of drug absorption from s.c. tissue is to record the clearance of injected drug from the local area. To date no investigators have used a direct method of studying drug absorption rate per unit area (p.u.a.) of s.c. tissue, very likely because of the difficulty of controlling the spreading of drug solution at the site.

In this work, benzyl alcohol (BA) was selected as a model drug for studying the kinetics of s.c. drug absorption because (*a*) it is a valuable pharmaceutical adjuvant in parenteral products intended for s.c. or intramuscular (i.m.) administration due to its local anesthetic and bacteriostatic properties; (*b*) it is relatively nontoxic (2–4) compared to other local anesthetics; (*c*) it is a lipid-soluble, neutral molecule not subject to the usual pH-partition effects expected of organic anionic and cationic drugs; and (*d*) it is quantitatively assayable in the biological fluids found at the s.c. region.

THEORETICAL

If one can assume the BA molecules penetrate the s.c. tissue by passive diffusion, then the penetration rate of the drug from the site of administration can be described by Fick's law in one direction:

$$dN/dt = \bar{D}\,A\,(dC/dx) \qquad \text{(Eq. 1)}$$

where dN/dt is the penetration rate, and N is the amount of drug penetrating the membrane. In a well-stirred system the rate is proportional to the mean diffusion coefficient (5) of drug in the membrane, $\bar{D}$, the area, A, of the solution exposed to the absorbing membrane, and the concentration gradient (dC/dx) of drug across the membrane. Under conditions of this experiment where the penetration rate is fairly rapid, Eq. 1 can be approximated (6) by:

$$dN/dt = \frac{\bar{D}AK}{\delta}(C_c - C_b) \qquad \text{(Eq. 2)}$$

where the dx term of Eq. 1 has been replaced by δ, the thickness of the thin s.c. membrane which is assumed to be constant for each animal, and the dC term of Eq. 1 has been replaced by the terms K and $(C_c - C_b)$. The term K is the equilibrium distribution ratio or partition coefficient of the drug between the membrane lipids and the aqueous phases of the absorption cell or body fluids. The term $(C_c - C_b)$ is the difference between the drug concentration in the absorption cell, C_c, and the drug concentration C_b, in the fluids, *e.g.*, blood and lymph, flowing past the absorption site at any time.

The term C_b can be ignored in these experiments, since it can be reasonably assumed that the drug's concentration in the fluids of distribution is negligible compared to its concentration at the absorption site at any time. Thus Eq. 2 can be written as:

$$dN/dt = \frac{\bar{D}AK}{\delta}(C_c) \qquad \text{(Eq. 3)}$$

When the mean volume of the drug solution at the absorption site, $\bar{V}_c$, remains nearly constant throughout the experiment, the rate of penetration will be equal to:

$$dN/dt = \left(\frac{\bar{D}AK}{\delta \bar{V}_c}\right)Ac \qquad \text{(Eq. 4)}$$

where Ac is the amount of drug in the cell at any time. The penetration rate per unit area (p.u.a.) equals:

$$\frac{dN/dt}{A} = \bar{P}\,\frac{Ac}{A} \qquad \text{(Eq. 5)}$$

where $\bar{P}$ is the mean penetration coefficient which includes the terms $\bar{D}$, A, K, δ, and $\bar{V}_c$, and has the

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units of time^{-1}. The mean penetration coefficient or absorption rate constant has the same magnitude as the clearance constant, but the opposite sign. Equation 5 may therefore be simplified to:

$$dB/dt = \bar{P}Bc \qquad \text{(Eq. 6)}$$

where dB/dt is the penetration rate p.u.a., and Bc is the amount of drug in the cell p.u.a. at any time. The term Bc equals the initial amount of drug p.u.a., Bi, less the amount of drug p.u.a., B, that has penetrated the membrane to any time, thus:

$$Bc = Bi - B \qquad \text{(Eq. 7)}$$

Substitution of Eq. 7 into Eq. 6 and integrating between the limits of zero and infinite time yields:

$$Bc = Bie^{-\bar{P}t} \qquad \text{(Eq. 8)}$$

When the same amount of BA p.u.a., B', is administered repeatedly at the times of zero, τ, 2τ, $3\tau \ldots$, the amount of drug in the cell p.u.a. after n doses will be:

$$Bc = B' + B'e^{-\bar{P}\tau} + B'e^{-\bar{P}2\tau} + B'e^{-\bar{P}3\tau} + \ldots + B'e^{-\bar{P}(n-1)\tau} \qquad \text{(Eq. 9)}$$

where the time τ is the dosing interval and is a constant. The amount of drug in the cell p.u.a. just after the administration of the nth dose p.u.a. can be calculated from:

$$B_c = \frac{B'(1 - e^{-\bar{P}n\tau})}{(1 - e^{-\bar{P}\tau})} \qquad \text{(Eq. 10)}$$

Equation 10 applies when Bi and the mean maintenance dose(s) p.u.a., $\overline{Bm}$, are both equal to B' in magnitude. When the initial and mean maintenance dose(s) p.u.a. are not equal in magnitude, then the term Bc at any time can be computed from Eq. 11:

$$Bc = Bie^{-\bar{P}t} + \left\{\overline{Bm}\left[\frac{1 - e^{-\bar{P}(n-1)\tau}}{1 - e^{-\bar{P}\tau}}\right]\right\}e^{-\bar{P}T} \qquad \text{(Eq. 11)}$$

where n, in this case, is an integer number of initial and maintenance doses p.u.a. administered, and T is another clock time starting at zero immediately after the administration of the last maintenance dose p.u.a.

When the number of doses p.u.a. approaches infinity (and the contribution of $Bie^{-\bar{P}t}$ becomes nil), the value of Bc as defined by Eq. 11 approaches a maximum limiting value, $Bc_{max.}$, just after the administration of the last mean maintenance dose p.u.a.:

$$Bc_{max.} = \overline{Bm}\left(\frac{1}{1 - e^{-\bar{P}\tau}}\right) \qquad \text{(Eq. 12)}$$

and a minimum limiting value, $Bc_{min.}$, just before the administration of the next mean maintenance dose p.u.a.:

$$Bc_{min.} = \overline{Bm}\left(\frac{e^{-\bar{P}\tau}}{1 - e^{-\bar{P}\tau}}\right) \qquad \text{(Eq. 13)}$$

Most of the equations described above have been discussed in connection with blood or serum drug concentration changes following intravenous, i.m., and oral drug administration (7–10), but have not been discussed in connection with changes in drug concentration at the s.c. absorption site as a function of time.

An equation for calculating the limiting value for $\overline{Bc}$, the asymptotic mean amount of drug in the cell p.u.a., can be derived using the values of $Bc_{max.}$ and $Bc_{min.}$ (11). The area under the curve from $Bc_{max.}$ to $Bc_{min.}$ over the time interval of one τ can be calculated from:

$$\text{area} = \frac{(Bc_{max.} - Bc_{min.})\tau}{\ln (Bc_{max.}/Bc_{min.})} \qquad \text{(Eq. 14)}$$

Substitution of Eqs. 12 and 13 into Eq. 14, and subsequent division by the dosing time interval, τ, yields:

$$\overline{Bc} = \frac{\overline{Bm}}{\bar{P}\tau} \qquad \text{(Eq. 15)}$$

Since $\bar{P}$ equals $(\ln 2/\overline{t_{0.5}})$, where $\overline{t_{0.5}}$ is the drug's mean absorption half-life in the cell, Eq. 15 can be written:

$$\overline{Bc} = \frac{\overline{Bm}}{(\tau/\overline{t_{0.5}})\ln 2} \qquad \text{(Eq. 16)}$$

A more general expression than Eq. 16 was derived by Wagner and co-workers (12) for drug blood levels at the equilibrium state after multiple dosing.[1] The term, $\overline{Bc}$, can also be calculated from:

$$\overline{Bc} = Bc_{max.}e^{-\bar{P}Z\tau} \qquad \text{(Eq. 17)}$$

where Z is the fractional part of τ at which time $\overline{Bc}$ is reached following the last maintenance dose p.u.a. given at a time infinite in terms of the experiment. Substitution of Eq. 12 into Eq. 17 yields:

$$\overline{Bc} = \overline{Bm}\left(\frac{1}{1 - e^{-\bar{P}\tau}}\right)e^{-\bar{P}Z\tau} \qquad \text{(Eq. 18)}$$

Substitution of Eq. 15 into Eq. 18, and solving for the term Z yields:

$$Z = \frac{1}{\bar{P}\tau}\ln\left(\frac{\bar{P}\tau}{1 - e^{-\bar{P}\tau}}\right) \qquad \text{(Eq. 19)}$$

Equation 19 can also be written in terms of the drug's absorption half-life:

$$Z = \frac{1}{(\tau/\overline{t_{0.5}})\ln 2}\ln\left[\frac{(\tau/\overline{t_{0.5}})\ln 2}{1 - e^{-(\tau/\overline{t_{0.5}})\ln 2}}\right] \qquad \text{(Eq. 20)}$$

The magnitude of the unitless Z term is a complex function of the ratio $\tau/\overline{t_{0.5}}$ and is dose independent. The significance of the term Z will be indicated under *Results and Discussion*.

[1] Equation 16 of this paper and Eq. 6 found in Wagner and co-workers' report (12) are mathematically similar, but are different in some important respects. Their Eq. 6 is $V\bar{C} = 1.44\,F\,D\,t_{1/2}/\tau$, where V is the apparent volume of distribution of the drug, $\bar{C}$ is the average asymptotic blood concentration, F is the fraction of each dose which is absorbed, $t_{1/2}$ is the biological half-life of the drug in a particular animal or individual, and τ is the length of the dosing interval (12). The two equations differ in terms of their definitions of half-life. In Eq. 16 the half-life is for absorption, while in Eq. 6 above it is the biological half-life. In Eq. 16 the term $\overline{Bm}$ equals the magnitude of the product of the terms F and D found in Eq. 6 above only when the fraction of each dose absorbed is equal to one. The term (VC) is the average amount of the drug in the "body" during a dosage interval at the steady state, whereas $\overline{Bc}$ is the asymptotic mean amount of drug in the s.c. cell p.u.a. during a dosing interval.

MATERIALS AND METHODS

Animals—Large Sprague-Dawley female rats weighing between 400 and 500 Gm. were deliberately chosen for this study because the drug's distribution volume in them would be large. After the animals were anesthetized with ethyl ether, a circular portion of the ventral skin approximately 3 cm. in diameter was carefully excised, exposing the s.c. connective tissue. The exposed region was anatomically at about the same location as that described in previous work with s.c. implants (13). As the experiment progressed, the amount of anesthetic had to be decreased to prevent death.

Reagents—The BA used was reagent grade (Merck) with a boiling point of 205°/752 mm. which agrees closely with that reported in the literature (14, 15). The complete solution of BA in 0.9% (w/v) aqueous sodium chloride or normal saline solution (NS) was effected by warming and stirring the mixture on a steam bath for about 1 hr. and then allowing it to cool to room temperature. The *n*-hexane used as the extraction solvent in the assay was of spectrographic grade[2] with an ultraviolet cut-off of 199 mμ or less.

Subcutaneous Absorption Cell—A cylindrical glass absorption cell was constructed to hold the drug solutions in contact with the s.c. tissue. It was about 21 mm. high and had an inside diameter of 17.2 mm. At one end of this cell was a rim having an outside diameter of about 26 mm. The part of the rim adjacent to the s.c. tissue was ground flat. The inner cross-sectional area of the cell was 2.322 cm.^{2}.

Adhesive—The rim of the absorption cell was attached to the moist s.c. tissue by a medical silicone adhesive[3] commercially available as a developmental aerosol formulation in a pressurized container. The use of silicones in biological research has been recently reviewed (16). According to the manufacturer, this adhesive appears to be completely inert to living tissue and will not cause irritation or sensitization. The adhesive qualities are retained in the presence of moisture. The only solvent present is a volatile fluorocarbon propellent,[4] which evaporates after a few minutes. The adhesive could not be sprayed accurately onto the cell's narrow glass rim. A special nozzle supplied by the company was inserted into the spray orifice. The nozzle was directed into an inverted 9.52 mm. diameter ophthalmic ointment tube with cap in place. The tube was nearly filled with the adhesive, allowed to stand for about 5 min., and placed in a dry oven at 104° until it reached a suitable semisolid consistency which usually took about 15 min. The ointment tube was cooled to room temperature and crimped in the usual manner. The adhesive could now be applied directly to the rim from the small orifice at the tube tip. Under experimental conditions this adhesive made the glass rim adhere to the moist tissue and formed a water-tight seal which prevented the solution from spreading onto the surrounding regions.

Assay—A method for determining the amount of BA in NS has been reported previously (15). However, the procedure is too tedious to perform routinely and not sufficiently sensitive for the present purposes. Instead, an assay procedure was developed which involved the extraction of BA from a small sample of the fluids present in the absorption cell into a suitable solvent and analyzing the resulting mixture by ultraviolet spectrophotometry. The extraction step was required because proteins qualitatively similar to those which could be present in the absorption cell shortly after the experiment had begun have chromophores that absorb in the same 205–210 mμ region of the U.V. as does BA in aqueous solution (17). According to the literature BA has a $\lambda_{max.}$ of 210 mμ (log ϵ = 3.88) in methanol (18) and 208 mμ (log ϵ = 3.83) in ethanol (19). Preliminary determinations using a Cary spectrophotometer, model 11, having a continuous recording attachment showed that BA dissolved in distilled water and spectrographic grade *n*-hexane had a $\lambda_{max.}$ of 208 mμ in both solvents. The values for log ϵ in water and *n*-hexane, respectively, were 3.82 and 3.88. Stock solutions were prepared containing 1 to 4% (w/v) BA dissolved in NS. Standard curves for the absorbance of BA extracted into *n*-hexane *versus* the BA concentration in NS were made by the method of least squares. The two instruments used were the Cary spectrophotometer, model 11, and the Beckman spectrophotometer, model DU, with a hydrogen lamp accessory. A 20-μl. sample of a saline blank and each stock solution was added to 50-ml. glass-stoppered volumetric flasks using a 25-μl. Hamilton syringe.[5] Three milliliters of NS was then added in order to rinse the drug sample down from the sides of the flask. Duplicate samples were run in each case. About 25 ml. of spectrographic grade *n*-hexane was added to each flask. It was stoppered and shaken for 0.5 hr. on a mechanical shaker. Afterward, sufficient *n*-hexane was added to bring the total volume up to the 50-ml. mark, and the shaking was repeated for another 0.5 hr. Samples of the *n*-

TABLE I—ULTRAVIOLET ABSORBANCE OF BENZYL ALCOHOL

Concn. of BA in NS, mg./ml.	Amt. of BA in Flask, μm.[a]	Absorbance in Hexane Extract at 208 mμ Cary Model 11[b]	Absorbance in Hexane Extract at 208 mμ Beckman Model DU[b]	Calcd. Concn. of BA in NS, mg./ml.[c]
9.9	1.83	...	0.223	9.6
10.0	1.85	0.205	...	10.0
15.1	2.79	...	0.342	15.1
19.5	3.61	...	0.454	20.1
20.0	3.70	0.425	...	19.9
24.6	4.55	...	0.554	24.7
30.0	5.55	0.655	...	30.1
35.0	6.47	...	0.778	34.9
40.0	7.40	0.875	...	40.0

[a] The amount of BA contained in 20 μl. of the solution listed in column 1 partitioned between 3 ml. of NS and sufficient *n*-hexane to make 50 ml. of total solution. [b] The equation for the standard curve by the least squares method in the Cary instrument is: concn. (mg./ml.) BA = (absorbance + 0.020)/0.0224 and that in the Beckman instrument is: concn. (mg./ml.) BA = (absorbance − 0.011)/0.0220. [c] The concentrations were computed using the appropriate standard curve equations for the respective instruments.

[2] Spectroquality reagent, Matheson, Coleman and Bell, East Rutherford, N. J.
[3] Dow Corning Medical Adhesive, type B, Midland, Mich.
[4] Freon. E. I. duPont de Nemours and Co., Wilmington, Del.
[5] Hamilton Co., Whittier, Calif.

hexane layer were removed from each flask and were read in the respective instruments at 208 mμ against the *n*-hexane blank similarly treated with NS solution. The absorbances in *n*-hexane were proportional to BA concentration in NS from 9.9 to 40 mg./ml. (See Table I.) Potential problems of stray radiation at this wavelength were investigated (20). Using the method described by Ofler (21), calculations were made with the intent to improve the linearity of the absorbance *versus* concentration standard curves mentioned above. No improvement in linearity could be achieved. Column 5 of Table I shows with both spectrophotometers that the calculated values of drug concentration using the respective standard curves agreed closely with the actual concentrations listed in column 1 of Table I.

The question of whether the silicone adhesive might interfere with the BA assay was investigated. The insides of two 1.9 cm. diameter test tubes were sprayed with a thin film of the silicone adhesive and allowed to stand until the volatile solvent had evaporated. Two milliliters of a 2.04% (w/v) BA stock solution in NS was added to each tube. One tube was shaken by hand intermittently for 4 hr. and the other for 24 hr. Then 20-μl. samples of these solutions, the original stock solution, and the NS blank solution were transferred to 50-ml. volumetric flasks and assayed according to the procedures described above. All determinations were made in triplicate. There was no significant difference between the absorbance values for the BA stock solutions exposed to the silicone adhesive and the BA stock solution not so exposed. It was concluded that the adhesive did not interfere with the assay at the BA concentration used.

Penetration Coefficient Determinations—Shortly after the absorption cell was securely affixed to the s.c. tissue, 0.5 ml. of NS preheated to 28° was introduced into the cell to ensure that the exposed tissue would remain moist before the introduction of the BA solution. A hollow glass rod 5.1 mm. in diameter and 34 cm. long was fastened by a rubber band to the metal spatula of a vibrating stirrer.[6] To avoid splashing of the test solution and injury to the s.c. tissue, one end of a piece of glass wool was twisted to a fine tip and the other end was attached to the glass rod by another rubber band. The strand of vibrating glass wool was introduced into the test solution. After about 10 min., 1.0 ml. of 2.04% (w/v) BA dissolved in NS also preheated to 28° was pipeted into the absorption cell. This was zero time in terms of this experiment. Four to five 20-μl. samples of solution were withdrawn each 0.5 hr. indicated in Table II. Each 20-μl. sample was transferred to a 50-ml. glass-stoppered volumetric flask and assayed for BA content according to the method described previously. At the end of each 0.5-hr. period, 0.1 ml. of 3.42% (w/v) BA in NS solution was added to the cell. From time to time the accuracy of the sampling technique with the Hamilton syringe was checked. The syringe containing the aliquot was weighed on a Sartorius analytical balance, and reweighed after the sample was transferred for the U.V. assay. In all cases the difference between the two weights corresponding to the volume of sample delivered (1 μl. = 1 mg.) agreed with that estimated from the syringe barrel markings within ±1%.

The magnitude of both the mean diffusion and partition coefficients and the resulting penetration coefficient as defined by Eq. 5 may be temperature dependent. It was therefore necessary to determine what the mean solution temperature was in the cell and the magnitude of possible fluctuations during the experiment. The temperature of the BA solution in one anesthetized animal was continuously recorded,[7] using a small thermocouple probe introduced into the cell. The thermocouple and accompanying equipment was specially designed[8] for routine pyrogen testing of parenteral solutions. After about 15 min., thermal equilibrium between the animal, the absorption cell contents, and the room environment was established. The temperature of the solution oscillated about a mean value of 28.3 ± 0.2°. In a parallel *in vitro* experiment the same absorption cell was affixed to a metal block heated to constant temperature, and no measurable amount of evaporation from the stirred solution used above was found when maintained at 28° for 3 hr. For each 0.5-hr. period, an estimate of the penetration coefficient, P, was made by plotting the logarithm of the amount of BA p.u.a. in the cell at any time, against the sampling times shown in Table II and by multiplying by -2.303 the resultant slope obtained by the least squares method. In this study all computations were programmed for the Wyle calculator.[9] In some cases in Tables II through IV extra digits obtained by computational means and indicated by subscripts, are listed to minimize rounding off errors in subsequent calculations, and do not imply that four significant figure experimental accuracy was achieved.

In a few cases each reading for the amount of BA p.u.a. was determined according to the weighting method described by Paradine and Rivett (22) to determine the value for P again. This latter method was discontinued, since the values for P calculated by this method seldom differed from the previous one by more than one digit in the third decimal place. The half-life values reported in Table III were calculated from the relationship: $t_{0.5} = \ln 2/P$.

Volume Determinations—The calculated intercepts of drug concentration in the cell at the beginning and end of each 0.5-hr. period were used to estimate any possible volume changes in the cell as a function of time. The values in Table II are expressed in terms of amount of BA p.u.a. and are reconvertible to units of concentration (mg./ml.) by the equation:

$$\text{concn. BA (mg./ml.)} = Bc\ (\text{mg./cm.}^2) \times \frac{A\ (\text{cm.}^2)}{\bar{V}_c\ (\text{ml.})} \quad \text{(Eq. 21)}$$

where A is the tissue area of 2.322 cm.^{2} exposed to the drug solution, and $\bar{V}_c$ is the mean volume of solution tabulated in column 9 of Table III for the respective animals. One could calculate the final concentration, C_f, of BA at the *end* of a given 0.5-hr. time from the 0.5-hr. intercepts of the regression line computed from the logarithm of BA concentra-

[6] Vibro Spatula, catalog No. 57900, Van Waters and Rogers, Brisbane, Calif.

[7] Speedomax, type G, Leeds and Northrup.

[8] Research and Development Laboratory, University of California, San Francisco Medical Center.

[9] Wyle Laboratories, El Segundo, Calif.

TABLE II—ABSORPTION DATA ON BENZYL ALCOHOL IN SOLUTION FROM THE SUBCUTANEOUS SITE

Clock Time, hr.	mg. BA at the Absorption Site/Unit Area of Exposed Tissue			
	Animal A	Animal B	Animal C	Animal D
		Second 0.5-hr. Run		
0.500[a]	7.52[b] (7.39$_4$; 6.86$_9$)[c]	6.67 (6.73$_6$; 6.55$_4$)	5.72 (5.68$_0$; 5.49$_5$)	5.51 (5.47$_4$; 5.31$_8$)
0.583	...	6.50 (6.43$_9$; 6.31$_1$)	...	...
0.600	...	...	5.24 (5.32$_5$; 5.19$_5$)	...
0.617	...	...	...	5.09 (5.15$_8$; 5.05$_3$)
0.633	6.95 (7.11$_1$; 6.75$_6$)	...	...	...
0.717	...	5.97 (5.98$_5$; 5.87$_3$)	5.01 (4.93$_7$; 4.84$_1$)	...
0.767	...	...	...	4.84 (4.77$_9$; 4.70$_1$)
0.800	6.74 (6.77$_0$; 6.44$_5$)	...	...	...
0.833	...	5.61 (5.61$_9$; 5.45$_8$)	4.55 (4.58$_0$; 4.47$_3$)	...
0.867	...	...	...	4.51 (4.54$_3$; 4.45$_2$)
0.967	6.50 (6.44$_6$; 5.96$_3$)	...	4.21 (4.19$_9$; 4.05$_4$)	...
0.984	...	...	...	4.29 (4.28$_2$; 4.16$_3$)
		Third 0.5-hr. Run		
1.000	7.44 (7.39$_9$; 6.93$_9$)	6.28 (6.33$_7$; 6.11$_0$)	5.32 (5.33$_1$; 5.31$_3$)	5.26 (5.24$_2$; 4.99$_9$)
1.100	...	...	...	4.86 (4.96$_6$; 4.79$_4$)
1.117	...	6.05 (6.04$_6$; 5.89$_1$)	4.92 (4.91$_7$; 4.90$_5$)	...
1.150	7.17 (7.14$_1$; 6.83$_7$)	...	...	...
1.233	...	...	...	4.71 (4.62$_2$; 4.49$_4$)
1.250	...	5.78 (5.73$_2$; 5.61$_3$)	...	...
1.267	...	...	4.43 (4.43$_2$; 4.42$_4$)	...
1.317	6.72 (6.86$_3$; 6.57$_7$)	...	...	...
1.350	...	...	...	4.39 (4.33$_8$; 4.19$_2$)

(*Continued on next page.*)

tions *versus* sampling times. This equals

$$C_f = B_f/V_f \qquad \text{(Eq. 22)}$$

where B_f is the final amount of BA dissolved in the final volume, V_f, at that time. The initial concentration, C_i, of drug at the *beginning* of the next 0.5-hr. period following the addition of more BA in the amount ΔB dissolved in a NS solution with a volume, ΔV, may be calculated from the zero intercept of the regression line for the next 0.5-hr. period. This equals:

$$C_i = (B_f + \Delta B)/(V_f + \Delta V) = B_i/V_i \qquad \text{(Eq. 23)}$$

where B_i is the initial amount of BA dissolved in the initial volume, V_i, at the initial time for the next 0.5-hr. period. If the values for ΔB and ΔV are

TABLE II—(*Continued.*)

Clock Time, hr.	mg. BA at the Absorption Site/Unit Area of Exposed Tissue			
	Animal A	Animal B	Animal C	Animal D
1.367	...	5.56 (5.46_9; 5.33_0)	4.14 (4.13_5; 4.12_6)	... ...
1.467	...	...	...	4.01 (4.07_1; 3.88_1)
1.484	6.68 (6.59_7; 6.17_6)	5.13 (5.21_8; 5.03_4)	3.81 (3.81_5; 3.80_2)	...
Fourth 0.5-hr. Run				
1.500	7.68 (7.68_3; 7.58_4)	6.05 (6.08_9; 5.94_5)	4.81 (4.79_6; 4.72_5)	5.09 (5.12_9; 4.90_4)
1.600	...	5.86 (5.87_2; 5.76_8)	4.41 (4.44_2; 4.39_2)	...
1.633	7.38 (7.38_9; 7.32_3)	...	...	4.81 (4.79_6; 4.65_2)
1.717	...	...	4.09 (4.06_0; 4.02_4)	...
1.734	...	5.64 (5.59_4; 5.51_4)	...	4.66 (4.56_2; 4.44_7)
1.800	7.07 (7.03_7; 6.97_6)	...	...	...
1.850	...	5.42 (5.36_2; 5.27_1)	3.66 (3.66_6; 3.62_5)	4.22 (4.30_5; 4.17_6)
1.967	6.68 (6.70_2; 6.61_3)	...	3.35 (3.35_2; 3.29_9)	...
1.984	...	5.05 (5.10_7; 4.98_2)	...	4.04 (4.02_6; 3.84_9)
Fifth 0.5-hr. Run				
2.000	7.58 (7.58_8; 7.46_6)	6.17 (6.14_4; 6.02_0)	4.43 (4.42_8; 4.33_5)	5.19 (5.18_2; 5.09_5)
2.100	...	...	4.06 (4.09_1; 4.02_6)	...
2.117	...	5.83 (5.88_8; 5.80_3)	...	...
2.133	7.32 (7.33_5; 7.25_2)	...	...	4.79 (4.82_6; 4.77_2)
2.233	...	5.64 (5.64_3; 5.57_7)	3.69 (3.68_1; 3.63_6)	4.61 (4.57_6; 4.53_2)
2.300	7.07 (7.03_1; 6.95_5)	...	...	...
2.334	...	5.50 (5.44_3; 5.36_8)	...	...
2.350	...	...	...	4.31 (4.30_1; 4.25_8)
2.367	...	...	3.35 (3.31_1; 3.25_9)	...
2.467	6.72 (6.73_8; 6.63_9)	...	3.03 (3.05_9; 2.99_5)	...
2.484	...	5.13 (5.15_3; 5.04_6)	...	3.99 (4.00_5; 3.93_9)

[a] The experimental points for the first and last 0.5-hr. periods are not included in this table. Consult text for discussion. [b] This value may be converted back to the original concentration of drug in the cell by Eq. 21. [c] The first number within the parentheses is the value for the amount of BA per cm.^{2} of absorption site as calculated from the equation for the regression line derived from the experimental values. The second number is the value for the lower limit of the 95% confidence limit. The upper and lower 95% confidence limits are equidistant from the first number in the parenthesis on a logarithmic scale (23).

known in Eq. 23, one can calculate the values for V_f and B_f from:

$$V_f = \left[\frac{\Delta B - (\Delta V)(C_i)}{C_i - C_f}\right] \quad \text{(Eq. 24)}$$

and

$$B_f = C_f\left[\frac{\Delta B - (\Delta V)(C_i)}{C_i - C_f}\right] \quad \text{(Eq. 25)}$$

The amounts of ΔB and ΔV added to the cell were kept constant and were 3.42 mg. and 0.10 ml., respectively. The mean volume, $\bar{V}'$, during any 0.5-hr. interval is:

$$\bar{V}' = (V_i' + V_f')/2 \quad \text{(Eq. 26)}$$

where the primes over each quantity indicate that

TABLE III—HALF-LIVES OF BA, MEAN VOLUMES OF SOLUTION IN THE ABSORPTION CELL, AND MEAN VALUES FOR Bm

Animal		0.5-hr. Intervals 1	2	3	4	5	6	Mean	$\overline{Bm}$[i] (mg./cm.²)
A	$t_{0.5}$[a]	(2.15)[b]	2.36	2.92	2.37	2.73	(2.88)[b]	2.58_4[c]	1.00_5
	$\overline{V}'$[d]	...	1.60	1.47	1.49	1.59	...	$1.53_8(0.10_4)$[e]	
	$B_{max.}$[f]	...	(7.39_4)	7.39_9	7.68_3	7.58_8	...	7.55_6[g]	
	$B_{min.}$[h]	...	6.46_6	6.47_1	6.71_7	...	...	6.55_1	
B	$t_{0.5}$	(1.37)	1.27	1.73	1.91	1.91	(1.96)	1.79_5	0.92_3
	$\overline{V}'$	...	1.28	1.45	1.56	1.41	...	$1.42_6(0.18_4)$	
	$B_{max.}$	...	(6.73_6)	6.33_7	6.08_9	6.14_4	...	6.19_0	
	$B_{min.}$	...	5.55_4	5.22_5	5.02_1	...	...	5.26_7	
C	$t_{0.5}$	(1.06)	1.07	1.00	0.90	0.79	(0.82)	0.95_7	1.18_3
	$\overline{V}'$	...	1.46	1.48	1.51	1.40	...	$1.46_2(0.07_9)$	
	$B_{max.}$	...	(5.68_9)	5.33_1	4.79_6	4.42_8	...	4.85_1	
	$B_{min.}$	...	3.95_5	3.71_1	3.33_9	...	...	3.66_8	
D	$t_{0.5}$	(1.10)	1.37	1.28	1.39	1.30	(1.61)	1.33_4	1.11_1
	$\overline{V}'$	...	1.31	1.30	1.24	1.23	...	$1.27_4(0.07_3)$	
	$B_{max.}$	...	(5.47_4)	5.24_2	5.12_9	5.18_2	...	5.18_4	
	$B_{min.}$	...	4.22_2	4.04_2	3.95_6	...	...	4.07_3	

[a] Absorption half-life (hr.) of drug in the cell. [b] The half-lives for the first and last 0.5-hr. runs appear in parentheses and were calculated on the assumption that the mean volume in the cell at these time periods was identical to the mean volume listed in column 9 for the 2nd through 5th half-hour intervals. [c] Mean half-life, $\overline{t_{0.5}}$, of intervals two through five. The mean penetration coefficient, $\bar{P}$, for animal D, for example, equals $\ln 2/1.33$ hr. or 0.52 hr.$^{-1}$. [d] Mean volume (ml.), $\overline{V}'$, calculated according to Eq. 26 for each 0.5-hr. interval. [e] Mean volume, $\overline{Vc}$, for intervals two through five followed by the plus and minus values for the 95% confidence limits about the mean in parentheses. [f] The values of $B_{max.}$ (mg./cm.²) are the calculated intercepts of the equation for the regression line and are identical to values shown in Table II at the beginning of the respective 0.5-hr. intervals. [g] Mean of the three values of $B_{max.}$. The value in parentheses is not included in this mean, but was used to calculate $B_{min.}$ at the end of the second interval. [h] The values of $B_{min.}$ (mg./cm.²) were calculated from Eq. 27. [i] The value of $\overline{Bm}$ was calculated by subtracting the mean of $B_{min.}$ from the mean of $B_{max.}$ shown in column 9.

TABLE IV—COMPUTED AMOUNTS OF BENZYL ALCOHOL IN THE ABSORPTION CELL AT VARIOUS TIMES AND OTHER RELEVANT INFORMATION

Constant		Animal A	B	C	D
$\tau/\overline{t_{0.5}}$[a]		0.193	0.278	0.522	0.375
Bi (mg./cm.²)[b]		7.39	6.74	5.68	5.47
$\overline{Bm}$ (mg./cm.²)[c]		1.00_5	0.92_3	1.18_3	1.11_1
$Bi/\overline{Bm}_{expt.}$[d]		7.35	7.30	4.80	4.92
$Bi/\overline{Bm}_{calcd.}$[e]		7.97	5.70	3.29	4.37
$\overline{Bc}_{max.}$ (mg./cm.²)[f]		8.00_3	5.25_8	3.89_4	4.85_7
$\overline{Bc}$ (mg./cm.²)[g]		7.49	4.78	3.27	4.28
Z[h]		0.494	0.493	0.485	0.490
$B_{min.}$ (mg./cm.²)[i] At end of	1τ	6.46	5.55	3.96	4.22
	2τ	6.53	5.34	3.58	4.11
	4τ	6.64	5.02	3.13	3.96
$Bc_{min.}$ (mg./cm.²)[j]		7.00_3	4.33_5	2.71_1	3.74_6
No. of total doses p.u.a. needed to approach within 1% of $Bc_{min.}$ shown above[k]		>16	>18	>11	>10

[a] The value of τ is the experimental one of 0.5 hr. as shown in Table II, and the value of $\overline{t_{0.5}}$ is the mean value found in column 9 of Table III. [b] The initial amount of drug/cm.² at the absorption site as calculated from the regression line equation. This is the first number within the parentheses at 0.5 hr. in column 2 of Table II. [c] See column 10 of Table III. [d] The experimental value of the ratio calculated from items 2 and 3 of this column. [e] The calculated value of the ratio at infinite time from Eq. 31 using the ratio of $\tau/\overline{t_{0.5}}$ tabulated as item 1 in this column. [f] The calculated maximum value at infinite time from Eq. 12. [g] The calculated value for the asymptotic mean value at infinite time from Eq. 16, using the ratio of $\tau/\overline{t_{0.5}}$ found as item 1 of this column. [h] Calculated from Eq. 20. [i] Calculated minimum value using Eq. 11 up to four τ. [j] Calculated according to Eq. 13. [k] Calculated from the appropriate form of Eq. 11.

the initial and final calculated volumes are from the same 0.5-hr. time interval.

RESULTS AND DISCUSSION

The results of the *in vivo* absorption experiments are presented in Tables II through IV and Fig. 1. The experiment for each animal lasted 3 hr. Table II shows the experimental values for the amount of BA in the absorption cell p.u.a. of s.c. tissue exposed to the drug solution as a function of time. It also lists the calculated values of the amount of BA p.u.a. from the regression line for each 0.5 hr., and the lower value for the 95% confidence limit (23) for each calculated point all at the same sampling times. In Table II no data points are presented for the zero to 0.5 and the 2.5 to 3-hr. time intervals, although four to five samples were withdrawn from the absorption cell and analyzed for BA content in the same manner as samples taken during the intervening 0.5-hr. intervals. The main reason for excluding details of the data for the first and sixth 0.5-hr. intervals is that mean volume calculations for these two runs could not be made according to Eq. 26, because values for V_i and V_f were not determined. At zero time no estimate of V_i could be made according to the experi-

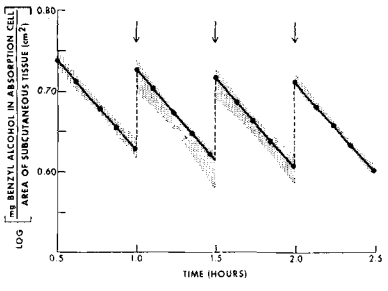

Fig. 1—The calculated logarithmic amount of BA present at the subcutaneous site per unit area as a function of dosing regimen and time for animal D. The solid lines were calculated using Eq. 11 where the value for $\bar{P}$ *is 0.52 hr.*$^{-1}$, B_i *is 5.47 mg./cm.*^{2}, $\bar{B}_m$ *is 1.11 mg./cm.*^{2}, τ *is 0.5 hr., and* n *is four. The arrows indicate the times of administration of maintenance doses. The upper and lower boundaries of the shaded areas are the logarithmic values for the 95% confidence limits* vs. *time about the regression line for the experimental points for each 0.5 hr.* (*See Table II.*)

mental procedures used, and at the end of 3 hr. no estimate of V_f could be made according to Eq. 24, since another maintenance dose was not given at that time.

In Table III the half-life of BA for each animal was calculated during each 0.5-hr. period from 0.5 to 2.5 hr. and is shown in columns 4 through 7. For each animal, the mean half-life, $\overline{t_{0.5}}$, shown in column 9 was calculated using *all* data points for the intervals from 0.5 to 2.5 hr. To do this, the data given in Table II were transposed in the following manner. The ordinate values at 0.5, 1.0, 1.5, and 2.0 hr. as obtained from the least square fit of the points for the 0.5-hr. intervals were divided into all the experimental points for that 0.5 hr. Thus, there was a common reference point for each of the four runs. Then, for purposes of calculation, the times of 0.5, 1.0, 1.5, and 2.0 hr. were all made equal to a zero clock time. For each animal $\overline{t_{0.5}}$ was computed by the least squares method from the slope of the plot of the logarithm of *Bc versus* time using all transposed experimental points listed in Table II. This mean half-life differed only slightly from the arithmetic mean half-life of the four runs because of weighting effects (22) and possible errors due to numerical rounding off. The half-lives for the first and last 0.5-hr. time periods are also included in Table II in parentheses. They were calculated on the assumption that the mean volume in the cell during these periods was identical to the mean volume shown in column 9 for the 0.5 to 2.5-hr. periods. The values of $\overline{t_{0.5}}$ for BA at the s.c. absorption site for all animals range from 0.96 to 2.58 with an average of about 1.6 hr. This mean is little affected even when one includes the values in parentheses.

In general, the variations in half-lives for the four runs in any one animal are less than those found among all animals. Probably the larger inter-animal variations in $\overline{t_{0.5}}$ are due to differences in membrane thickness. With the surgical procedures used in this study there was no way to ensure that all animals had the same membrane thickness at the absorption site. In Eq. 4, the penetration coefficient is inversely proportional to the membrane thickness, and therefore the half-life is directly proportional to membrane thickness. There is no easy way to explain the variations in half-lives with successive doses in any one animal, since membrane thickness in each case would be constant. Apart from analytical errors, the one possible reason for the half-life differences in successive 30-min. periods might be traceable to flow rate changes in the microcirculation supplying the s.c. site (24). It is not known at present how and to what extent the regional blood and lymph flows near the absorption site are affected at various times by changes in blood concentrations of ethyl ether and BA in the animal.

Table III shows the mean volume, $\bar{V}'$, of the solution in the cell during each 0.5-hr. period from 0.5 to 2.5 hr. in columns 4 through 7 as calculated from Eq. 26. The arithmetic mean values, $\bar{V}_c$, of these volumes for each animal are given in column 9 along with the plus and minus 95% confidence limits about the mean. The range of the 95% confidence limits for these volumes for the four animals is 0.07 to 0.18 ml. These small variations indicate that the volume of the BA solution in the cell remains essentially constant throughout the experiment. Equation 4 is therefore valid in so far as constancy of volume is concerned.

Table III also shows the calculated values of $B_{max.}$ and $B_{min.}$ The terms $B_{max.}$ are the initial intercepts of the equation for the regression line calculated from the logarithm of the BA amounts p.u.a. *versus* time at the beginnings of the third through fifth time intervals (see Table II). The terms $B_{min.}$ are the final intercepts during the second through fourth 0.5-hr. periods using the mean penetration coefficient, $\bar{P}$, in the equation:

$$B_{min.} = B_{max.}e^{-\bar{P}\tau} \qquad \text{(Eq. 27)}$$

where τ is equal to 0.5 hr. The mean value of the maintenance dose p.u.a., $\overline{Bm}$, was calculated by subtracting the mean values of $B_{min.}$ and $B_{max.}$ shown in column 9 of Table III. These values for $\overline{Bm}$ for each animal were used in Table IV to calculate the asymptotic values for $Bc_{max.}$ and $Bc_{min.}$ at times infinite in terms of these experiments.[10]

Multiple Dosing—Multiple dosing of the drug was used in this study for the following reasons: (*a*) the concentration of BA in the absorption cell could be maintained at a level much higher than that found at all times in the animal's fluids of distribution so that the assumption made in connection with Eq. 3 would be valid; (*b*) the volume of drug solution in the cell could be maintained nearly constant by replacing solution lost through the frequent sampling; (*c*) the drug could be used as its own "marker" for detecting possible volume changes in the cell as a function of time, and (*d*) several independent determinations of absorption half-life of BA in the cell could be made in the same animal under comparable experimental conditions.

There may be instances under multiple dosing

[10] Note that $Bc_{max.}$ and $Bc_{min.}$ are constant and can be calculated from Eqs. 12 and 13, respectively, whereas both $B_{max.}$ and $B_{min.}$ are not constant except at infinite time when $B_{max.} = Bc_{max.}$ and $B_{min.} = Bc_{min.}$ provided that the original maintenance dosing regimen is continued.

conditions where it is desirable to establish constant values for the maximum and minimum amounts of drug p.u.a. at the s.c. site at all times starting with the initial dose. At the beginning of the experiment, let:

$$Bc_{max.} = Bi \qquad \text{(Eq. 28)}$$

and at the end of the initial dosing interval of one τ, let:

$$Bc_{min.} = Bie^{-\bar{P}\tau} \qquad \text{(Eq. 29)}$$

Immediately after an infinite number of mean maintenance doses p.u.a. have been administered, the value for $Bc_{max.}$ can be calculated using Eq. 12. Substitution of Eq. 28 into Eq. 12 yields:

$$Bi = \overline{Bm}\left(\frac{1}{1 - e^{-\bar{P}\tau}}\right) \qquad \text{(Eq. 30)}$$

The same equation results when Eq. 29 is substituted into Eq. 13. The ratio of the respective doses p.u.a. is:

$$R = \frac{Bi}{\overline{Bm}} = \left(\frac{1}{1 - e^{-\bar{P}\tau}}\right) = \left[\frac{1}{1 - e^{-(\tau/\overline{t_{0.5}})\ln 2}}\right] \qquad \text{(Eq. 31)}$$

and is constant as long as the terms $\bar{P}$ and τ are constant.

Constant values for the maximum and minimum amounts of drug p.u.a. of tissue at the s.c. site can be maintained at all times by adjusting the ratio $Bi/\overline{Bm}$ in accordance to any fixed value of the ratio $\tau/\overline{t_{0.5}}$. For example, if the ratio $\tau/\overline{t_{0.5}}$ is fixed at unity, the ratio $Bi/\overline{Bm}$ must equal the value of two. In this case if the ratio $Bi/\overline{Bm}$ is greater than two, the amount of drug p.u.a. in the cell will decrease with time until the appropriate asymptotic values for $Bc_{max.}$ or $Bc_{min.}$ are reached. On the other hand, if the $Bi/\overline{Bm}$ ratio is less than two, the amount of drug p.u.a. in the cell will increase with time until the appropriate asymptotic values for $Bc_{max.}$ or $Bc_{min.}$ are reached.

One can see examples of this type of descending or ascending behavior in the data compiled for Table IV. With animal A the $\tau/\overline{t_{0.5}}$ ratio was 0.193 and the experimental $Bi/\overline{Bm}$ ratio was 7.35. This ratio was not large enough to maintain constant values for $B_{min.}$ (and $B_{max.}$) from the first dose on. Values for $B_{min.}$ increase gradually from a value of 6.46 at the end of the first dosing interval to the asymptotic value of 7.00 at the end of an infinite number of dosing intervals. If the $Bi/\overline{Bm}$ ratio of 7.97 calculated according to Eq. 31 had been used, then values for $Bc_{max.}$ and $Bc_{min.}$ would have remained at constant levels throughout the experiment on to an infinite number of doses. With animal D, the $\tau/\overline{t_{0.5}}$ ratio was 0.375 and the experimental $Bi/\overline{Bm}$ ratio was 4.92. This ratio was too large to maintain constant values for $B_{min.}$ (and $B_{max.}$) from the first dose on. Values for $B_{min.}$ decrease from a value of 4.22 at the end of the first dosing interval to the asymptotic value of 3.75 at the end of an infinite number of dosing intervals. According to calculations that can be made from Eq. 31, the correct $Bi/\overline{Bm}$ ratio should have been 4.37 instead of 4.92 to effect constant values for $Bc_{max.}$ or $Bc_{min.}$ from the first dose on. Results for animals B and C are similar in this regard to those for animal D.

The asymptotic mean amount of drug p.u.a., $\overline{Bc}$, can be calculated according to Eq. 15 when the number of doses p.u.a. approaches infinity. For animal A this value is 7.49. The value of $\overline{Bc}$ can be closely approximated by Eq. 32 only when the $\tau/t_{0.5}$ ratio is small:

$$\overline{Bc} \simeq \frac{Bc_{max.} + Bc_{min.}}{2} \qquad \text{(Eq. 32)}$$

From Eq. 17 one should be able to determine when the value of $\overline{Bc}$ is reached after the administration of the last mean maintenance dose p.u.a. at a time infinite in terms of the experiment. The value of $\overline{Bc}$ should be reached at some time, $Z\tau$, after $Bc_{max.}$ is reached. Solution of Eqs. 17 and 18 for Z yielded Eqs. 19 and 20. It should be evident from Eq. 20 that when the ratio $\tau/\overline{t_{0.5}}$ tends toward zero, or the drug is being administered by continuous infusion at a constant rate, the term Z tends toward a value of 0.5. On the other hand, when the ratio $\tau/\overline{t_{0.5}}$ tends toward infinity, or the drug is administered only once, the term Z tends toward zero. In Table IV the calculated values of Z for all animals is nearly equal to 0.5, since the $\tau/\overline{t_{0.5}}$ ratios are all small. Table IV also shows the total number of doses p.u.a. needed to approach within 1% of the values of $Bc_{min.}$ after the administration of an infinite number of doses p.u.a. By the use of Eq. 11, one can see that the value approaching 1% of $Bc_{min.}$ is reached with animals A through D in the range of more than 10 to 18 total doses.

Figure 1 represents some of the data given in Table II for animal D. The boundaries of the shaded regions represent plots of the logarithms of the calculated upper and lower 95% confidence limits of *Bc versus* time. The actual experimental points and the regression lines calculated from these points for each 0.5 hr. are omitted from this figure. The solid dots represent times at which samples were withdrawn from the absorption cell for analysis. The solid lines are plots of the logarithm of *Bc versus* time and were calculated from Eq. 11, where the initial dose p.u.a., Bi, is 5.47 mg./cm.^{2}, the mean value for the penetration coefficient, $\bar{P}$, is 0.52 hr.$^{-1}$, t is clock time from 0.5 to 2.5 hr., the mean value for the maintenance doses p.u.a., $\overline{Bm}$, is 1.11 mg./cm.^{2}, n is the total number of doses and ranges in whole numbers from one to four, τ is the dosing time interval of 0.5 hr., and the time, T, ranges from zero to 0.5 hr. after the last maintenance dose p.u.a. The arrows represent times at which the three mean maintenance doses p.u.a. were administered. The vertical dashed lines in Fig. 1 represent the magnitude of logarithm of $\overline{Bm}$. Figure 1 shows with animal D, when values for $\overline{Bm}$ and $\bar{P}$ are substituted into Eq. 11, that the straight lines generated by plotting the logarithm of *Bc versus t* all fall within the shaded areas. Qualitatively similar results are obtained with data treated in the same manner for animals A through C.

In conclusion, the procedures outlined in this report illustrate some quantitative methods of studying drug absorption from the s.c. site. Although BA in NS was used as a model drug system

for this work, one can reasonably expect that other drugs in the same or different vehicles could be similarly studied in various animal species in order to discover fundamental factors affecting drug absorption rate from the s.c. site.

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Technical Articles

Automatic Determination of Ethinyl Estradiol in Pharmaceutical Preparations

By A. J. KHOURY* and L. J. CALI†

An automated method for determining ethinyl estradiol or ethinyl estradiol methyl ether is described. The method is based on the fluorescence exhibited by ethinyl estradiol when it is treated with 90 per cent sulfuric acid. The nonvarying time, temperature, and volume characteristics of an automatic analyzer (AutoAnalyzer) system have been used to study a number of variables which might affect the data. Specificity of the fluorescence method with respect to degradation products, interference from other estrogens and progestational agents, as well as repeatability and sensitivity of the method are discussed. Also presented are data on trade packages of a number of antifertility formulations. Samples can be analyzed using the automated method at a rate of 20 samples per hour as opposed to one or two samples per hour by manual techniques.

SINCE THE USE of progesterone and ethisterone (17α-ethinyltestosterone), was first described by Inhoffen and Hohlweg (1) a number of antifertility products have become available. All of these products contain ethinyl estradiol (EE) or its methyl ether (EEME) in combination with a progestational steroid hormone. EE is usually formulated into tablets at concentrations as low as 0.05% of the total tablet weight along with another steroid whose concentration is 50 to 500 times greater than that of EE. The analysis of EE in this environment has presented a difficult analytical problem. The U.S.P. method of analysis (2) is very time consuming, while the colorimetric method described by Urbanyi and Rehm (3) does not distinguish between EE and other estrogens, and also is not applicable to the methyl ether of EE. Paper chromatographic

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