These results support the view that the catatoxic action of different steroids is not subordinate to any presently known specific hormonal action (1).

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Subcutaneous Absorption Kinetics of Benzyl Alcohol II

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Abstract □ Under multiple-dosing conditions at a subcutaneous site, equations were derived which permit one to estimate the number of doses, n, required to approach within ± 1% (or any other fixed fraction) of the asymptotic minimum level: $n \geq 3.3219 [(t_{0.5})/\tau] \log_{10} Q$, where $Q > +1$. Here $t_{0.5}$ is the absorption half-life of benzyl alcohol from a subcutaneous absorption cell, τ is the dosing interval, Q (always positive) equals $(\beta - 1)/(\alpha - 1)$, α equals $B^{(n)}_{\text{min.}}/B^{\infty}_{\text{min.}}$ (and is 0.99 or 1.01 in this example), and β equals $B''/B^{\infty}_{\text{min.}}$, where B'' equals $Bi - Bm$. Definitions: $B^{(n)}_{\text{min.}}$ = amount of benzyl alcohol in the cell per unit area of subcutaneous tissue one τ after the nth dose, $B^{\infty}_{\text{min.}}$ = asymptotic minimum amount of benzyl alcohol in the cell per unit area, Bi = initial dose of benzyl alcohol per unit area, and Bm = constant maintenance dose of benzyl alcohol per unit area ($Bi \geq Bm$). The benzyl alcohol disappears from the cell in an apparent monoexponential manner.

Keyphrases □ Benzyl alcohol—subcutaneous absorption □ Kinetics—benzyl alcohol, subcutaneous absorption □ Equations—dosage numbers/subcutaneous area for asymptotic minimum level

In a recent report from this laboratory (1), a multiple-dosing procedure was used in connection with a study of the subcutaneous (s.c.) absorption kinetics of benzyl alcohol (BA) dissolved in normal saline (NS). The BA in NS solution was contained in a glass absorption cell affixed to the moist s.c. tissue of an anesthetized rat by a silicone adhesive. The solution was kept homogeneous by means of a vibrating stirrer. The use of this cell made it possible to control the area of s.c. tissue exposed to the drug solution at all times and to sample the cell's contents periodically.

Although the number of doses per unit area (p.u.a.) of s.c. tissue needed to approach within ±1% of the asymptotic minimum value were shown in Table IV of the previous report (1), details of the mathematical calculations were omitted. The purpose of this note is to derive the equations needed to make this estimation.

THEORETICAL

Under multiple-dosing conditions, where the mean volume of drug solution in the cell was nearly constant throughout the experiment, and the drug was administered at the times zero, τ, 2τ, 3τ, ..., the following equation can be derived (1):

$$Bc = B' \frac{(1 - e^{-Pn\tau})}{(1 - e^{-P\tau})} \qquad \text{(Eq. 1)}$$

where Bc in this case is the amount of drug in the cell p.u.a. just after the administration of the nth dose p.u.a. In Eq. 1 it is assumed that the drug disappears from the cell in an apparent monoexponential manner and the initial dose p.u.a., Bi, equals the constant maintenance dose(s) p.u.a., Bm, in magnitude. Thus

$$B' = Bi = Bm \qquad \text{(Eq. 2)}$$

The term P in Eq. 1 is the mean penetration coefficient having the units of time^{-1}, n is the integer number of initial and maintenance doses administered, and τ is the constant dosing time interval.

When Bi is larger than Bm, then

$$Bi = B'' + Bm \qquad \text{(Eq. 3)}$$

where B'' is the amount of drug p.u.a. in the cell administered along with Bm as a part of the initial dose p.u.a. and $Bi \geq Bm$. The amount

of drug in the cell p.u.a. at any time, Bc, can be calculated from

$$Bc = B''e^{-Pt} + Bm\left[\frac{(1 - e^{-P\tau})}{(1 - e^{-P\tau})}\right]e^{-PT} \qquad \text{(Eq. 4)}$$

where t is clock time starting at zero following the administration of the first dose p.u.a., and T is a new clock time starting just after the administration of the last maintenance dose.

The minimum amount of drug p.u.a. in the cell,[1] $B^{(n)}_{\text{min.}}$, one τ after the administration of the nth dose p.u.a., can be calculated from

$$B^{(n)}_{\text{min.}} = B''e^{-Pn\tau} + Bm\left[\frac{(1 - e^{-Pn\tau})}{(1 - e^{-P\tau})}\right]e^{-P\tau} \qquad \text{(Eq. 5)}$$

After a very large (or infinite) number of maintenance doses p.u.a. has been administered, according to Eq. 5, the asymptotic minimum amount of drug p.u.a.,[1] $B^{\infty}_{\text{min.}}$, can be calculated from

$$B^{\infty}_{\text{min.}} = Bm\left(\frac{e^{-P\tau}}{1 - e^{-P\tau}}\right) \qquad \text{(Eq. 6)}$$

Let

$$\alpha_n = \frac{B^{(n)}_{\text{min.}}}{B^{\infty}_{\text{min.}}} = \frac{B^{(n)}_{\text{min.}}/V}{B^{\infty}_{\text{min.}}/V} \qquad \text{(Eq. 7)}$$

where α_n is a ratio of amounts or concentrations of drug with V being the constant for cell volume (or apparent distribution volume for the body). In this paper the value of $B^{(n)}_{\text{min.}}$ or $(B^{(n)}_{\text{min.}}/V)$ was arbitrarily set equal to $\pm 1\%$ of $B^{\infty}_{\text{min.}}$ or $(B^{\infty}_{\text{min.}}/V)$, so that the value of α_n in Eq. 7 would be either 0.99 or 1.01, depending upon whether $B^{\infty}_{\text{min.}}$ or $(B^{\infty}_{\text{min.}}/V)$ was being approached from below or above.

Substitution of Eq. 7 into Eq. 5 yields

$$\alpha_n B^{\infty}_{\text{min.}} = B''e^{-Pn\tau} + Bm\left[\frac{(1 - e^{-Pn\tau})}{(1 - e^{-P\tau})}\right]e^{-P\tau} \qquad \text{(Eq. 8)}$$

Rearranging Eq. 8 yields

$$\alpha_n = \left(\frac{B''}{B^{\infty}_{\text{min.}}}\right)e^{-Pn\tau} + \left(\frac{Bm}{B^{\infty}_{\text{min.}}}\right)\left(\frac{1 - e^{-Pn\tau}}{1 - e^{-P\tau}}\right)e^{-P\tau} \qquad \text{(Eq. 9)}$$

Let

$$\beta = \frac{B''}{B^{\infty}_{\text{min.}}} = \frac{B''/V}{B^{\infty}_{\text{min.}}/V} \qquad \text{(Eq. 10)}$$

where β is a ratio of amounts or concentrations of drug. Substitution of Eqs. 6 and 10 into Eq. 9 yields

$$\alpha_n = \beta e^{-Pn\tau} + 1 - e^{-Pn\tau} \qquad \text{(Eq. 11)}$$

or

$$(\alpha_n - 1) = e^{-Pn\tau}(\beta - 1) \qquad \text{(Eq. 12)}$$

For α arbitrarily close to 1, say $|\alpha - 1| = 0.01$, it is desired to find the smallest n such that

$$|\alpha_n - 1| = |e^{-Pn\tau}(\beta - 1)| \leqslant |\alpha - 1| \qquad \text{(Eq. 13)}$$

Solving Eq. 13 for n gives

$$n \geqslant \frac{1}{P\tau}\ln\left(\frac{\beta - 1}{\alpha - 1}\right) \qquad \text{(Eq. 14)}$$

Since $P = \ln 2/t_{0.5}$, substitution of P into Eq. 14 gives

$$n \geqslant \left(\frac{t_{0.5}}{\tau}\right)\left(\frac{1}{\ln 2}\right)\ln\left(\frac{\beta - 1}{\alpha - 1}\right) \qquad \text{(Eq. 15)}$$

Let

$$Q = \left(\frac{\beta - 1}{\alpha - 1}\right) \qquad \text{(Eq. 16)}$$

where Q is always positive and greater than 1.[2] Substitution of Eq. 16 into Eq. 15 yields

$$n \geqslant 3.3219\left(\frac{t_{0.5}}{\tau}\right)\log_{10} Q \qquad \text{(Eq. 17)}$$

The time, t, needed for amounts or concentrations to reach within $\pm 1\%$ of the corresponding asymptotic values one τ after the nth dose is

$$t = n\tau \qquad \text{(Eq. 18)}$$

RESULTS AND DISCUSSION

Table I summarizes the data needed to estimate the doses, n, and the time, t, to approach within $\pm 1\%$ of the asymptotic value $B^{\infty}_{\text{min.}}$ or $(B^{\infty}_{\text{min.}}/V)$.

The methods described are exact when it has been demonstrated that monoexponential loss of drug occurs from a compartment such as a subcutaneous absorption cell. However, when the concentration–time course of drug in a compartment is best described by a polyexponential function (2), the methods described for calculating n and t must be used with caution. Also, other phenomena discussed by Wagner *et al.* (3) may invalidate these procedures for multiple-dosing calculations. Two examples using literature data indicate how Eqs. 17 and 18 can be used to solve for the quantities n and t. The first example involves multiple intramuscular injection of drug; the second involves continuous intravenous infusion of drug.

Example 1—Multiple Intramuscular Injection—Boxer *et al.* (4) injected streptomycin hydrochloride solutions intramuscularly into dogs at regular dosing intervals and followed changes in the plasma drug concentration with time. They derived an equation which permitted them to use the serum drug concentrations obtained at a given time after the initial dose was administered, but before the second dose was administered, to predict the serum drug concentration in the body at the same time interval after the last dose given after an infinite number of doses had been administered. The equation is

$$C_H = C_h\left(\frac{1}{1 - e^{-k\tau}}\right) \qquad \text{(Eq. 19)}$$

where C_H is the actual concentration to be expected at the corresponding time interval after a steady state has been established, C_h is an experimentally determined concentration at a time in the interval between the initial and second doses; k is the rate constant for the disposition of drug in the body, assuming it to be a single compartment; and τ is the dosing interval.

In the derivation of Eq. 19, Boxer *et al.* (4) assumed that the absorption rate of streptomycin hydrochloride from the intramuscular site was extremely (or infinitely) rapid, so that the disappearance of drug from the serum could be described by a monoexponential function. There is evidence to indicate that drug absorption rate from this site is not instantaneous (5–8). However, Eq. 19 can be a useful first approximation for the mathematical analysis of some clinical data involving blood or serum levels of drug.

Data in the left half of Table IV of the Boxer *et al.* paper (4) show that the calculated value of C_h 3 hr. after the initial dose, and before the second dose was given, was 26.3 mcg./ml.; τ is 3 hr., the mean value of k is 0.39 (± 0.028) hr.$^{-1}$; and the biological half-life in the dog is 1.78 hr. According to Eq. 19, the calculated value of C_H is 38.1 mcg./ml. The initial dose equals the maintenance dose ($\beta = 0$), and substitution of these values into Eq. 17 gives the following value for n:

[1] The notation in this paper differs somewhat from that used previously (1). The term $B_{\text{min.}}$ has been changed to $B^{(n)}_{\text{min.}}$ because $B_{\text{min.}}$ in these derivations depends in part upon n. The term $Bc_{\text{min.}}$ has been changed to $B^{\infty}_{\text{min.}}$ to indicate more clearly that the asymptotic minimum amount of drug p.u.a. is evaluated at infinite time.

[2] By appropriate substitution into Eq. 17, where $\beta \geqslant 0$, it follows that when $\alpha > \beta$, then $\alpha = 0.99$ and $Q > +1$. When $\alpha < \beta$, then $\alpha = 1.01$ and $Q > +1$. It also follows that $\alpha \neq \beta$, because Q can never equal 1. Should $Q = 1$, then no doses would be required to reach the desired level, which is an obvious impossibility.

Table I—Number of Total Doses Necessary to Approach within ±1% of $B^{\infty}_{min.}$, and the Constants Needed for Its Calculation[a]

Constant	Animal A	Animal B	Animal C	Animal D
$t_{0.5}$ (hr.)[b]	2.58_4	1.79_5	0.95_7	1.33_4
$(t_{0.5})/\tau$[c]	5.16_8	3.59_0	1.91_4	2.66_8
$B^{\infty}_{min.}$ (mg./cm.²)[d]	7.00_3	4.33_5	2.71_1	3.74_6
Bi (mg./cm.²)[e]	7.39	6.74	5.68	5.47
Bm (mg./cm.²)[f]	1.00_5	0.92_3	1.18_3	1.11_1
B'' (mg./cm.²)[g]	6.38_5	5.81_7	4.49_7	4.35_9
$(\alpha - 1)$[h]	−0.01	+0.01	+0.01	+0.01
$(\beta - 1)$[i]	-0.0882_5	$+0.3418_7$	$+0.6588_7$	$+0.1636_4$
Q[j]	8.82	34.2	65.9	16.4
n[k]	>16 (16.28)	>18 (18.33)	>11 (11.57)	>10 (10.75)
t (hr.)[l]	8	9	5.5	5

[a] For some constants in this table, extra digits were obtained by computational means and are indicated by subscripts. Subscripts are listed to minimize rounding-off errors in subsequent calculations, and do not imply that four significant figure experimental accuracy was achieved. [b] BA absorption half-life in cell. [c] The dosing interval, τ, is 0.5 hr. [d] Calculated from Eq. 6. [e] Initial dose p.u.a. [f] Mean maintenance dose p.u.a. [g] Calculated from Eq. 3. [h] The term α is defined in Eq. 7. [i] The term β is defined in Eq. 10. [j] Calculated from Eq. 16. [k] Number of total doses p.u.a. needed for $B^{(n)}_{min.}$ to approach within ±1% of $B^{\infty}_{min.}$ one τ after the nth dose as calculated from Eq. 17. [l] Time needed for amounts in the cell to reach within ±1% of the asymptotic value of $B^{\infty}_{min.}$ calculated from Eq. 18.

$$n \geq 3.3219\,(1.78/3)\log_{10}(-1/-0.01) \geq 3 \quad \text{(Eq. 20)}$$

Thus, about 3 hr. after three doses have been administered, the concentration of drug in the serum should be within 1% of that found 3 hr. after the last dose given after an infinite number of doses had been administered. While the experimental value of 39.4 mcg./ml. found 3 hr. after three doses had been given does not quite fall within ±1% of the theoretical value of 38.1 mcg./ml., it is close, particularly if one takes into account the uncertainty in the value for k.[3] The time needed to reach this level can be calculated from Eq. 18 and is 9 hr.

Example 2—Continuous Intravenous Infusion—Wagner and Alway (9) studied data obtained from the continuous intravenous infusion of lincomycin hydrochloride to humans. Consider the case where the infusion rate, k_0, is 50,000 mcg./hr.; the apparent half-life for the disappearance of the drug from the body by all processes, assuming that it is a single compartment, is 3.91 hr. (k = 0.177 hr.$^{-1}$); and the volume constant, V, or the apparent volume of distribution is 26,000 ml. Using Eq. 21, it is possible to calculate the concentration, C, of lincomycin in the fluids of distribution at any time:

$$C = \frac{k_0}{Vk}(1 - e^{-kt}) \quad \text{(Eq. 21)}$$

The concentration, C^{∞}, in the body at infinite time is

$$C^{\infty} = \frac{k_0}{Vk} \quad \text{(Eq. 22)}$$

Substituting the appropriate values into Eq. 22, the value of C^{∞} is 10.9 mcg./ml.,[4] and a concentration 1% less than this is 10.8 mcg./ml.

How long would the infusion apparatus have to run to reach the value of 10.8 mcg./ml.? As a first approximation, a continuous intravenous infusion of a drug can be thought of as a process where a large number of small doses are administered at very frequent time intervals (*e.g.*, intravenous drip). For the present discussion, let $\tau = 3.91 \times 10^{-4}$ hr. (1.4 sec.), $t_{0.5}/\tau = 10^4$, and $\alpha = 0.99$. The first dose is equal in magnitude to all succeeding doses, so that $\beta = 0$. The amount of drug administered in the first dose equals $k_0\tau$, and is 19.6 mcg. The number of doses needed to reach the 10.8-mcg./ml. level can be calculated from Eq. 17:

$$n \geq 3.3219\,(10^4)\log_{10}(-1/-0.01) \geq 6.64 \times 10^4 \quad \text{(Eq. 23)}$$

and the time needed to reach the serum concentration of 10.8 mcg./ml. calculated from Eq. 18 is 26.0 hr.

If 200,000 mcg. of lincomycin hydrochloride was injected intravenously in an instantaneous manner into the same subject, and the "continuous" infusion was then begun immediately (1.4 sec. later), how long would it take for the serum concentration of the drug to reach 10.8 mcg./ml.? The value of β can be calculated by substitution into Eq. 10:

$$\beta = \frac{B''/V}{B^{\infty}_{min.}/V} = \frac{(200{,}000 \text{ mcg.} - k_0\tau)/26{,}000 \text{ ml.}}{10.9 \text{ mcg./ml.}} = 0.706 \quad \text{(Eq. 24)}$$

To be mathematically exact in Eq. 24, one maintenance dose, $k_0\tau$, must be subtracted from the 200,000-mcg. initial dose. From a practical standpoint in this example, the $k_0\tau$ term can be ignored because it was made negligibly small compared to the 200,000-mcg. first dose. The α-term remains at 0.99. Substitution of α and β and $t_{0.5}/\tau = 10^4$ into Eqs. 16 and 17 gives a value of n of 4.88×10^4. The time needed for the serum concentration to reach 10.8 mcg./ml. as calculated from Eq. 18 is about 19 hr. Thus, the time needed to approach within −1% of C^{∞} was reduced about 7 hr. when the initial dose was set at a value four times greater than the hourly amount delivered by the infusion apparatus.

[3] The value of k in this report is related to the K found by Boxer *et al.* (4) by the following equation: k = (K ± one standard error) (−2.303) = (−0.17 ± 0.012 hr.$^{-1}$) (−2.303) = 0.39 ± 0.028 hr.$^{-1}$.

[4] This value of C^{∞} has been reached or exceeded in some clinical studies with this drug (10–13).

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Ethylene Oxide Penetration of the Silicone Coating Used as a Lubricant on Disposable Syringe Rubber Plunger Tips and Hypodermic Needles

YU YIN CHEN

Abstract □ The ability of 100% ethylene oxide to penetrate the silicone coating used on disposable hypodermic needles and syringe rubber plunger tips has been determined. It was shown that ethylene oxide has the ability to penetrate through the silicone coating and thus kill the spores of *Bacillus subtilis* var. *niger*, ATCC 9372, which were introduced underneath the silicone coating.

Keyphrases □ Ethylene oxide penetration—silicone lubricant □ Silicone lubricant effect—ethylene oxide bactericidal action □ *Bacillus subtilis*—ethylene oxide penetration determination

With the introduction of medical devices made from comparatively low melting point plastics, the ability to sterilize these instruments by autoclaving was no longer feasible; subsequently, ethylene oxide has been utilized and found effective (without high temperature) as a chemical sterilant.

Recent usage of silicone as a lubricant for disposable hypodermic needles and syringe rubber plunger tips raised the question of whether the ethylene oxide gas can penetrate the silicone coating and sterilize the surface beneath. It was, therefore, the purpose of this study to test the effectiveness of 100% ethylene oxide [4-hr. cycle, 48.8° (120° F.)] as a penetrating sterilant through a silicone coating. A number of preliminary tests were necessary to establish procedures and suitable materials. In the selection of materials it was necessary to obtain a nonbacteriostatic solvent capable of dissolving silicone (to expose the organisms which were covered by it), due to its immiscibility with the water present in the culture medium. Initial tests indicated *n*-hexane (1), purified (Curtin Co.), was an effective solvent. In turn, methanol (Baker analyzed reagent) was added to increase the miscibility of the *n*-hexane. The bacteriostatic properties of *n*-hexane had to be determined to assure that there was no killing factor other than ethylene oxide.

MATERIALS AND METHODS

Bacillus subtilis var. *niger*, ATCC 9372, was selected as the test organism since its spores are known to be highly resistant to ethylene oxide gas [Beeby and Whitehouse (2); Ernst and Shull (3, 4); Kelsey (5); and Phillips (6)]. Sterile trypticase soy broth (TSB) was used as the culture medium (20 ml. per culture tube), and disposable hypodermic syringes (2.5 ml.) and needles [16 gauge × 3.81 cm. (1.5 in.)] were used as the test samples. Commercial silicone fluid M360 (Dow Corning Co.), a colorless, highly water-repelling, nontoxic, nonvolatile, low-surface tension, and chemically thermally inert substance (1) was used as the coating agent.

Spore Strips Treated with *n*-Hexane—To determine whether *n*-hexane had a bacteriostatic effect on *B. subtilis* var. *niger* spores, three different concentrations (10^3, 10^5, and 10^7) of spore strips (American Sterilizer Co.) were immersed in *n*-hexane for 1 hr. These were then divided into two groups. The first group was cultured in TSB; the other was mixed with 1 ml. of 90% methanol (shaken for 20 min. on an automatic shaker) and then cultured in TSB for 48 hr. at 32°.

Vegetative Cells Treated with *n*-Hexane—To determine the bacteriostatic properties of *n*-hexane on vegetative cells of *B. subtilis* var. *niger*, four separate amounts (0.1, 0.2, 0.5, and 1.0 ml.) of *n*-hexane were mixed with 0.5 ml. of 90% methanol and 0.06 ml.

Table I—Effect of *n*-Hexane on Spore Strips of *B. subtilis* var. *niger*

Concentration of Spores	Total No. of Samples	24-hr. Growth	48-hr. Growth
10^3	5 Without methanol	1 Light growth 4 No growth	1 Heavy growth 4 No growth
10^3	5 With methanol	4 Light growth 1 No growth	4 Heavy growth 1 No growth
10^5	5 Without methanol	5 Medium growth	5 Heavy growth
10^5	5 With methanol	5 Heavy growth	5 Heavy growth
10^7	5 Without methanol	5 Heavy growth	5 Heavy growth
10^7	5 With methanol	5 Medium growth	5 Heavy growth