An alternate procedure has been developed for calculations of adrenal gland ascorbic acid based on absorbance readings. Absorbance paper is used in conjunction with a single 8.0-mcg./ml. level of ascorbic acid standard. A computer program is used that provides for the subtraction of recorded absorbance readings of standard and test samples from the absorbance of the color reagent +2.5% metaphosphoric acid base line. The program also provides for the computation of milligrams ascorbic acid per 100 Gm. of paired adrenal glands based on the relative absorbances of the 8-mcg./ml. standard and test samples and on the weights of paired adrenal glands. The computer program places ascorbic acid values in their proper dosage groups and provides for statistical calculations for the assay as directed by U.S.P. XVII.

SUMMARY

An automated procedure is described for the determination of ascorbic acid in rat adrenal gland homogenates in the bioassay of corticotropin. The procedure, based on that of the U.S.P. XVII, permits the analysis of 60 samples/hr. with a coefficient of variation of approximately 1%. Ascorbic acid was recovered satisfactorily from standard samples spiked with varying amounts of adrenal gland homogenate. Corticotropin bioassays using ascorbic acid results obtained with manual and automated procedures compare favorably.

The automated ascorbic acid procedure and the computer program should have application in the Parlow ovarian ascorbic acid depletion bioassay of luteinizing hormone, chorionic gonadotropin, and pregnant mare's serum (2).

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Notes

Estimation of Volume of Distribution and Half-Life of a Compound After Rapid Intravenous Injection

By JOHN G. WAGNER and JACK I. NORTHAM

It is common practice to plot the plasma or serum concentrations of a compound obtained following rapid intravenous injection on semilogarithmic graph paper, then estimate the rate constant ($\hat{\beta}$) for loss of compound from the body or half-life ($0.693/\hat{\beta}$) from the slope of the terminal linear segment, extrapolate the linear segment to time zero, and divide the intercept into the dose to obtain the apparent volume of distribution ($\hat{V}_d$). Under certain conditions the estimates obtained by this procedure may be valid. However, this report shows that if the model which applies is a two compartment open system, then the $\hat{V}_d$ estimated by this method is always an overestimate of the true total volume ($V_1 + V_2$), and the error depends upon the relative values of V_1/V_2 and K_1/K_2 where K_1 is the first-order rate constant for distribution and K_2 is the first-order rate constant for loss from the inner compartment. The half-life estimates ($0.693/\hat{\beta}$) will always be greater than the true half-life ($0.693/K_2$), and $\hat{\beta}$ as an estimator of K_2 also depends on the ratios V_1/V_2 and K_1/K_2.

IT IS COMMON practice[1] (1–5) to plot the plasma or serum concentration of a compound obtained following rapid intravenous injection on semilogarithmic graph paper, then estimate the rate constant for loss of compound from the body ($\hat{\beta}$) or the half-life ($0.693/\hat{\beta}$) from the slope ($\hat{\beta}/2.303$) of the terminal linear segment; and extrapolate the linear segment to time zero and divide the intercept ($\hat{B}_1$) into the dose (D) to obtain the apparent volume of distribution, pool size, or "space" ($\hat{V}_d$). If the model which applies to the particular system involves a single compartment and the initial nonlinearity of the semilogarithmic plot is assumed to be due to mixing, then the estimates $\hat{\beta}$ and $\hat{V}_d$ would be very close to those expected on the basis of the appropriate mathematical expression.

Nelson (6), however, pointed out that the error in the estimated volume of distribution may be substantial if there are two compartments with volumes V_1 and V_2 and the volume of the outer compartment (V_2) is large, and rate of attainment of equilibrium is slow. This report will show that if the model which applies is a two compartment open system, then $\hat{V}_d$ always overestimates the true total volume

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[1] The practice is so common that the authors considered it unnecessary to provide an extensive bibliography. However, *References 1–5* are a representative sample.

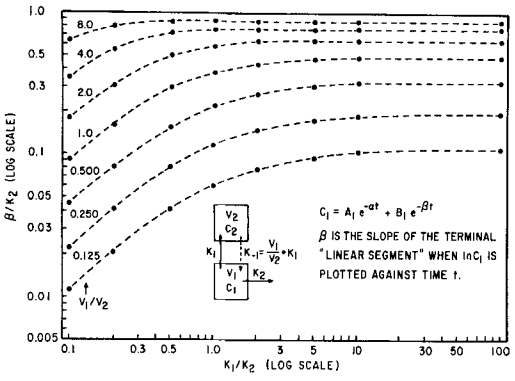

Fig. 1—Plot of the ratio β/K_2 versus *the ratio* K_1/K_2 *on a log-log scale.*

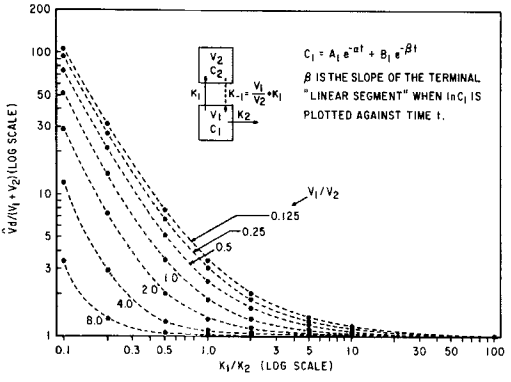

Fig. 2—Plot of the ratio $\hat{V}_d/(V_1 + V_2)$ versus *the ratio* K_1/K_2 *on a log-log scale.*

$(V_1 + V_2)$, and the error depends upon the relative values of V_1/V_2 and K_1/K_2, where K_1 is the first-order rate constant for distribution and K_2 is the first-order rate constant for loss from the inner compartment. That is, the errors involved in $\hat{V}_d$ as an estimator of $V_1 + V_2$ and $\hat{\beta}$ as estimator of K_2 depend on the ratios V_1/V_2 and K_1/K_2. If a compound is widely distributed in body water, then physiologically (7, 8) one might expect V_2 to be considerably greater than V_1; hence, $V_1/V_2 < 1$ and $\hat{V}_d$ will be a poor overestimator of $V_1 + V_2$ and $\hat{\beta}$ will be a poor underestimator of K_2.

The equations appropriate to the two compartment open system in which drug is lost only from the inner compartment are given under *Appendix* where all symbols are defined. Based on Eqs. 13 through 16 of the *Appendix*, a digital computer program was written which allowed print-out of the values β/K_2, α/K, and $\hat{V}_d/(V_1 + V_2)$ when the input data were V_1/V_2 and K_1/K_2. Values of β/K_2, α/K_1, and $\hat{V}_d/(V_1 + V_2)$ were obtained corresponding to $K_1/K_2 = 0.1$, 0.5, 1, 2, 5, 10, and 100 and $V_1/V_2 = 0.125$, 0.25, 0.5, 1, 2, 4, and 8. These data allowed preparation of Figs. 1 and 2.

Figure 1 is a plot of the ratio β/K_2 *versus* the ratio K_1/K_2 on a log-log scale. The figure illustrates that over the range considered β/K_2 ranges from 0.0113 when $K_1/K_2 = 0.1$ and $V_1/V_2 = 0.125$ to 0.889 when $K_1/K_2 = 100$ and $V_1/V_2 = 8$. As $V_1/V_2 \to \infty$, $\beta/K_2 \to 1$. Hence, half-life estimates $(0.693/\hat{\beta})$ will always be greater than the true half-life $(0.693/K_2)$.

Figure 2 is a plot of the ratio $\hat{V}_d/(V_1 + V_2)$ *versus* the ratio K_1/K_2 on a log-log scale. The figure illustrates that when $K_1/K_2 < 5$ and $V_1/V_2 < 1$ relatively large errors are made in the estimated volume. When $K_1/K_2 \gtrsim 100$, then V_d is within 1% of $(V_1 + V_2)$, provided V_1/V_2 is not extremely small.

When employing experimental data, estimates $\hat{B}_1$ and $\hat{\beta}$ of the intercept B_1 and the slope β, respectively, are usually obtained by the method of least squares using the terminal ln C_1 and time t values.[2] Table I gives an indication of the relatively small errors involved in this procedure in the absence of experimental error. In the case of some experimental data, however, the number of available points and the accuracy and precision of the assay would influence the estimates.

Additional observations about the two compartment open system may be of interest. During the interval $t < t'$, $C_1 > C_2$ but during the interval $t' < t < \infty$, $C_2 > C_1$ where $t' = \ln(\beta/\alpha)/(\beta - \alpha)$. When $10 < K_1/K_2 < 100$ and $t > t'$, C_2 is always only slightly greater than C_1 and for all practical purposes $C_1 \simeq C_2$. For a given ratio K_1/K_2 the smaller the ratio V_1/V_2 the more delayed is t'; for example, with $K_1/K_2 = 1$, $t' = 7.45$, 9.84, 11.4, and 13.4 hr. when $V_1/V_2 = 1$, 0.5, 0.33, and 0.2, respectively. For given values of V_1 and V_2 the smaller the ratio K_1/K_2, the more delayed is the time at which a semilogarithmic plot of C_1 *versus* time apparently becomes linear. Conversely, when $K_1/K_2 \geq 100$ a semilogarithmic plot of C_1 *versus* time becomes linear within an hour or less postadministration if the true half-life $(0.693/K_2)$ is 6 hr. or less.

It should be noted that if a compound is administered by any other route than intravenously (*e.g.*, orally, intramuscularly, *etc.*) and the terminal linear segment of the semilogarithmic plot of the serum or plasma concentration is extrapolated to zero time, the quotient obtained by dividing the intercept into the dose is not a "volume of distribution." The number obtained in such a case is a complex function of many variables among which are the following: (*a*) the true volume of distribution, (*b*) *all* the rate constants involved in the model which applies to the particular set of data, (*c*) the fraction of the dose absorbed, and (*d*) the lag time between time of administration and the time when absorption begins.

APPENDIX

The Two Compartment Open System with the Dose (D) Introduced into Compartment 1 at Zero Time.—Let V_1 be the volume and C_1 be the concentration of unchanged drug at time t in the inner compartment or pool and V_2 be the volume and C_2 be the concentration of unchanged drug at time t in the outer compartment or pool. Let K_1 be a first-order rate constant representing the instantaneous fraction of drug in compartment 1 being transferred to compartment 2 and K_2 be a first-order rate constant representing the instantaneous fraction of drug in compartment 1 which is being lost

[2] This approximation is valid since

$$-\beta = \lim_{t \to \infty} \left\{ \frac{d \ln C_1(t)}{dt} \right\}$$

TABLE I—INDICATION OF THE SMALL ERRORS INVOLVED WHEN THE INTERCEPT B_1 AND THE SLOPE β ARE ESTIMATED BY THE METHOD OF LEAST SQUARES USING THE TERMINAL ln C_1, t VALUES[a]

K_1/K_2	Time Range, hr.	B_1	$\hat{B}_1$[b]	β	$\hat{\beta}$	$\hat{V}_d$ $100/B_1$	$\hat{V}_d$ $100/\hat{B}_1$
1	17–24	.00553	.00609	.0441	.0482	18,100	16,400
2	13–24	.00758	.00760	.0506	.0508	13,200	13,150
5	11–24	.00901	.00899	.0549	.0548	11,100	11,120
10	9–24	.00950	.00950	.0563	.0563	10,530	10,530
100	1–24	.00995	.00995	.0576	.0576	10,050	10,050

[a] For all examples shown $V_1 = V_2 = 5{,}000$, $K_2 = 0.1155$, and a dose of 100 units was placed in compartment 1 at time zero. [b] $\hat{\beta}_1$ is the antilogarithm of the estimated intercept and $\hat{\beta}$ is the estimated slope obtained by the method of least squares for the regression of ln C_1 on time where $C_1 = A_1e^{-\alpha t} + B_1e^{-\beta t}$, and t was taken at 0.5-hr. intervals over the time range indicated.

from compartment 1. For convenience refer to the compartmental diagram shown in Fig. 1. Let D be the dose of drug introduced into the inner compartment at time zero.

The appropriate differential equations, which incorporate Fick's law, are:

$$V_1 \frac{dC_1}{dt} = -V_1K_1 (C_1 - C_2) - V_1K_2C_1 \quad \text{(Eq. 1)}$$

and

$$V_2 \frac{dC_2}{dt} = V_1K_1 (C_1 - C_2) \quad \text{(Eq. 2)}$$

The time-concentration curves resulting from the solution are:

$$C_1 = A_1e^{-\alpha t} + B_1e^{-\beta t} \quad \text{where } \beta < \alpha \quad \text{(Eq. 3)}$$

and

$$C_2 = A_2e^{-\alpha t} + B_2e^{-\beta t} \quad \text{(Eq. 4)}$$

A semilogarithmic (base e) plot of C_1 *versus* t is terminally linear with slope-β and intercept ln B_1 (by extrapolation). The residuals give a linear semilogarithmic plot with slope-α and intercept ln A_1. It follows that $(C_1)_{t=0} = A_1 + B_1$.

In Eq. 3,

$$A_1 = \frac{(K_1 + K_2 - \beta)(C_1)_{t=0}}{\alpha - \beta} \quad \text{(Eq. 5)}$$

and

$$B_1 = \frac{(K_1 + K_2 - \alpha)(C_1)_{t=0}}{\beta - \alpha} \quad \text{(Eq. 6)}$$

In Eq. 4,

$$A_2,B_2 = \pm \frac{K_1V_1(C_1)_{t=0}}{(\beta - \alpha)V_2} \quad \text{(Eq. 7)}$$

By appropriate algebraic manipulation we find

$$K_1 = \frac{A_1\alpha + B_1\beta}{A_1 + B_1} \quad \text{(Eq. 8)}$$

$$K_2 = \frac{\alpha\beta(A_1 + B_1)}{A_1\beta + B_1\alpha} \quad \text{(Eq. 9)}$$

$$V_1 = \frac{D}{A_1 + B_1} \quad \text{(Eq. 10)}$$

$$V_2 = V_1 \cdot \frac{K_1K_2}{\alpha \cdot \beta} \quad \text{(Eq. 11)}$$

$$C_1 = C_2 \text{ and } C_2 = \text{a maximum at } t' = \frac{\ln \beta - \ln \alpha}{\beta - \alpha} \quad \text{(Eq. 12)}$$

Equations 1 through 11 have been reported in similar or modified form by Teorell (9), Gaudino (10), Dominguez (11), Tait *et al.* (12), and Cornfield *et al.* (13).

In preparing Figs. 1 and 2 the following equations were employed:

$$\beta/K_2 = \left[1 + \frac{K}{f} - \sqrt{\left(1 - \frac{K}{f}\right)^2 + 4K}\right]/2 \quad \text{(Eq. 13)}$$

where $K = K_1/K_2$, $V = V_1/V_2$, and $f = V_2/(V_1 + V_2) = \frac{1}{V+1}$.

$$\frac{\hat{V}_d}{V_1 + V_2} = (1 - f) \cdot \left[\frac{\alpha/K_2 - \beta/K_2}{\alpha/K_2 - K - 1}\right] \quad \text{(Eq. 14)}$$

The latter is conveniently calculated since, when getting β/K_2, one can easily calculate:

$$\alpha/K_2 = \left[1 + \frac{K}{f} + \sqrt{\left(1 - \frac{K}{f}\right)^2 + 4K}\right]/2 \quad \text{(Eq. 15)}$$

In addition one can calculate α/K_1 as follows:

$$\alpha/K_1 = (V_1/V_2)/(\beta/K_2) = V/(\beta/K_2) \quad \text{(Eq. 16)}$$

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