Some Possible Errors in the Plotting and Interpretation of Semilogarithmic Plots of Blood Level and Urinary Excretion Data

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Semilogarithmic plots of amount (or per cent) not excreted against time will be curved either if (a) the wrong asymptote is used, or (b) absorption is still proceeding during the interval observations are made. Factor (a) causes "convex decreasing" type of curvature, whereas factor (b) causes "concave decreasing" type of curvature. If both factors are operative, it is possible to obtain an apparently linear semilogarithmic plot; however, the rate constant estimated from the linear segment is meaningless. Models and actual data from the literature are used to illustrate these points. Using a simple model, it is shown that rate constants estimated from terminal segments of semilogarithmic plots of amount not excreted or of blood levels may be appreciably lower than the true values. The implications of these factors in making in vitro–in vivo correlations are discussed.

VAN LIEW (1) indicated that concavity[1] of a semilogarithmic curve may result from operation of one of four types of systems. Application of the "backward projection" technique to "convex decreasing" semilogarithmic plots of blood level and urinary excretion data can lead to linear components from which rate constants between assumed independent compartments may be estimated. Van Liew showed that another interpretation may be the operation of a continuum of exponential processes.

Assuming the independent compartment model applies to blood level and urinary excretion data, this report will discuss (*a*) other causes of curvature of semilogarithmic plots, (*b*) possible errors in plotting and interpreting such data, and (*c*) the implications of these factors in making *in vitro–in vivo* correlations.

Several types of semilogarithmic plots are commonly utilized. In these, time is plotted as the abscissa and the ordinates plotted on the logarithmic scale are (**A**) urinary excretion rate (amount/unit time or per cent of dose/unit time), (**B**) amount (or per cent) not excreted, and (**C**) blood (serum or plasma) concentration. Data plotted as the ordinate of Cartesian coordinate paper frequently are (**D**) cumulative amount or per cent of the dose excreted, (**E**) urinary excretion rate, and (**F**) blood (serum or plasma) concentration; again, time is plotted as the abscissa in each of these. On the semilogarithmic plots, curvature of the type discussed by Van Liew (1) is termed "convex decreasing" (analogous to Curve A in Fig. 3) to distinguish it from another type of curvature termed "concave decreasing" (analogous to segments AB in Figs. 1 and 2).

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[1] The terminology used in this report would describe the type of curvature Van Liew discussed as "convex decreasing." See Fig. 12 for the descriptions.

EXPERIMENTAL

Consider Model I:

$$X \xrightarrow[(0.180\,\text{hr.}^{-1})]{k_1} Y \xrightarrow[(0.090\,\text{hr.}^{-1})]{k_2} Z$$

Let us assume that (*a*) the rate constants, k_1 and k_2, are first-order rate constants and have the values shown; (*b*) 100% of the "drug" is initially in compartment X at time, $t = 0$; (*c*) the volumes of compartments X, Y, and Z are equal. This is an idealized model for first-order absorption and first-order elimination if X is considered to be the reservoir of "drug" at the absorption sites, Y is drug in the volume of distribution, and Z is drug lost by various processes from the volume of distribution. Figure 1 is a semilogarithmic plot of per cent of "drug" in Y against time based on the above model; it is analogous to type (**C**) plot, above. The plot has pronounced "concave increasing" curvature during the interval when most of the drug is leaving X. Although the curve is actually continuous, only some of the values have been calculated and the points joined; this is the case in other examples shown too. At point A only 13.8% and at point B only 2.28% of the "drug" is left in X. It would be easy to interpret segment AB as a straight line, especially if the assay error for the drug were relatively large as is often true with blood assays. The segment BC appears linear even with the idealized values obtained from the model. Estimation of k_2 from the segment BC gave a value of 0.080 hours^{-1} which is 11% lower than the actual value of 0.090 hours^{-1}.

Using Model I, a type (**B**) plot is shown as Curve I in Fig. 2. The per cent of "drug" not in compartment Z would be analogous to per cent of drug not excreted if all the drug initially in X were excreted unchanged in the urine, Z. Note that Curve I is "concave decreasing" from A to B. For times beyond B the plot appears linear. Estimation of k_2 from the segment BC gave a value of 0.0866 hours^{-1} which is 3.8% lower than the actual value of 0.0900 hours^{-1}. This example indicates that estimation of k_2 from "urinary data" (sampling in Z) is more accurate than estimation of k_2 from "blood level data" (sampling in Y) in the same time interval. In actual practice the higher concentrations of drug in urine than in blood and the greater accuracy and precision of urine assays compared with blood assays also contribute to greater accuracy from urine data than blood data.

When constructing type (**B**) plots, values of ($Ae°$-Ae) must be calculated where $Ae°$ is the amount of drug excreted in the urine at "infinite time" (about ten half-lives is usually an adequate collection time). The ($Ae°$-Ae) values or the percentages $Ae°$-$Ae/Ae° \times 100$, are plotted on the logarithmic axis. $Ae°$ is called the asymptote of the first-order plot. Some authors incorrectly use the administered dose for the value of $Ae°$ when they do not show experimentally that the dose is excreted completely in the urine. An example of use of such an incorrect asymptote is shown as Curve II in Fig. 2. Curve II was constructed by assuming that the "dose" initially in X of Model I was 125 mg. but that only 100 mg. of the intact "drug" appeared at infinite time in Z; however, rather than using the correct value for $Ae°$, namely 100 mg. as in Curve I, the incorrect value of 125 mg. (the "dose") was utilized to calculate the percentages. Curve II is "concave decreasing" from D to E but becomes apparently linear from E to F; from F to G the curve is "convex decreasing." One could interpret that in such a plot the segment EF represents first-

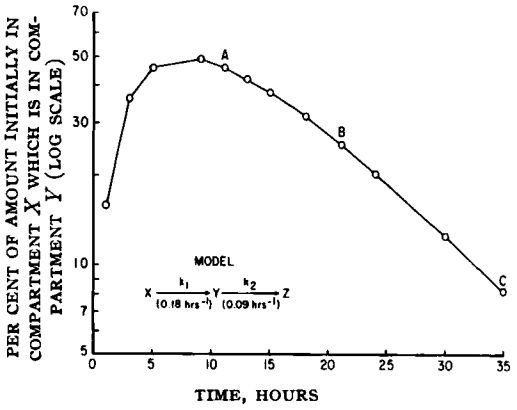

Fig. 1.—Semilogarithmic plot of per cent of "drug" in Y against time based on Model I.

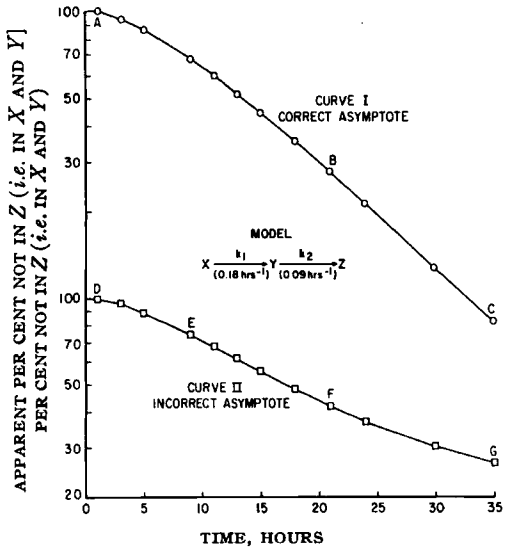

Fig. 2.—Semilogarithmic plot of per cent (-o-) and apparent per cent (-□-) not in z against time based on model I.

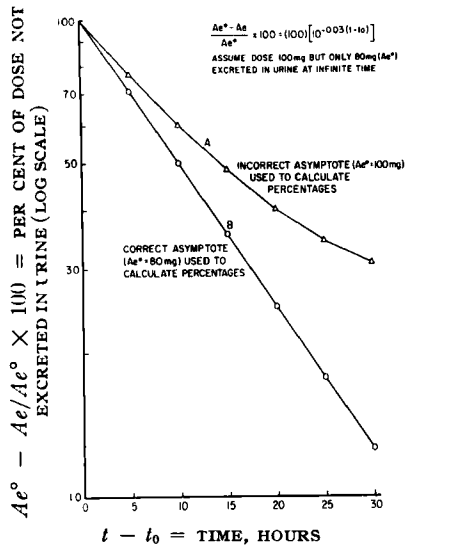

Fig. 3.—Semilogarithmic plot of Eq. 1; k = 0.03 for both Curves A and B.

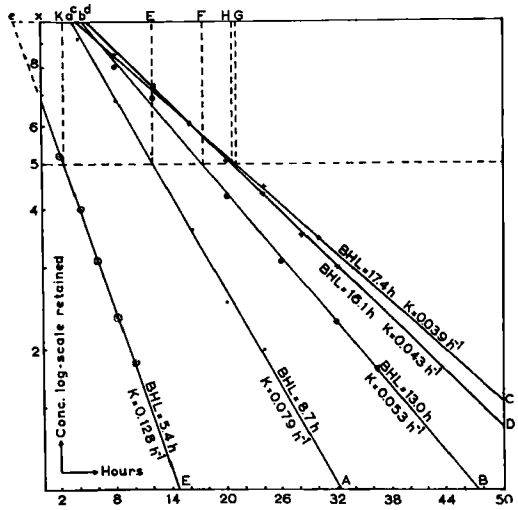

Fig. 4.—Reproduction of Fig. 31 of Simoons (2).

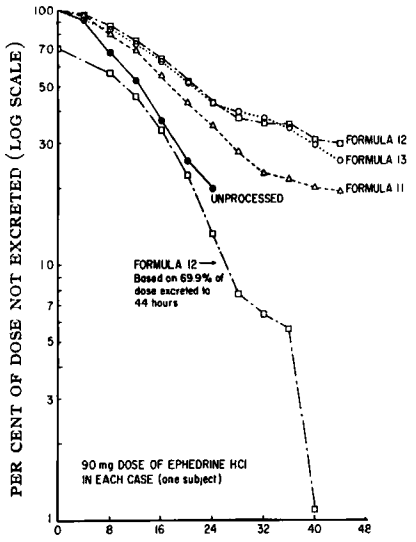

Fig. 5.—Tabular data (2) of Fig. 4 replotted.

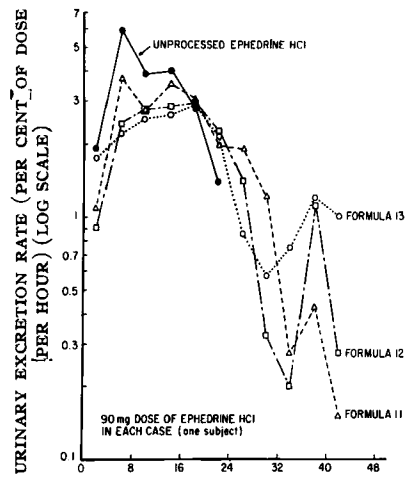

Fig. 6.—Type (A) plots derived from data in Figs. 4 and 5.

order loss of drug from the body or appearance in the urine, particularly if little or no data were collected beyond point F. The rate constant estimated from segment EF is 0.0481 hours^{-1} which is approximately one-quarter and one-half of the actual values of k_1 and k_2, respectively. The rate constant derived from segment EF is purely "artificial" and bears no real relationship to either of the rate constants k_1 and k_2. In this example the effect of "absorption still proceeding" (*i.e.*, transfer of significant amounts of "drug" from X to Y), which causes "concave decreasing" curvature,[2] and the effect of using the wrong asymptote, which causes "convex decreasing" curvature, cancel each other. The net effect is an apparently linear segment which is completely artificial.

Consider Eq. 1

$$\frac{Ae^\circ - Ae}{Ae^\circ} \times 100 = (100)\{10^{-k(t-t_0)}\} \quad \text{(Eq. 1)}$$

This equation, according to theory, should fit urinary excretion data in two situations: (*a*) where t_0 is the end of the distribution-tissue equilibration phase following intravenous administration of the drug, or (*b*) where t_0 is the end of the absorption and tissue equilibration phase following oral administration and where $t > t_0$ in both cases. Figure 3 is a semilogarithmic plot of Eq. 1 with k equal to 0.03 for both Curves A and B. Assume the dose is 100 mg. but that only 80 mg. of drug appears in the urine in infinite time. Curve B was constructed using the correct asymptote, namely $Ae^\circ = 80$; the line is straight and has a slope of -0.03. Curve A was constructed using Ae° = dose = 100. Note that Curve A is "convex decreasing." Rate constants estimated from the points between 0 and 15 hours or between 15 and 30 hours would be greatly in error.

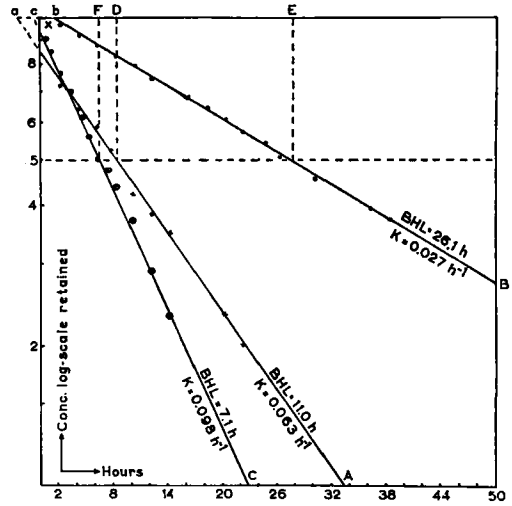

Fig. 7.—Reproduction of Fig. 32 of Simoons (2).

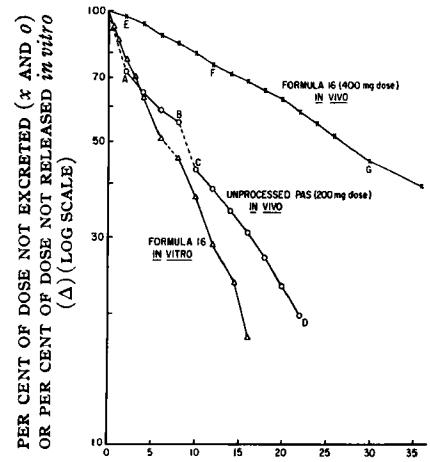

Fig. 8.—Tabular data of Fig. 7. replotted.

[2] "Concave decreasing" curvature may also be observed if the drug elimination process is zero order (rather than first order) and the blood concentration is plotted against time on semilogarithmic paper. In such a case, replotting the data on Cartesian coordinate paper should yield a linear plot of blood level against time.

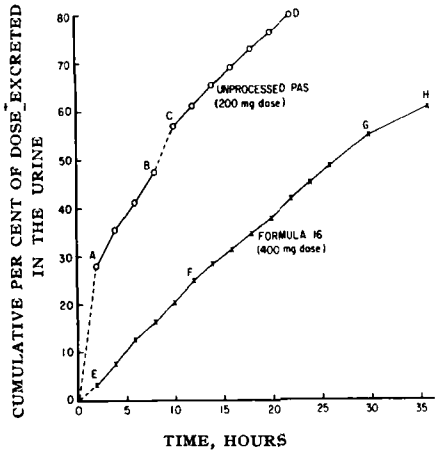

Fig. 9.—Type (**D**) plots derived from data in Figs. 7 and 8.

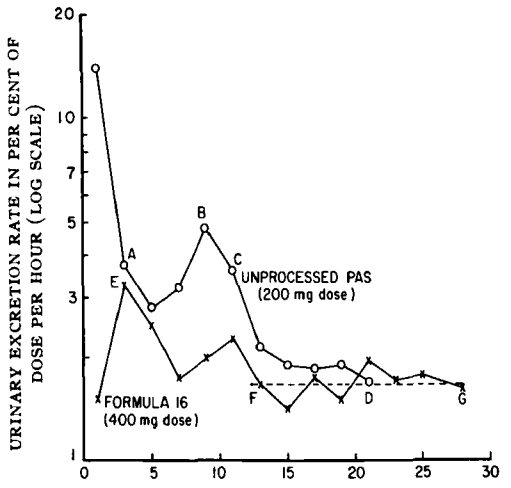

Fig. 10.—Type (**A**) plots for data shown in Figs. 7, 8, and 9.

Practical examples of the operation of some of the factors discussed above are found in the plots and data of Simoons (2). At different times a human subject was administered 90-mg. doses of ephedrine hydrochloride as unprocessed drug and in the form of three different sustained-release formulations. Urine was collected at regular intervals and analyzed for drug content. Formula 13 was tested *in vitro* using a new apparatus.[3] Figure 4 is a reproduction of Fig. 31 of Simoons (2). The tabular data (2) on which Fig. 4 was based were replotted and are shown in Fig. 5. In Fig. 5 the data points were merely joined, whereas in Fig. 4 straight lines were drawn through the points and extrapolated; data points have been left off the extrapolated regions of Fig. 4. The situation in Fig. 5 is analogous to Curve II in Fig. 2. No meaningful rate constant(s) can be calculated from any of the plots shown in Fig. 5. Also, since Ae° was not determined in any of the experiments (since urine was not collected long enough), a type (**D**) plot (such as Figs. 4 and 5) should not be made at all for data. Type (**A**) plots, derived from the same data, are shown in Fig. 6. The excretion rates are plotted against the midpoints of the urinary collection intervals over which the rates were calculated. Simoons (2) and Cummings and Martin (3) plotted the rates against the times at the end of the collection intervals; this distorts the plots and leads to erroneous conclusions. The peculiar curvature at the tail ends of the plots shown in Fig. 6 are not readily explained but may be because of continued irregular absorption and/or a recycling phenomenon. Although Fig. 6 is a "valid" plot for these data, it is obvious that no meaningful rate constants can be estimated.

Another example is taken from Simoons (2). At different times a human subject was administered 200 mg. of unprocessed *p*-aminosalicylic acid (PAS) and 400 mg. of PAS in the form of ten sustained-release tablets (formula 16). The latter were also tested *in vitro* using the new apparatus.[3] Figures 7–11 are derived from the same data given by Simoons (2). Figure 7 is a reproduction of Fig. 32 of Simoons (2). The tabular data (2), on which Fig. 7 were based, were replotted and are shown in Fig. 8. In Fig. 8 the data points were merely joined, whereas in Fig. 7 straight lines were drawn through the points. Inspection of the plot of the *in vitro* data in Fig. 8 indicates there are two essentially linear segments, one between 0.5 and 6 hours, the other between 8 and 16 hours, with a discontinuity between them from 6 to 8 hours; this is a different interpretation than the single linear plot shown in Fig. 7. Inspection of the plots of the *in vivo* data in Fig. 8 indicates curvature in the segments EF, FG, AB, and CD, with a discontinuity from B to C. No meaningful rate constants can be estimated from these *in vivo* data as was done by Simoons in Fig. 7. Again, since Ae° was not determined in either of the *in vivo* experiments, type **B** plots (such as Figs. 7 and 8) should not be made at all for these data. The type **D** plots for these data, shown in Fig. 9, support this statement; at times D and H, when urine collection ceased, excretion of drug in the urine was incomplete, but one could not predict that the entire dose (100%) would be excreted. Note that the segmentation of the plots shown in Fig. 8 is also apparent in Fig. 9. A type **A** plot is more "sensitive" than either types **B** or **D**. The type **A** plots for the same data are shown in Fig. 10. These plots show double peaks indicative of a recycling phenomenon. The plot for the unprocessed PAS did not become linear during the observation period; hence, no rate constant for loss of drug from the body can be calculated. Formula 16 gave an essentially constant excretion rate averaging 1.67% of dose/hour or 6.68 mg. of PAS/-hour in the 13 to 28-hour interval. This is a considerably different interpretation than the first-order rate constant of 0.027 hours^{-1} shown on Fig. 7. The variability about the mean rate in the 13- to 28-hour interval is exaggerated due to the logarithmic scale in Fig. 10; this variability is not nearly so evident in the type (**E**) plot of the same data shown in Fig. 11. Figure 11 has a considerably different appearance than Fig. 25 of Simoons (2), yet both were derived from the same tabular data.

[3] The apparatus is described in Reference 2. A commercial model, the Erweka Tester Type AT-3, is distributed by the Chemical and Pharmaceutical Industry Co., Inc., New York, N. Y.

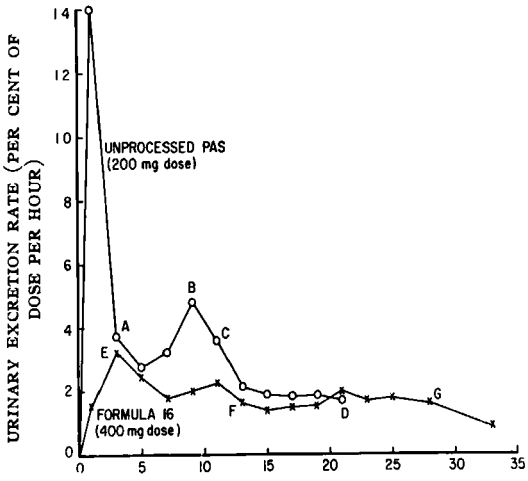

Fig. 11.—Type (E) plots derived from tabular data of Fig. 25 of Simoons (2).

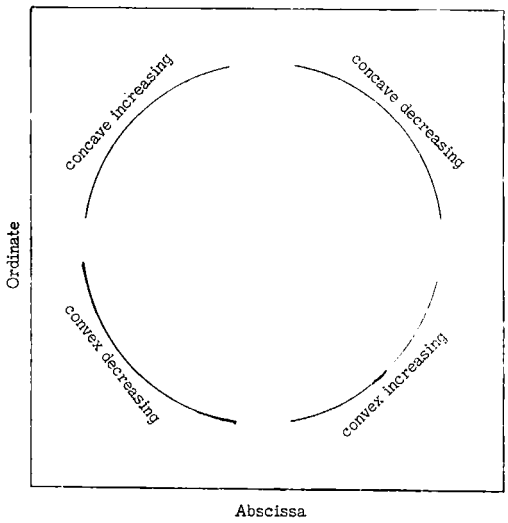

Fig. 12.—Description of concavity of semilogarithmic curve. The definitions assigned to the curve are those of James and James (6).

DISCUSSION

Given a set of urinary excretion data for interpretation, one should first make two types of plots: (*a*) a plot of cumulative amount excreted to time *t* against time *t*, and (*b*) a semilogarithmic plot of excretion rate (amount/unit time) against the midpoints of the urinary collection intervals.[4] If the first plot indicates an asymptote was reached, then (*c*) a semilogarithmic plot of amount not excreted against time should also be made. Theory (4) predicts that plots (*b*) and (*c*) should have terminal linear segments and that the rate constant derived from these linear segments should be same and equal to the rate constant for loss of drug from the volume of distribution. Care should be used to insure that the segments used do not show curvature and that "artifacts" are not produced by using the wrong asymptote in the third type of plot. Comparison of the three types of plots prepared from the same set of data allows more accurate interpretation and permits comparison of different segments as has been done in the figures in this report. Even in the absence of evidence of curvature in the terminal segments of the semilogarithmic plots one should be aware that the most likely situation is that the estimated rate constant will be less (the half-life greater) than the true value. This is also true of semilogarithmic plots of blood level against time.

The rate constant for clearance (or biological half-life) of a drug in the body is independent of dosage form effects if the data are properly collected and interpreted. Apparent deviation from this independence is purely an artifact due to inadequate data or misinterpretation of data. It is the author's experience that the rate constant for clearance (or biological half-life) of a drug in the body is almost always independent of the route of administration in a given species if the data are properly collected and interpreted. However, there are physiologic reasons for differences with some drugs.

The magnitude and duration of blood levels and urinary excretion of a drug can be markedly altered by dosage form effects, as many investigators have shown (5). An interesting and necessary area of research is to try to correlate *in vivo* and *in vitro* results obtained with different dosage forms. This research is "interesting" since correlation of *in vivo* results with physical constants such as rate of dissolution, solubility, and partition coefficient may someday lead to the ability to predict behavior of new drugs and old drugs in new dosage forms or at minimum reduce the number of biological experiments necessary and aid in experimental designs. Such research may someday be necessary for adequate control of pharmaceutical products, but we do not have enough knowledge today to apply such criteria adequately and rigorously.

It is obvious from the experimental part of this report that the apparent correlations of *in vitro* and *in vivo* results of Simoons (2) were only apparent and not real. In fact, it is completely spurious to correlate rate constants derived from *in vitro* dissolution tests with rate constants for clearance (or biological half-lives) of the drugs derived from blood level and urinary excretion data. If dissolution of the drug from its dosage form *in vivo* partly, or completely, rate limits its absorption, then the "correlation" should involve the use of blood level or urinary excretion data in the time interval shortly after administration. However, it is necessary to know the rate constant for clearance to perform the calculations (4, 5). Quantitative correlations are needed in this research area. The author trusts this report illuminates some of the pitfalls.

[4] If the cumulative curve is fitted by a polynomial and the derivatives estimated at the data points, then the excretion rate (the derivative) should be plotted against the time at the end of the collection intervals.

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