Use of computers in pharmacokinetics

Analogue, digital, and hybrid computers may be used in two principal ways in the analysis of data from pharmacokinetic studies: (1) for rapid numerical analysis of data and (2) for pharmacokinetic simulation. Simulation may be defined as the act of building a model of a system and observing its performance. In pharmacokinetics, the equations and models elaborated are always oversimplifications and their appropriateness can only be judged by their ability to describe the observed data and the accuracy with which they make predictions of future observed data. Five examples are employed to illustrate the use of computers; the paths from the original observed data to the final answers are shown in detail. The examples are as follows: (1) the relationship between the area under the lincomycin serum concentration curve and the dose of lincomycin administered intramuscularly; (2) the distribution of the half-lives of the antibiotics lincomycin and novobiocin; (3) prediction of multiple dose serum levels of lincomycin observed after constant rate intravenous infusions with an analogue computer; (4) the fitting of serum levels of tetracycline observed after a single oral dose with a two-term exponential equation; (5) simulation of serum levels produced by depo-intramuscular preparations of lincomycin with a digital computer.

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Analogue, digital, and hybrid computers may be used in two principal ways in the analysis of data from pharmacokinetic studies: (1) for rapid numerical analysis of data and (2) for pharmacokinetic simulation. Simulation may be defined as the act of building a model of a system and observing its performance. A pharmacokinetic simulation is usually a physical embodiment of a mathematical model, although it is possible for the mathematical model to be unknown or unknowable prior to the development of the simulation.

When applying the methods of kinetics to data derived from blood level and urinary excretion studies in man, interest is in how fast the drug enters the system, i.e., in the rate of absorption, and in the efficiency of absorption. Furthermore, how fast the drug is metabolized and how fast the unchanged drug and metabolites are excreted are also of interest. Sometimes the all-over rate of loss of drug from the body can be determined from either blood level or urinary excretion data. The blood level at various times is a resultant of how fast the drug was absorbed and how fast the drug was lost from the body.

Fig. 1 is a naive approach but is instructive in explaining the kinetic approach. Consider three vessels in series. The top one represents the drug in the gastrointes-

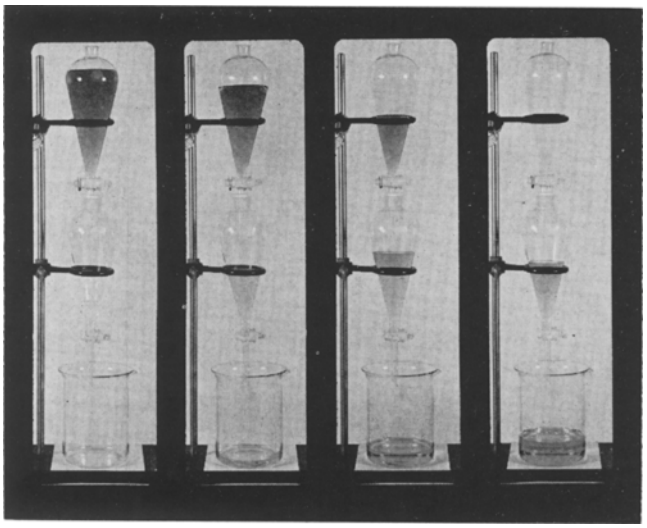

Fig. 1.

tinal tract. The middle one represents drug in the body or apparent volume of distribution of the drug. The bottom one represents the metabolites and unchanged drug excreted. If the instantaneous rate of flow from the top vessel to the middle vessel is more rapid than the instantaneous rate of flow out of the middle vessel to the bottom vessel, then the fluid level, analogous to the blood level of drug, will build up gradually in the middle vessel. Eventually a maximum level is reached in the middle vessel. When all the drug has left the absorption site or top vessel, the blood level (i.e., the middle vessel level) will gradually decrease with time. In the human body this latter phase is usually exponential relative to time.

Why try to fit data with equations and derive kinetic models? (1) It helps to summarize observed data. For example, one number such as biological half-life will replace a whole series of observed blood levels. (2) It increases understanding of the process or processes involved. (3) One can make predictions such as the prediction of multiple-dose blood levels from single-dose blood level data or predicted areas under the single-dose blood level curve and predicted average equilibrium state blood levels for doses which were not administered clinically. (4) Comparisons may be made of several drugs with similar pharmacologic action. (5) One can sometimes quantitatively relate the biological activity or response to the concentrations of unchanged drug or metabolites in the blood.

An analogue computer is primarily a device for solving differential equations, using time as the independent variable, and for plotting the dependent variable as a continuous function of time. The analogue computer uses voltage values to represent variables. Analogue computers are used two ways in pharmacokinetics. (1) They are used to plot the dependent variable continuously as a function of time when the analogue computer is programmed on the basis of an equation with known parameters. The lincomycin intravenous studies given later are examples. Used in this way an analogue computer offers no particular advantage over a digital computer which is equipped with an automatic curve-plotting device. The lincomycin intramuscular example later will show one can perform simulations with a digital computer also. (2) Analogue computers are used to build compartment models by curve-fitting methods. The experimenter has one or more sets of discrete observations such as blood levels measured at dif-

ferent times. He starts with a preconceived compartment model and has the computer generate a curve which is compared to the observed points. He then adds or deletes compartments, changes the values of the rate constants and volumes, and so forth until he generates a curve which smoothly fits the observed points. He then looks at the dials of the analogue computer when a match is attained and writes the final compartment model corresponding to the dial readings and connections made. Such model building must be guided by known physiology, pharmacology, and all the information available from absorption, metabolism, and excretion studies. The greatest problem with this type of model building is that the models built are not unique and it is difficult to decide which of several possible models is the correct one. Usually the studies necessary to resolve the problem are impossible to perform in man, although, frequently, comparable studies may be performed in animals.

A digital computer deals with discrete observations expressed as integers or binary numbers in the memory of the computer. It is capable of addition, subtraction, multiplication, and division with amazing speed, hence it is excellent for rapid numerical analysis of data. Digital computers may be employed for pharmacokinetic simulations also, as indicated previously. The usefulness of digital computers in pharmacokinetics is indicated in several of the examples below.

Hybrid computers have both analogue and digital components. They have a great potential in the analysis of pharmacokinetic data but, to my knowledge, there has been no publication to date concerning the application of a hybrid computer to a pharmacokinetic problem.

There have been a number of papers on application of the analogue computer and the digital computer to pharmacokinetic problems. Rather than review some or all of these applications, I thought it would be more instructive to take a few examples from my own work, present the raw data, and show in detail the path leading to the final answers. I wish to emphasize that for the particular examples, the path chosen and the methods employed are not the only approaches which could have been applied. In fact, in pharmacokinetics, the equations and models elaborated are always oversimplifications. The appropriateness of them can be judged only by their ability to describe the observed data and the accuracy with which they make predictions of future observed data.

Relationship between area under serum concentration curve and dose administered

The raw data for this example consisted of 470 serum concentrations of the antibiotic, lincomycin, (microbiological assay), observed in 60 human volunteers after intramuscular administration of the commercial preparation in sterile solution,* 300 mg. per cubic centimeter. The studies are summarized in Table I.

*Lincocin.

Table I. *Summary of intramuscular lincomycin studies*

Study No.	*No. of subjects*	*Dose (mg.)*	*Time in hours blood samples taken*														
			0	*¼*	*½*	*1*	*1½*	*2*	*3*	*4*	*6*	*8*	*12*	*14*	*17*	*20*	*24*
A	10	100	X			X		X		X	X	X					
B1	10	200	X			X		X		X	X	X	X				
B2	10	200	X			X		X		X	X	X	X				
C1	10	600	X					X	X	X			X	X	X	X	X
C2	10	600	X			X		X		X			X	X	X		X
C3	10	600	X	X	X	X	X	X		X		X	X				X

The problem posed is how to relate the results of the six studies and obtain some additional information not evident from the analyses of the data of the individual studies. A material balance equation, useful in pharmacokinetics, which may be applied to this problem is as follows:

$$FD = VK\int_0^\infty C(t)\ dt \qquad (1)$$

where F is the fraction of the dose, D, which is absorbed, V is the apparent volume of distribution of the drug, K is the first order rate constant for loss of drug from the body, and the integral is the area under the serum concentration curve, C(t), from zero to infinite time. Rearrangement of this equation yields:

$$\int_0^\infty C(t)\ dt = \frac{F}{VK}(D) \qquad (2)$$

Substitution of $0.693/t_{1/2}$ for K where $t_{1/2}$ is the half-life yields:

$$\int_0^\infty C(t)\ dt = \frac{F}{0.693V}[D.t_{1/2}] \qquad (3)$$

The infinite areas may be estimated by means of the equation:

$$\int_0^\infty C(t)\ dt = \int_0^T C(t)\ dt + \hat{C}_T/K \qquad (4)$$

where the second integral is estimated by trapezoidal rule from the observed serum concentrations, T is the time the last observed serum concentration was measured, and $\hat{C}_T$ corresponds to the serum concentration at time T estimated from the least squares line used to estimate K.

Each set of serum levels of the 60 individual subjects was plotted on semilogarithemic graph paper. Notations were made of those terminal C(t), t values which appeared to be randomly distributed about a straight line; these data as well as all serum concentration data were then used as input for an IBM 1620 computer program with the output shown in Fig. 2. The doses shown in the third column are in milligrams per kilogram and were calculated by the computer from the milligram doses and the body weights in pounds, which were part of the input data. The *area O-T* and *area INF* correspond to $\int_0^T C(t)dt$ and $\int_0^\infty C(t)dt$ in equation 4, respectively. The *K* values were estimated by the method of least squares from the ln C(t), t values chosen by the preliminary graphical technique indicated above; *SEK* is the standard error of *K*, and the *half-life* is 0.693/K. The computer also calculates and prints the individual ratios $\int_0^\infty C(t)dt/D$ and $\int_0^\infty C(t)dt/D.t_{1/2}$ indicated by the headings *area/dose* and *area/dose/half-life* in the last two columns. The computer also calculates and prints the averages and coefficients of variation of six of the columns of data. Table II lists the coefficients of variation of these six parameters which

Table II. *Coefficients of variation for various parameters estimated from serum concentrations observed in six studies following intramuscular administration of Lincocin, 300 mg. per cubic centimeter*

Study	Dose (mg.)	Coefficients of variation (%) based on 10 subjects per study					
		Dose (mg./Kg.)	Area ($0 \rightarrow T$)	Area ($0 \rightarrow \infty$)	K ($hours^{-1}$)	Area/ dose	Area/ dose/half-life
A	100	14.3	12.5	12.1	32.2	23.9	42.4
B1	200	14.3	24.0	24.4	23.9	29.2	25.9
B2	200	15.7	12.5	13.4	37.7	17.7	30.7
C1	600	14.0	22.2	22.4	14.1	26.7	25.2
C2	600	17.3	21.2	21.3	27.6	31.6	36.8
C3	600	16.3	24.3	24.3	35.4	30.7	36.9

ADME STUDY 0177 PROTOCOL MONITOR 12
BORGESS

SERUM CONCNS. AFTER IM ADMINISTRATION
MCG/ML

SUBJ. NO.	TREATMT. NO.	DOSE	AREA O - T	AREA INF.	K	SEK	HALF-LIFE	AREA/DOSE	AREA/DOSE/ HALF-LIFE
1	A	.9	16.20	17.59	.134	.014	5.14	19.15	3.72
2	A	1.2	14.50	15.49	.091	0.000	7.60	12.30	1.61
3	A	1.2	11.16	12.16	.079	.013	8.76	9.93	1.13
4	A	1.5	13.00	13.94	.084	.137	8.23	9.17	1.11
5	A	1.3	15.60	16.59	.091	0.000	7.60	12.42	1.63
6	A	1.5	14.50	15.67	.173	.048	3.99	10.31	2.57
7	A	1.2	12.70	13.96	.075	.151	9.22	11.40	1.23
8	A	1.4	13.60	14.54	.159	.062	4.34	9.76	2.24
9	A	1.1	12.62	13.61	.117	.003	5.89	11.43	1.93
10	A	1.4	16.70	17.85	.158	.095	4.36	12.55	2.87
AVERAGES		1.3	14.05	15.14	.116			11.84	2.01
COEFF. OF VAR.		14.34	12.54	12.10	32.19			23.92	42.41

Fig. 2. Print-out of a program which calculates dose in milligrams per kilogram from milligram dose and body weight in pounds and several parameters from blood level data. Particular example constitutes results from one of the six lincomycin intramuscular studies.

were obtained for groups of 10 subjects in the six studies. For example, one can see that giving uniform milligram doses to a panel of subjects leads to a 14 to 17 per cent standard deviation of the dose on a milligrams per kilogram basis. There is reasonably good consistency in the coefficients of variation for the other variables from study to study.

According to equation 2, a plot of the area against dose in milligrams per kilogram should yield a straight line with slope F/VK. This plot for the lincomycin data is shown in Fig. 3. The line with equation $\hat{Y} = 12.05X$ is the least squares line forced through the origin. This line was employed on the basis of the next two figures. Fig. 4 shows the print-out of the least squares regression program for these data. The intercept is not significantly different from zero. From the corrected sums of the squares at the bottom, the correlation coefficient, *r*, was calculated and added by pen. The regression is highly significant ($p < .001$). The $100r^2$ value is *74 per cent*, indicating that 74 per cent of the variability of the area under the serum concentration curve may be accounted for by differences in the milligram per kilogram doses.

Since the intercept was not significantly different from zero the least squares line forced through the origin was calculated. The print-out is shown as Fig. 5. It is from the *slope* of *12.05* shown on this print-out that the line shown in Fig. 3 is based.

According to equation 3, a plot of area against the product of the dose in milligrams per kilogram and the half-life should yield a straight line with slope F/0.693V. This plot for the lincomycin data is shown as Fig. 6. The intercept of the least squares line free to pass through any intercept was found to have an intercept not significantly different from zero. The $100r^2$ value was 75 per cent, which when compared with the previous value of 74 per cent indicated that the milligrams per kilogram dose alone accounted for about the same percentage variability of the area as did the product of the dose and the half-life. This is probably a reflection of the low variability of half-life of lincomycin in adult subjects, as will be indicated in the next example to be considered. Since the intercept was not significantly different from zero, the least squares line forced through the origin was calculated and is shown.

The least squares method assumes equal standard deviations at all values of the independent variable. However, the points in Fig. 6 are arranged in a fan-shaped ar-

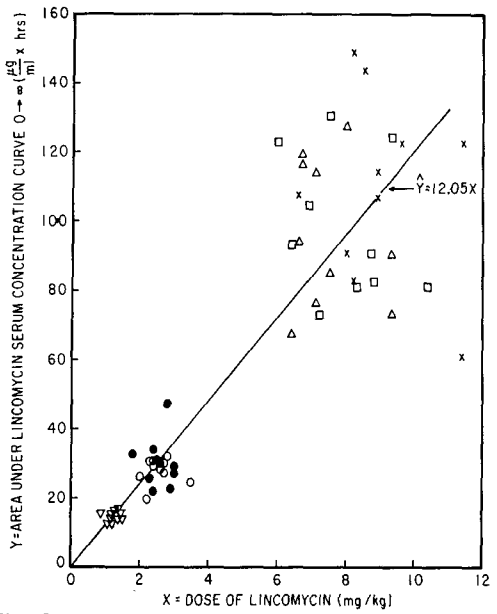

Fig. 3.

ray and theory predicts the per cent standard deviation (or coefficient of variation) of the area will be the same at all doses. Hence, rather than use the slope, 2.134, and its confidence interval to estimate $F/0.693V$, the individual ratios of area $0 \rightarrow \infty / D.t_{½}$ were employed. The ratios were calculated by means of another IBM 1620 computer program and a partial print-out is shown as Fig. 7. The print-out contained all the input data and each individual ratio, but only a few of them are shown. At the bottom, the average ratio, the standard deviation, standard error, and 95 per cent confidence limits of the average ratio are shown. The 95 per cent confidence interval of individual ratios was

AVERAGE X	=	5.13166
AVERAGE Y	=	63.09100
SLOPE	=	11.41754
STD ERROR OF SLOPE	=	.89150
T X STD ERROR OF SLOPE	=	1.78454
INTERCEPT	=	4.49995
STD ERROR OF INTERCEPT	=	5.39009
T X STD ERROR OF INTERCEPT	=	10.78951
VARIATION	=	487.41137
STD DEVIATION	=	22.07739
SSXX	=	613.26990
SSYY	=	108215.95000
SSXY	=	7002.03800
$r = \frac{SSXY}{\sqrt{SSXX \cdot SSYY}}$	=	0.860 (P < .001)
$100 r^2$	=	74%

Fig. 4. Print-out of "Least Squares Linear Regression Program," where Y equals the area under lincomycin serum concentration curve following intramuscular administration and *X* equals the dose of lincomycin in milligrams per kilogram.

AVERAGE X	=	5.13166
AVERAGE Y	=	63.09100
SLOPE	=	12.04925
STD ERROR OF SLOPE	=	.47019
T X STD ERROR OF SLOPE	=	.94086
VARIATION	=	484.90813
STD DEVIATION	=	22.02062

Fig. 5. Print-out of "Least Squares Linear Regression Program" for line forced through the origin, where *Y* equals the area under lincomycin serum concentration curve following intramuscular administration, and *X* equals the dose of lincomycin in milligrams per kilogram.

found to be 0.895 to 3.939. From the average ratio, we have $F/0.693V = 2.417$ or $V/F = 1/0.693 \times 2.417 = 0.597$ of body weight. The 95 per cent confidence interval of V/F is 0.366 to 1.61 of body weight, based on the 95 per cent confidence interval of the individual ratios above. The average body weight of the 60 subjects was 76 kilograms, corresponding to $V/F = 0.597 \times 76 = 45$ liters. Hence, if all the antibiotic was absorbed (i.e., $F = 1$), the average apparent volume of distribution ($\overline{V}$) was 45,000 ml.; if only 75 per cent was absorbed, the $\overline{V}$ was 34,000 ml. The latter figure agrees well with the V estimated from the constant rate infusion studies described in another section of this paper.

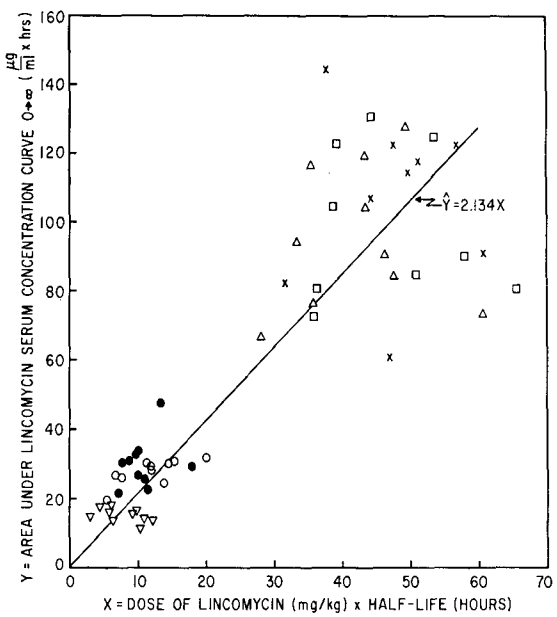

Fig. 6.

Distribution of the half-lives of two antibiotics

The raw data of this example consisted of two parts: (a) half-lives of lincomycin estimated from serum concentrations of lincomycin observed in 60 adult volunteers given lincomycin intramuscularly and (b) half-lives of novobiocin estimated from serum concentrations of novobiocin in 58 adult volunteers administered one capsule of a commercial preparation,* 250 mg. orally.

Preliminary graphical estimations indicated the logarithms of the half-lives were normally distributed. A computer program was written to convert the half-lives to their logarithms (base e) and print-out the latter suitable as input for the "Chi-Square Program." Normal distribution of the half-lives was also tested by using the half-lives themselves as input for the "Chi-Square Program." The computer arranges the in-

*Albamycin.

X	Y	RATIO
4.6300	17.6000	3.8012
9.1200	15.5000	1.6995
10.5000	12.2000	1.1619
12.3000	13.9000	1.1300
.	.	.
.	.	.
.	.	.
47.0000	60.9000	1.2957
37.6000	144.0000	3.8297
49.7000	114.2000	2.2977
44.1000	106.6000	2.4172

NUMBER OF RATIOS	=	58.
AVERAGE RATIO	=	2.4170
ST DEV OF RATIOS	=	.7608
ST ERR OF AVE R	=	.0999
95 LOWER LIMIT	=	2.2170
95 UPPER LIMIT	=	2.6171

Fig. 7. Partial print-out of "Ratio Program." Here X is dose (milligrams per kilogram) × half-life (hours), and Y is the area under serum concentration curve from 0 to ∞ for lincomycin administered intramuscularly to 58 adult volunteers.

put data in class intervals, based on the standard deviation it calculates, and prints out the low, midpoint, and high value of each class interval along with the observed or actual frequencies, fo, and the theoretical frequencies, f_i, based on a normal distribution. The computer then calculates chi-square.

Lincomycin half-lives. Two half-lives of 18.5 and 18.9 hours were excluded since the next highest half-life of the remaining 58 was 9.22 hours. The print-out of the "Chi-Square Program" for the logarithms of the half-lives of lincomycin is shown as Fig. 8. The number of points, *N*, the average log $_e$ $t_{1/2}$ (*XBAR* on the print-out and in this case the median) and the *class interval* is given at the top of the print-out. All the data necessary to plot the histogram are shown in the middle of the print-out and the calculated value of χ^2, *1.641*, is shown at the bottom. A similar print-out was obtained for the 58 half-lives.

The histograms are shown as Fig. 9. At the top is the distribution based on the *half-lives*. The χ^2 value is *7.781* ($p \cong 0.10$); hence, one cannot reject the hypothesis that the half-lives are normally distributed. At the bottom is the distribution based on the *logarithms* of the *half-lives*. There is a much better fit of the theoretical (dotted line) to the observed data, as indicated by the much lower χ^2 value, *1.641* ($0.8 < p < 0.9$). The estimated parameters of the log-normal distribution are shown in Table III. The coefficient of variation, 29.7 per cent, is low for an antibiotic and is indicative that the intersubject variation of the half-life of lincomycin is considerably less than that of many other drugs. The median half-life of 5.13 hours agrees very well with the median half-life estimated from serum concentration data obtained after oral administration of lincomycin hydrochloride.[5] The cumulative distribution plot will be shown later in Fig. 11, along with that for the novobiocin data.

Novobiocin half-lives. The histograms are shown as Fig. 10. At the top is the distribution based on the *half-lives*; the χ^2 value is *44.3* ($p < 0.001$). Hence, one can reject the hypothesis that the half-lives

N	XBAR	STAND DEV.	CLASS INTERVAL			
58	1.6355710	.29076096	.11630438			
INT.	LOW	MIDPOINT	HIGH	OBS F SUM	OBS F	EXPECT. FREQ.
1	.0000000	.00000000	.87959270	0	0	.27260000
2	.8795927	.93774489	.99589708	2	2	.53360000
3	.9958972	1.05404930	1.11220140	2	4	1.27600000
4	1.1122015	1.17035360	1.22850570	1	5	2.60420000
5	1.2285058	1.28665790	1.34481000	2	7	4.51820000
6	1.3448101	1.40296220	1.46111430	7	14	6.70480000
7	1.4611144	1.51926650	1.57741860	10	24	8.49120000
8	1.5774187	1.63557080	1.69372290	8	32	9.19880000
9	1.6937230	1.75187510	1.81002720	10	42	8.49120000
10	1.8100273	1.86817940	1.92633150	8	50	6.70480000
11	1.9263316	1.98448370	2.04263580	4	54	4.51820000
12	2.0426359	2.10078800	2.15894010	2	56	2.60420000
13	2.1589402	2.21709230	2.27524440	2	58	1.27600000
14	2.2752445	2.33339660	2.39154870	0	58	.53360000
15	2.3915487			0	58	.27260000

ADJUSTED TABLE

INT.	HIPOINT	OB. FREQ.	EXP. FREQ.	CHI SQUARE
1	1.3448100	7.0000000	9.2046000	.52802524
2	1.4611143	7.0000000	6.7048000	1.2997112E-02
3	1.5774186	10.0000000	8.4912000	.26809843
4	1.6937229	8.0000000	9.1988000	.15622922
5	1.8100272	10.0000000	8.4912000	.26809843
6	1.9263315	8.0000000	6.7048000	.25020030
7	2.0426358	8.0000000	9.2046000	.15764522
58		58.0000000	58.0000000	1.6412939 (0.8 < P < 0.9)

NORMAL DISTRIBUTION AT .05 LEVEL, CHI SQ = 9.4817852

Fig. 8. Print-out of "Chi-Square Program" to test the goodness of fit of data to a theoretical distribution. In this particular example the data are the logarithms (base e) of the half-lives of lincomycin estimated from terminal serum concentrations of lincomycin observed after intramuscular administration of the antibiotic to 58 adult volunteers.

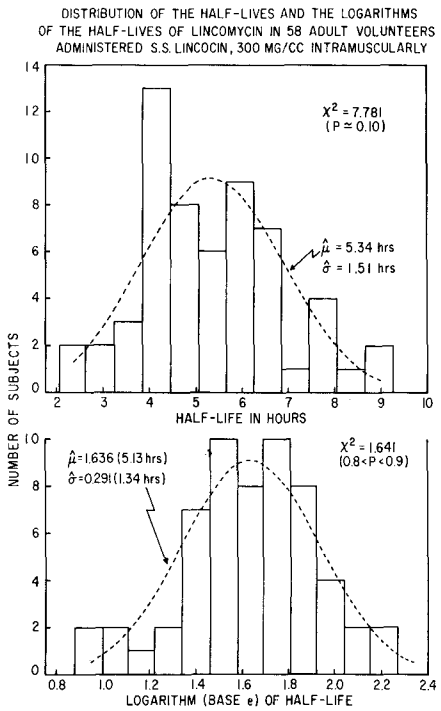

Fig. 9.

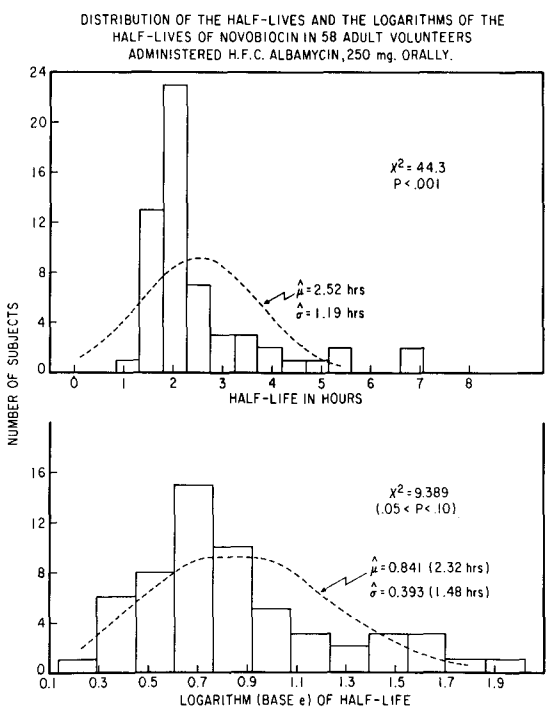

Fig. 10.

Table III. *Estimated parameters of the log-normal distribution of half-lives of lincomycin in 58 adult volunteers. The half-lives were estimated from the terminal portions of serum concentration curves following intramuscular administration of Lincocin, 300 mg. per cubic centimeter, at doses equivalent to 100, 200, and 600 mg. of lincomycin base*

Parameter	*Symbol and components*	*Hours*	*Log $_e$ units*
Median (50% point)	$\hat{\mu}$	5.13	1.6356
Standard deviation	$\hat{\sigma}$	1.34	0.2908
Mean	$e^{\mu+\sigma^2/2}$	5.35	1.6779
84% point	$e^{\mu+\sigma}$	6.86	1.9264
16% point	$e^{\mu-\sigma}$	3.84	1.3448
Coefficient of variation (%)	$100\sqrt{e^{\sigma^2}-1} = 29.7\%$		

are normally distributed. The poor fit of the theoretical dotted line to the observed data is easily observed. At the bottom of Fig. 10 is the distribution based on the *logarithms* of the *half-lives*. Here χ^2 equals *9.389 (0.05 < p < 0.10)* and the better fit of the theoretical dotted line to the observed data is readily seen. The estimated parameters of the log-normal distribution are shown in Table IV. One can see that 68 per cent of the subjects had half-lives between 1.57 and 3.43 hours, with the median being 2.32 hours and the mean being 2.52 hours. The coefficient of variation, 40.7 per cent, is about the usual order of magnitude calculated from data on other drugs. The cumulative distribution plots for the log-normal distributions of half-lives of both lincomycin and novobiocin are shown as Fig. 11. From such plots one can read directly the median (50 per cent point) and the percentage of subjects with half-lives below any given value. For example, on the *lincomycin* plot one can read that 94 per cent of the subjects have half-lives below 8 hours.

Table IV. *Estimated parameters of the log-normal distribution of half-lives of novobiocin in 58 adult volunteers. The half-lives were estimated from the terminal portions of serum concentration curves following oral administration of Albamycin, 250 mg.*

Parameter	*Symbol and components*	*Hours*	*Log $_e$ units*
Median (50% point)	$\hat{\mu}$	2.32	0.8409
Standard deviation	$\hat{\sigma}$	1.48	0.3926
Mean	$e^{\mu+\sigma^2/2}$	2.52	0.9256
84% point	$e^{\mu+\sigma}$	3.43	1.2335
16% point	$e^{\mu-\sigma}$	1.57	0.4483
Coefficient of variation (%)	$100\sqrt{e^{\sigma^2}-1} = 40.7\%$		

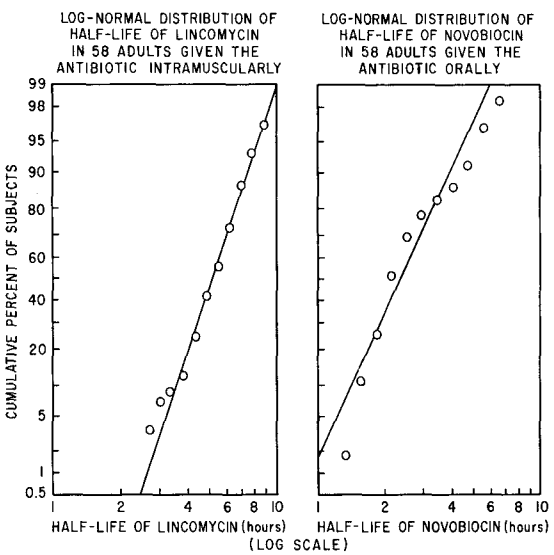

Fig. 11.

Prediction of multiple dose serum levels with an analogue computer

Wagner and Alway[2] predicted multiple dose serum levels of the antibiotic lincomycin from serum levels observed after the first infusion when a solution of lincomycin hydrochloride was administered at regular intervals by constant rate intravenous infusion. Predictions were based on the assumption of the model shown at the bottom of Fig. 12. This model has a single vascular compartment, C, with volume, V. Compartment A represents the infusion apparatus, and drug is delivered from this apparatus at a constant rate k_0 in units of micrograms per hour over the infusion time of T hours. In terms of serum level of lincomycin, the rate of delivery is k_0/V in units of micrograms per milliliter times hours. We assume drug is lost from the body by a first order rate, and the first order rate constant is designated by K on the arrow for the transfer from compartment C to compartment D. Here compartment D represents accumulated unchanged drug and metabolites.

The upper equation shown in Fig. 12 gives the serum concentration at the end of the first infusion, C_T. Rearrangement of this equation yields the equation giving V as a function of k_0, C_T, K, and T. After cessation of the first infusion, the serum concentration, C, will fall off according to the equation shown.

For a particular set of data, the value of K is estimated from the observed serum concentrations after the first infusion of drug when $t > T$; $k_0 = D/T$ where D is the dose in micrograms given over T hours. The print-out of a digital computer program which calculates K and V when the input data are k_0, T, C_T, and C values after first infusion is shown as Fig. 13. The input data correspond to the three examples

Table V. *Parameters programmed on analogue computer to generate curves shown in Figs. 1, 2, and 3**

Figure No.	*D/V*	*ko/V*	*K*	*τ†*
1	11.54	5.77	0.177	12
2	9.14	4.57	0.177	12
3	18.4	9.2	0.217	6

*Refer to Figs. 1, 2, and 3 from Wagner and Alway.[2]

†τ, the time interval between the start of repetitive infusions, so that τ—T is the interval between infusions.

shown in Figs. 1, 2, and 3, of Wagner and Alway.[2]

An analogue computer was programmed with the model shown in Fig. 12. The initial amount (but in terms of serum concentration units) placed in compartment A was D/V. A dose box was built as supplementary equipment for the analogue computer, so that a voltage corresponding to D/V could be placed in compartment A at times corresponding to the start of each infusion. The A to C transfer rate was set at k_0/V and the C to D transfer rate was set at K.

The analogue computer plotted the concentration in the C compartment as a function of time which corresponded to the continuous serum level plot expected for the multiple infusions given over T hours every τ hours. This example is discussed in more detail in Wagner and Alway.[2]

It was fortunate that the simple model with only one vascular compartment gave such good agreement in the examples cited. In other cases a more complicated model may be necessary.

D = DOSE
T = INFUSION TIME
ko = D/T

C_T = SERUM OR PLASMA LEVEL AT END OF FIRST INFUSION

$$C_T = \frac{ko}{V.K.}(1-e^{-KT}) \text{ and } V = \frac{ko}{C_T K.}(1-e^{-KT})$$

AFTER INFUSION CEASES:

$$\hat{C} = C_T \cdot e^{-K(t-T)}$$

PROGRAM FOR ANALOG COMPUTER IS:

D/V AT START OF EACH DOSAGE INTERVAL, τ → A —ko/V, for T hrs.→ C —K→ D

DETERMINE V AND K FROM SINGLE (FIRST) DOSE DATA ONLY.

Fig. 12. Multiple-dose constant rate intravenous infusion of lincomycin hydrochloride.

The fitting of blood levels obtained after single oral doses with a two-term exponential equation

Many sets of observed blood concentrations obtained following the administration of single doses of a drug may be fitted with a two-term exponential equation of the type

$$\hat{C} = Be^{-\beta t} - Ae^{-\alpha t} \qquad (5)$$

A typical set of data is shown in Table VI.

The observed serum levels, C, are plotted as the X's on semilogarithmic graph paper in Fig. 14. The terminal six points yield an apparent straight line. As a first approximation, these six points are fitted by the equation $\hat{C} = 2.4e^{-0.130t}$ with the slope being estimated as shown on Fig. 14. Residuals (in this case, $2.4e^{-0.130t}$-C) were estimated graphically and are plotted as the open circles. The three residuals, R, are approximately fitted by the equation $R = 3.0e^{-0.810t}$. Hence, as a first approximation

```
3.
150000.        2.        9.7
2.             9.7
6.             3.6
12.            1.6
999.
150000.        2.        7.7
2.             7.7
6.             3.6
12.            1.3
999.
300000.        2.       15.
2.            15.
6.             6.3
999.
```

SUBJECT	KO	T	CT	K	V
1.	150000.000000	2.	9.700000	.176655	26054.682000
2.	150000.000000	2.	7.700000	.177244	32803.936000
3.	300000.000000	2.	15.000000	.216875	32454.243000

Fig. 13. Print-out of "Constant Rate Intravenous Injection Program" which calculates the rate constant, K, and the apparent volume of distribution, V, from serum concentrations observed after the first infusion.

Table VI. *The lowest serum levels of tetracycline hydrochloride activity observed in a panel of 8 subjects administered Panmycin, 250 mg., after specified breakfast*

Time (hours)	*Serum concentration of tetracycline HCl (mcg./ml.)*
1	0.7
2	1.2
3	1.4
4	1.4
6	1.1
8	0.8
10	0.6
12	0.5
16	0.3

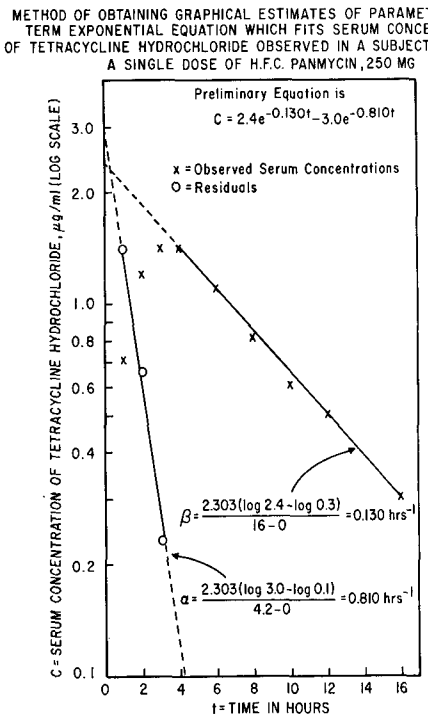

Fig. 14.

the observed serum levels are fitted by the equation:

$$\hat{C} = 2.4e^{-0.130t} - 3.0e^{-0.810t} \quad (6)$$

These preliminary graphical estimates were used as input data to an iterative IBM 1620 computer program which, if convergence is obtained, will yield a set of parameters providing a least squares fit of estimated to observed serum levels, i.e., the quantity $\sum_{i=1}^{n} (C_i - \hat{C})^2$ is minimized where C_i is the observed serum concentration, $\hat{C}$ is the estimated serum concentration, and n is the number of serum concentrations in the set.

The computer print-out for this particular set of data is shown as Fig. 15. The terminal set of parameters (under iteration No. 5 on the print-out) yield the equation:

$$\hat{C} = 2.817e^{-0.149t} - 3.559e^{-0.716t} \quad (7)$$

which is somewhat different than that obtained graphically. However, equation 7 provides a better fit of estimated serum levels to observed serum levels, as indicated by the reduction in the variance. Employing equation 7, the computer calculated $\hat{C}$ (*calc. Y* on print-out) for each time (*X* on print-out) corresponding to the observed serum concentration (*Y* on print-out).

If we assume that the model which applies is:

$$\frac{FD}{V} \text{ at } t = T_0 \Big\} \quad \underset{① \rightarrow ② \rightarrow ③}{K_1 \quad K_2} \quad \text{where } K_1 > K_2$$

then $\alpha = K_1 = 0.716$, $\beta = K_2 = 0.149$, and $\hat{C} = (FD/V)\,(K_1/K_1 - K_2)\,[e^{-K_2(t-T_0)} - e^{-K_1(t-T_0)}]$ (8)

where $\hat{C}$ is the estimated concentration in compartment ② at time t. The parameters we need to write the equation in the form shown as equation 8 are given by what we call the "Lag-Time Program." This leads to calculation of the following:

$$T_0 = \frac{\ln A - \ln B}{K_1 - K_2} \quad (9)$$

$$A_{NEW} = B\, e^{-KT_0} \quad (10)$$

$$FD/V = A_{NEW} \Big/ \frac{K_1}{K_1 - K_2} \quad (11)$$

A print-out of the "Lag-Time Program" corresponding to the parameters in equation 7 is shown as Fig. 16. The input figures are at the top and the output quantities, calculated by means of equations 9, 10, and 11 by the computer, are at the

```
N=  9          IW=  1          TEST=  .00001000          NP=  4

ITER. NO.                      SS                        VMIN

    B                          β                         A                         α
    0                      .235730E-01               .000000E-99
.240000E+01                .130000E-00               -.300000E+01              .810000E-00
    1                      .133917E-01               .541420E-00
.260375E+01                .139906E-00               -.325046E+01              .741523E-00
    2                      .102607E-01               .974163E-00
.280075E+01                .148369E-00               -.353417E+01              .713690E-00
    3                      .100454E-01               .998460E-00
.281439E+01                .148697E-00               -.355799E+01              .716705E-00
    4                      .100453E-01               .942307E-00
.281766E+01                .148813E-00               -.355903E+01              .715586E-00
    5                      .100453E-01               .618421E-00
.281734E+01                .148803E-00               -.355901E+01              .715715E-00

    X                          Y                         CALC Y                    Y-CALC Y
1.00000000                 .70000000                 .68802160                 .01197840
2.00000000                1.20000000                1.24165790                -.04165790
3.00000000                1.40000000                1.38712740                 .01287260
4.00000000                1.40000000                1.35037400                 .04962600
6.00000000                1.10000000                1.10513460                -.00513460
8.00000000                 .80000000                 .84512509                -.04512509
10.00000000                .60000000                 .63342922                -.03342922
12.00000000                .50000000                 .47177701                 .02822299
16.00000000                .30000000                 .26048654                 .03951346
```

Fig. 15. Print-out of computer program which automatically proceeded to obtain a least squares fit of the data (serum concentrations of tetracycline hydrochloride) by iterative adjustment of the values of the parameters.

```
1.
2.817          .149          3.559          .716

               A                            K1                        B                         K2
               2.81700000                   .14900000                 3.55900000                .71600000
               TO                           ANEW                      K1/(K1-K2)                FD/V

               .41235821                    2.64912950                1.26278650                2.09784430
```

Fig. 16. Print-out of "Lag-Time Program."

bottom. We may now write the final equation in the form of equation 8 and the model above. This is shown in Fig. 17. The two forms of the equation for the serum concentrations are shown at the top, and the model is beneath. The open circles are the observed serum concentrations shown in Table VI, and the solid line is based on the derived equation. If the model is correct, the derived parameters indicate the tetracycline started to be absorbed at *0.421* hour and entered the blood at a first order rate with rate constant *0.716* hour^{-1}, corresponding to an absorption half-time of 0.97 hour; the tetracycline was lost from the blood at a first order rate with rate constant *0.149* hour^{-1}, corresponding to a biological half-life of 4.7 hours.

It should be noted that several different models would provide a two-term exponential equation such as equation 7. Writing the model as shown in Fig. 17 is probably the most plausible one.

An example of simulation with a digital computer

Analysis of serum concentration curves[3] obtained in human subjects after intramuscular administration of 600 mg. of lincomycin as the commercial solution,* 300 mg. per cubic centimeter, indicated that about two thirds of the dose entered the blood so rapidly that this portion acted like an intravenous injection, and about one third of the dose formed a depot which was released at a first order rate over a period of 10 to 12 hours. Hence, the simulation model could be:

*Lincocin.

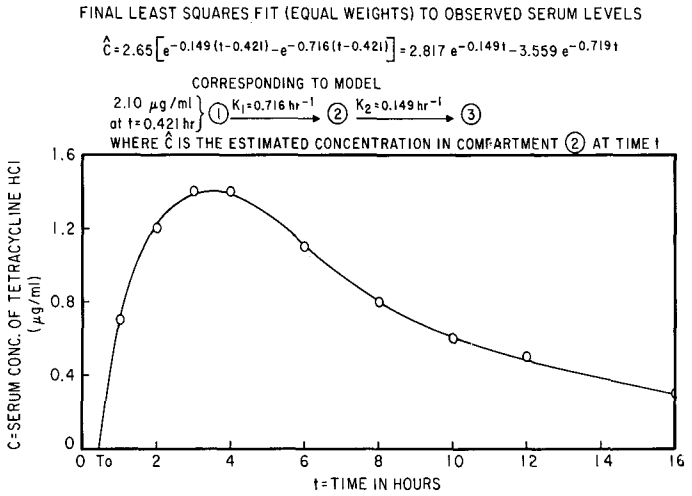

Fig. 17.

Drug in K_1 depot → Drug in blood K_2 and O.F.D.* → Drug metabolized and excreted

↑

Drug rapidly available

where K_1 is a first order rate constant for release and absorption from the depot, and K_2 is a first order rate constant representing all-over loss from the body. The average area under the lincomycin serum concentration curve from 0 to 24 hours following administration of 600 mg. of lincomycin as the hydrochloride intramuscularly in 30 subjects was 100 mcg. per milliliter times hours. The average serum concentration 24 hours after intramuscular administration of the 600 mg. dose was about 1 mcg. per milliliter.

We were interested in considering the possibility of a depot intramuscular preparation. By chemical or pharmaceutical manipulation, one may be able to keep the area under the lincomycin serum concentration curve constant but flatten the curve. Hence, the peak blood level would not be as high, but the time that the serum level remained above a certain value would increase, such as shown in Fig. 18. This approach was explored by a simulation technique first to see how much one would have to slow the release rate from the depot to make an appreciable difference in the serum concentration curves.

The appropriate material balance equation for the simulation is equation 1, which transposes to:

$$\frac{FD}{V} = K_2 \cdot \int_0^\infty C(t)dt \qquad (12)$$

Although in concentration units FD/V is the initial amount, corresponding to the dose, we need to generate serum concentration curves in order to keep the area under the serum concentration curve constant at, say, 100 units. I decided to run one curve corresponding to a half-life of lincomycin of 4.4 hours and one corresponding to a half-life of 6.4 hours for each change in K_1, since more than two thirds of individual subjects have half-lives of lincomycin in this range. Hence, for the 4.4 hour half-life curves $FD/V = 0.693/4.4 \times 100 = 15.7$ mcg. per milliliter and for the 6.4 hour half-life curves $FD/V = 0.693/6.4 \times 100 = 10.8$ mcg. per milliliter, in order to simulate injection of 600 mg. into a depot. When one wished to simulate intravenous-like injection of an additional 300 mg. of lincomycin, then one half of the above values or 7.85 and 5.4 mcg. per milliliter, respectively, were placed at $t = 0$ directly into the blood compartment.

The equation appropriate to the model above is:

*Other fluids of the distribution.

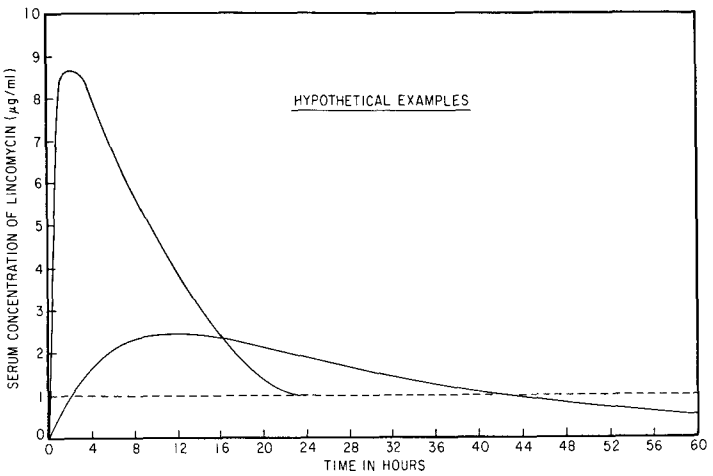

Fig. 18.

$$\hat{C} = (fs)\left(\frac{FD}{V}\right)\left(\frac{K_1}{K_1 \pm K_2}\right)\left[e^{-K_2 t} \pm e^{-K_1 t}\right] + (fi)\left(\frac{FD}{V}\right) e^{-K_2 t} \qquad (13)$$

where fs is the fraction of the dose in the depot initially, fi is the fraction of the dose placed directly in the blood at t = 0, and $\hat{C}$ is the estimated serum concentration at time t after administration.

A large number of curves were generated by using various sets of K_1, K_2, and total FD/V values corresponding to 600 and 900 mg. doses of lincomycin. Only three pairs of curves will be shown as examples in Figs. 20 to 22. These were based on the parameters and equations in Table VII.

An example of a print-out of the "Exponential Prediction Program" is shown in Fig. 19; the last equation in Table VII was the input data.

Curve *1* in Fig. 20 is not unlike that obtained from administration of 600 mg. dose of lincomycin, as the commercial formulation. With the commercial formulation the peak is higher and occurs at 0.5 to 2 hours. The curves shown in Fig. 21 are probably too flat, and serum levels may not be high enough to allow administration every 48 hours. The curves shown in Fig. 22 may be satisfactory for a depot preparation, but they were generated by assuming a *900 mg. dose* of lincomycin and half-absorption times of 8.8 and 12.8 hours. Whether such a pharmaceutical can be made is unknown at the present, but the simulation did specify an approximate target if we wished to tackle the job.

In general, the attitude of other researchers to the results of this simulation was to make them more satisfied with the commercial product.

Other IBM 1620 computer programs useful in pharmacokinetics

1. A program which gives a summary and statistical analysis of blood concentra-

10.80000000	.05400000
-5.40000000	.10800000
T	F (T)
0.00000000	5.40000000
1.00000000	5.38507800
2.00000000	5.34340730
3.00000000	5.27921370
4.00000000	5.19621070
5.00000000	5.09765790
6.00000000	4.98641160

Fig. 19. Example of print-out of "Exponential Prediction Program." *F (T)* corresponds to $\hat{C}$ and T to t for the equation $\hat{C} = 10.8\ [e^{-.054t} - e^{-.108t}] + 5.4\ e^{-.108t}$.

Table VII. *Parameters and equations used to generate the curves in Figs. 20, 21, and 22*

Fig. No.	*Curve No.*	*Total FD/V*	*fs*	*fi*	K_1	K_2	*Equation*
20	1	15.7	1	0	0.314	0.157	$\hat{C} = 31.4\ [e^{-0.157t} - e^{-0.134t}]$
20	2	10.8	1	0	0.216	0.108	$\hat{C} = 21.6\ [e^{-0.108t} - e^{0.216t}]$
21	9	15.7	1	0	0.0785	0.157	$\hat{C} = 15.7\ [e^{-0.0785t} - e^{-0.157t}]$
21	10	10.8	1	0	0.0540	0.108	$\hat{C} = 10.8\ [e^{-0.054t} - e^{-0.108t}]$
22	11	23.55	0.67	0.33	0.0785	0.157	$\hat{C} = 15.7\ [e^{-0.0785t} - e^{-0.157t}] + 7.85\ e^{-0.157t}$
22	12	16.2	0.67	0.33	0.0540	0.108	$\hat{C} = 10.8\ [e^{-0.054t} - e^{-0.108t}] + 5.4\ e^{-0.108t}$

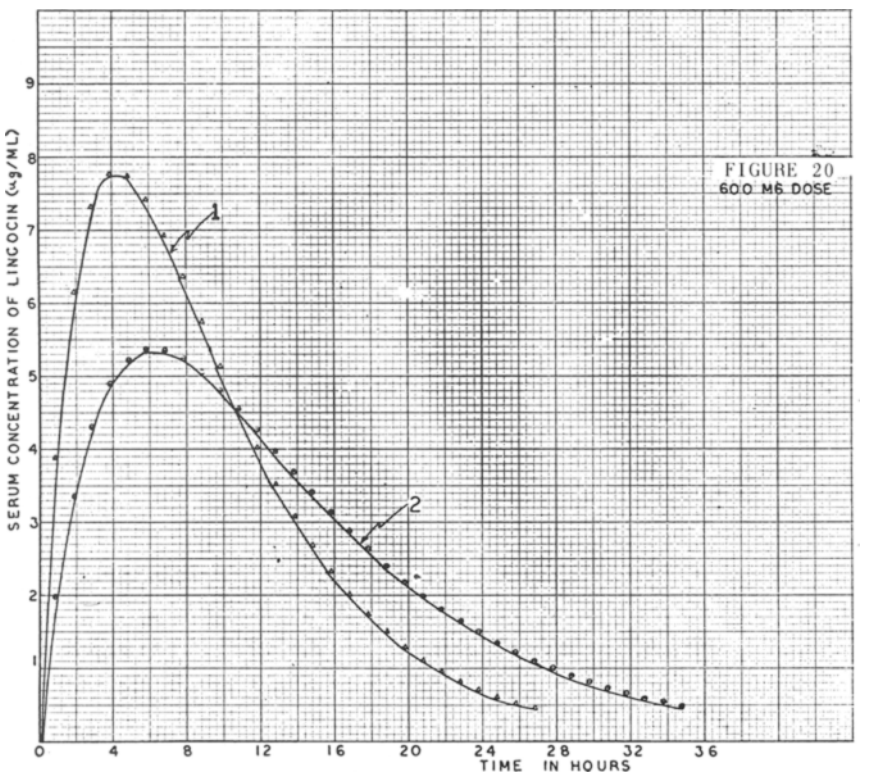

Fig. 20.

tion data available in a typical crossover study.

2. A program which gives a summary and statistical analysis of urinary excretion data available in a typical crossover study.

The types of information available from these programs include: (1) vital statistics; (2) blood concentrations and areas under blood concentration curves with averages and coefficients of variation; (3) analyses of variance; (4) rate constant and half-life from serum concentration data; (5) urine volume, concentration, and amount excreted in each collection period; (6) total amount excreted and per cent recovery.

3. A program which gives a least squares fit to observed amounts excreted in the urine in the postabsorptive period. The program yields an estimate of the asymptote, $Y\infty$, and the rate constant, k, for the equation $\hat{Y} = Y\infty\ (1-e^{-kt})$ where $\hat{Y}$ is the estimated amount excreted to time t.

4. A program which gives predictions of multiple-dose blood levels based on the equation:

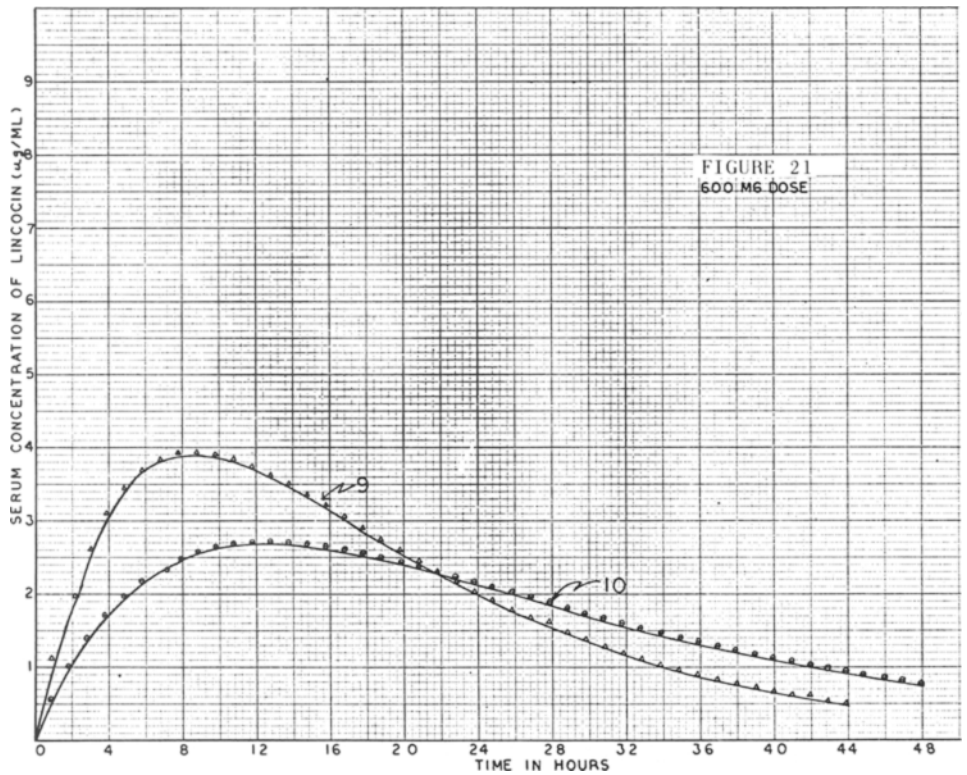
FIGURE 21
600 MG DOSE
SERUM CONCENTRATION OF LINCOCIN (µg/ML)
TIME IN HOURS
9
10

Fig. 21.

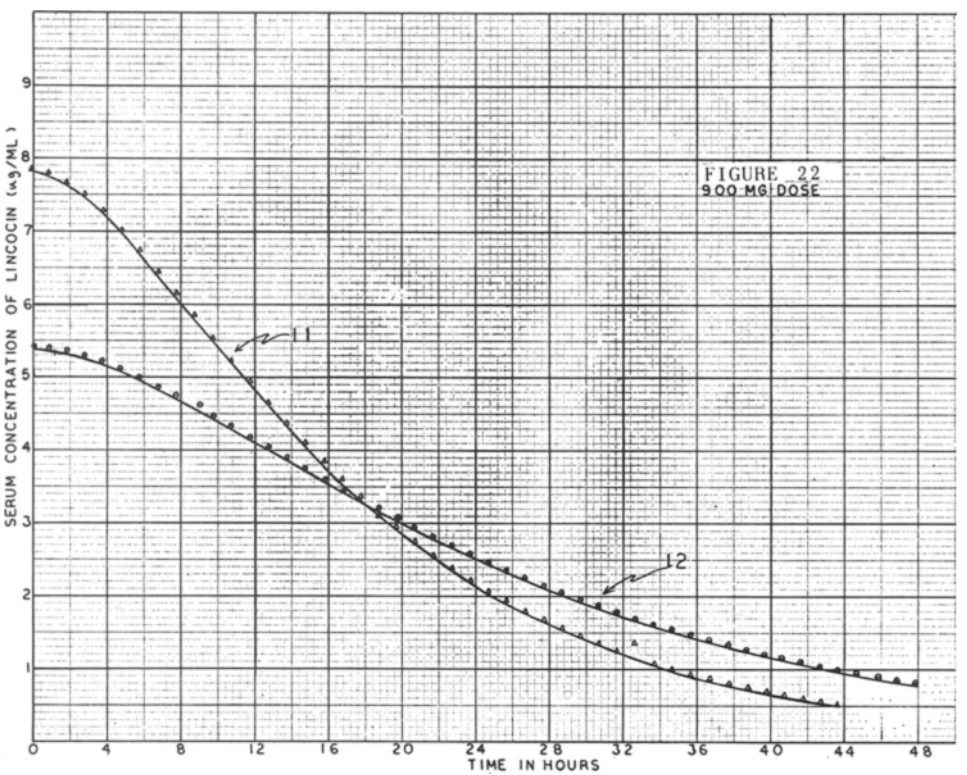
FIGURE 22
900 MG DOSE
SERUM CONCENTRATION OF LINCOCIN (µg/ML)
TIME IN HOURS
11
12

Fig. 22.

$$\hat{C}(t) = \left(\frac{FD}{V}\right)\left(\frac{K_1}{K_2-K_1}\right)\left[\left(\frac{1-e^{-nK_1\tau}}{1-e^{-K_1\tau}}\right)e^{-K_1(t-T_0)} - \frac{1-e^{-nK_2\tau}}{1-e^{-K_2\tau}}\,e^{-K_2(t-T_0)}\right] \quad (14)$$

which, in turn, is based on the model:

$$\left.\frac{FD}{V} \text{ at } t = T_0\right\} A \xrightarrow{K_1} C \xrightarrow{K_2} D$$

where T_0 is the lag time and K_1 and K_2 are first order rate constants.

5. A multiple linear regression program which prints out all possible correlation coefficients for the linear regressions between pairs of several variables used as input. This is useful to determine if various parameters such as FD/V are correlated with others.

6. A program to estimate per cent absorbed as a function of time based on the equation of Wagner and Nelson.[4]

7. A program to derive a two-compartment model from blood concentrations observed after rapid intravenous injection; the model is the same as that proposed by Tait and co-workers.[1]

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