Practical concepts of drug absorption, distribution and loss

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Summary: Serum concentrations of ampicillin were measured following administration of the drug orally and intravenously to nine normal volunteers. The results were analysed by graphic methods, and the effects of absorption, distribution and loss on the time serum concentration curve are discussed.

Résumé: *Des concepts pratiques sur l'absorption, la distribution et la perte des médicaments*

Nous avons mesuré les concentrations sériques d'ampicilline chez neuf volontaires normaux, auxquels nous avions administré l'antibiotique par voie orale et intraveineuse. Les résultats, analysés par des méthodes graphiques, nous ont permis d'évaluer les effets de l'absorption, de la distribution et des pertes sur la courbe chronologique de la concentration sérique.

The time course of drug action in relationship to the processes of absorption, distribution, metabolism and excretion is commonly described by compartment models that are derived using mathematics beyond the experience of most practising physicians. It would not be surprising if many readers were discouraged by the sight of complex formulas that are utilized to characterize the disposition of a drug, with the result that their appreciation of the usefulness of the pharmacokinetic data would be limited. However, it is important for all physicians to have an appreciation of these characteristics in order to understand optimal dose sizes and intervals of individual drugs. This paper describes studies of the disposition of a commonly used drug, ampicillin, and discusses the pharmacokinetic features derived from measurements made after intravenous and oral administration without employing extensive mathematics. Ampicillin was convenient to study because it could be given with relative safety by both routes of administration and because large numbers of serum concentration measurements could be made efficiently.

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Materials and methods

Nine healthy men aged 20 to 40 years, weighing between 58 and 95 kg, were studied at The Montreal General Hospital. Preliminary investigations included medical history (with care to exclude a history of penicillin allergy), physical examination, urinalysis, serum biochemistry and hematology. Signed informed consents were obtained. During the experiments each volunteer was observed and questioned for side effects. The ampicillin was supplied as 250-mg trihydrate capsules and as desiccated ampicillin trihydrate for intravenous administration. The desiccated preparation was dissolved in normal saline on each experimental day just prior to infusion.

The oral study was conducted on three experimental days separated by an interval of one week. Each day each subject received two 250-mg capsules of ampicillin at 8:30 a.m. with 250 ml of water during a fast from midnight until two hours after drug administration. Venous blood samples were obtained just prior to, and at ½, 1, 1½, 2, 3, 4, 5 and 6 hours after ampicillin ingestion. No antibacterial activity was found in any of the blood samples taken prior to drug administration. The serum concentration of ampicillin measured at each sample time during the three experimental days was averaged for each individual subject.

Twelve weeks later the volunteers received intravenous ampicillin during a fast from midnight until two hours after drug administration. Five hundred mg of ampicillin in 20 ml of normal saline was infused over a five-minute period through a superficial vein of the forearm. An indwelling catheter was inserted in a retrograde direction into the deep contralateral antecubital vein. A three-way stopcock was attached to the catheter to allow rapid and repeated blood sampling, and patency was maintained with intermittent infusions of a 5% dextrose-in-water solution containing 10 units of heparin per ml. Less than 40 ml of this solution was administered to each subject. In drawing venous samples, 2 to 3 ml was taken in one syringe to clear the tubing of the D/W solution, then 5 ml was drawn off immediately thereafter into a second syringe for ampicillin assay. Venous samples were obtained just prior to drug administration, and at 3, 6, 9, 12, 15, 20, 30 and 45 minutes and 1, 1½, 2, 3, 4, 5 and 6 hours after the termination of the drug infusion. The subjects remained recumbent throughout the experiment. No antibacterial activity was found in any of the serum samples obtained prior to drug administration.

On each experimental day the blood samples were allowed to clot at room

temperature for one hour, then centrifuged and the serum drawn off using sterile Pasteur pipettes. The samples were refrigerated at 4°C until all samples for each day had been taken, when the microbiological assays were done in triplicate. The measurements were averaged and reported as micrograms of ampicillin per ml of serum.

The ampicillin assay employed *Bacillus subtilis* in the modified microbiological method of Bennett *et al.*[1] Samples for an ampicillin standard curve were prepared in fresh, pooled human serum at the time of each experimental assay. A standard curve was obtained on each plate, using the mean of three samples at each concentration point. Because the accuracy of this assay was decreased at concentrations above 10 μg/ml, serum samples obtained in the first 45 minutes after intravenous ampicillin administration were diluted with the subject's own control serum (1:5 to 1:1) to obtain concentrations within the acceptable range of the standard curve.

Results

The study was completed as described and no adverse effects were encountered. The serum concentrations shown in Table I are averages of the individual values* after oral and intravenous administration of ampicillin to each volunteer. By recording graphically the results of the oral and intravenous studies, calculation of the various characteristics of drug handling necessary to predict drug dose and dosage schedules can be undertaken.

Intravenous administration

Fig. 1 represents the average serum ampicillin concentrations of the nine volunteers over the time period of the intravenous drug study, and is similar to the graph that can be drawn for many drugs to depict their disappearance from the serum after intravenous administration. The serum drug concentration is plotted on the ordinate on a logarithmic scale, while the time at which samples were drawn is plotted on the abscissa using an arithmetic scale. The graph shows an initial rapid decline of drug serum concentration and a subsequent more gradual decrease.

In assessing the pharmacokinetics of a drug, the terminal phase of the graph is first studied. This portion is termed the beta phase of drug disposition. The steepness of the slope of this phase measures the rate at which the drug is cleared from the serum by continuous distribution between serum and tissues, metabolism and excretion. Since this part of the graph for ampicillin and many other drugs is a straight line, the rate of disappearance is constant, and the rate constant is referred to as beta. The serum half life of a drug in the beta phase is determined from the slope and is the time required for any selected drug concentration along the line to decrease to one half its value. It can be read directly from the graph and averaged 1.0 hour for ampicillin.

A second important aspect of drug handling is the "apparent" volume of

*Tables of the individual subjects' serum concentrations are available from the authors upon request.

Table I—Serum concentrations of ampicillin (μg/ml) in nine men following oral and intravenous administration of 500 mg

Time after dose (hours)	Route of administration	
	Oral*	Intravenous†
0.05	—	40.26 ± 3.75
0.10	—	29.88 ± 1.93
0.15	—	28.02 ± 1.09
0.20	—	24.13 ± 2.14
0.25	—	23.56 ± 1.30
0.33	—	19.63 ± 1.19
0.50	0.94 ± 0.46	13.80 ± 0.68
0.75	—	9.67 ± 0.61
1.0	2.39 ± 0.42	7.24 ± 0.40
1.5	3.41 ± 0.43	3.69 ± 0.26
2.0	3.43 ± 0.40	2.42 ± 0.20
3.0	3.29 ± 0.28	1.13 ± 0.11
4.0	1.97 ± 0.20	0.57 ± 0.06
5.0	1.10 ± 0.12	0.27 ± 0.03
6.0	0.57 ± 0.08	0.13 ± 0.02

*Mean ± standard error of the mean following 500-mg oral doses on three different days
†Mean ± standard error

Table II—Pharmacokinetic parameters of ampicillin in nine men following intravenous administration of 500 mg

Subject	$AUC_0 \rightarrow \infty$ μg/ml × hr	V_C litres	V_{Dss} litres	K_{el} hours^{-1}	K_{12} hours^{-1}	K_{21} hours^{-1}	α hours^{-1}	$t_{\frac{1}{2}}\alpha$ hours	β hours^{-1}	$t_{\frac{1}{2}}\beta$ hours
1	24.22	9.1	13.2	2.271	0.516	1.149	3.092	0.22	0.844	0.82
2	28.59	12.4	18.9	1.413	0.530	1.014	2.346	0.30	0.611	1.13
3	27.66	11.1	15.5	1.629	0.492	1.232	2.573	0.27	0.780	0.89
4	24.80	14.8	20.2	1.363	0.359	0.991	2.056	0.34	0.657	1.05
5	22.88	12.0	18.8	1.814	0.696	1.225	2.992	0.23	0.743	0.93
6	33.23	8.3	12.9	1.810	0.932	1.686	3.574	0.19	0.854	0.81
7	19.94	19.2	26.3	1.309	0.364	0.986	2.020	0.34	0.639	1.08
8	24.64	9.0	17.2	2.257	1.369	1.497	4.345	0.16	0.778	0.89
9	28.83	12.2	18.7	1.422	0.571	1.078	2.444	0.28	0.627	1.11
Mean ± SE	26.09 ±1.30	12.01 ±1.13	17.97 ± 1.35	1.699 ±0.123	0.648 ±0.107	1.206 ±0.081	2.827 ±0.254	0.26 ±0.02	0.726 ±0.032	0.97 ±0.04

distribution of the drug. It is referred to as apparent since it does not represent a physiological or anatomical body compartment but a hypothetical space that facilitates the calculation of the amount of drug required to achieve specific serum concentrations. Many factors affect the magnitude of the volume of distribution, including the size of the drug molecule, its lipid solubility and its binding to plasma and tissue proteins. This parameter is easily calculated from the graph after intravenous administration by extrapolating the straight line of the beta phase back to the drug concentration axis. The value thus determined is an estimate of the concentration of drug at time 0 (Fig. 2). Since the total amount of drug given intravenously is known, the volume into which this total amount appears to have been distributed can be calculated by dividing the total quantity given by the concentration estimated at time 0. It is usually expressed in litres or litres per kilogram of body weight. From Fig. 2 the time 0 serum concentration for ampicillin is estimated at 10 μg/ml after a 500-mg intravenous dose. Therefore, the apparent volume of distribution is 50.0 litres. The larger the apparent volume of distribution, the greater is the amount of drug distributed to extravascular compartments. More accurate estimations of the distribution of a drug may be obtained by considering the body to exist as two compartments, and the size of the central compartment (V_C in Table II) can be calculated in relation to the volume of distribution at steady state (V_{Dss} in Table II).† The apparent volume of distribution greatly overestimates the volume of distribution because of failure to separate distribution from metabolism and excretion.

The beta phase of drug disappearance can be further analysed as representing losses of drug by metabolism and/or excretion, and those occurring by distribution factors. The rate at which metabolism and/or excretion occurs is characterized by the rate constant for elimination, K_{el}.

In order to gain an appreciation of the rate at which drug distribution into tissues occurs, we turn our attention to the initial portion of the intravenous curve. Here, drug concentration declines rapidly from peak concentrations after rapid injection owing to distribution of the drug between serum and tissues, and to excretion and metabolism of the drug. However, in contrast to the beta phase of the curve, the processes of distribution are predominant in this initial phase. In order to separate the distribution from the elimination phase, the beta phase points previously determined by extrapolation of the line back to time 0 are subtracted from the actual data points throughout the entire curve. The calculated differences, called residual concentrations (Fig. 2), are then plotted on the graph, and the line thus drawn represents the loss of drug from the serum by distribution between the serum and tissues. This calculated part of the graph is referred to as the alpha phase and is more rapid than the beta phase, as seen by its steeper slope. Similar to the beta phase, a straight line denotes a rate constant, which is called alpha. The half life of the alpha phase can be calculated in the same manner as

†For more detailed information on volumes of distribution readers are referred to the discussion of the subject by Portmann.[2]

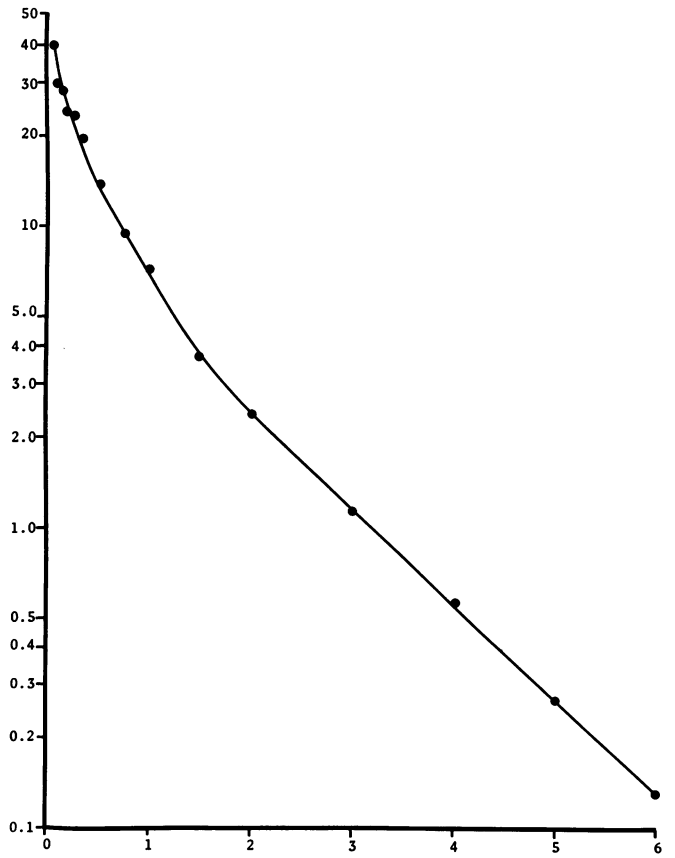

FIG. 1—Mean serum concentrations of ampicillin (μg/ml) in nine men following intravenous administration of 500 mg. Abscissa: time (hours) after administration. Ordinate: ampicillin serum concentration (μg/ml).

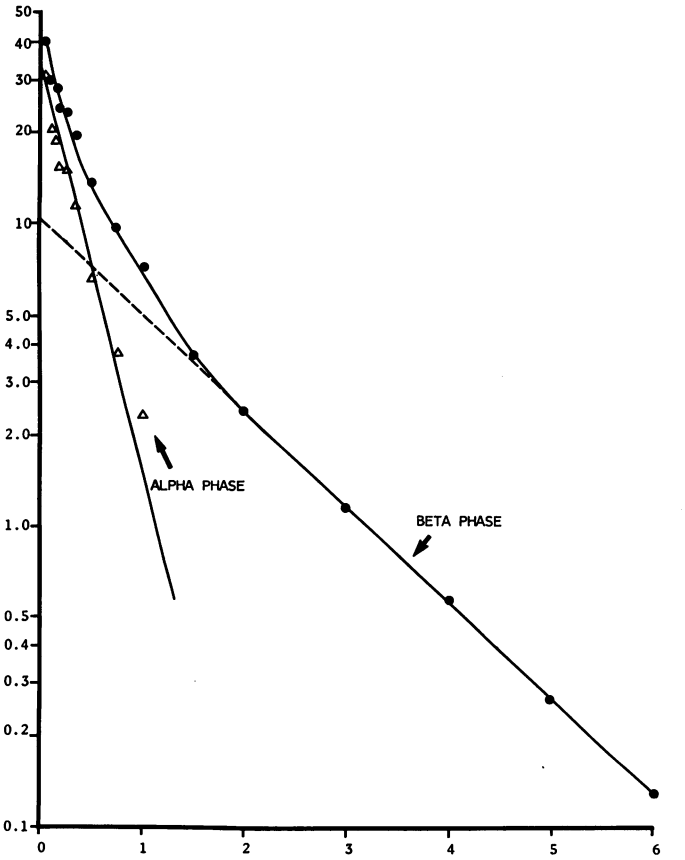

FIG. 2—Pharmacokinetic analysis of mean ampicillin serum concentrations in nine men following intravenous administration of 500 mg. Abscissa: time (hours) after administration. Ordinate: ampicillin serum concentration (μg/ml). Mean serum concentrations (•——•) as in Fig. 1. Refer to text for explanation of extrapolation of the beta phase to the ordinate (- - - -), determination of residual serum concentration (△——△), and definition of the alpha and beta phases.

the half life of the beta phase. Distribution of the drug occurs continuously in two directions, some drug molecules leaving the serum to enter the tissues and others leaving the tissues to re-enter the serum compartment. Each direction of drug flow has a separate rate constant, that from serum to tissue being termed K_{12} and that from tissue to serum K_{21}. The rate constant alpha is the net rate constant of distribution including K_{12} and K_{21}.

For ampicillin, the rate constant for distribution from serum to tissue is about one half that for tissue to serum, indicating that proportionately more ampicillin is in the serum compartment. The half life of the alpha phase, approximately 15 minutes, indicates that distribution occurs relatively rapidly as compared with elimination, where the half life of the beta phase was 1.0 hour.

The final important calculation that can be made from the graph is the total amount of drug that appears in the serum after intravenous administration. This amount is represented as the total area under the time versus serum drug concentration curve (abbreviated as AUC). While there are several methods of calculating this value, the simplest and most easily applied is the trapezoidal rule using the values obtained directly from the graph. Vertical lines are drawn from the horizontal (time) axis to the measured serum concentration curve at each sampling time. The area of each trapezoid so constructed is calculated in the usual way by dividing the sum of the two parallel sides by two and multiplying by the base (i.e. divide the sum of the drug concentrations at adjacent time intervals by two and multiply by the length of the time interval). This procedure is repeated until all the areas have been calculated as μg/ml x unit of time. Their sum represents the area under the curve from time 0 to the end of the experimental time, in this study six hours. In order to measure the length of the last interval, from six hours in this study to infinite time when serum concentrations fall to unmeasurable levels, the tail end of the beta phase is extrapolated to the horizontal axis and the area of the resultant triangle calculated and added to the previous total. This more complete area under the curve is referred to as $AUC_{0\rightarrow\infty}$ and since it was obtained after intravenous administration it serves as a basis for comparison with other routes of administration. For 500 mg of ampicillin the average value was 26.1 μg/ml x hours.

Oral administration

If we next consider ampicillin serum concentrations following oral administration (Fig. 3), the beta phase again can be identified and its rate constant and half life, in this case 1.0 hour, calculated from the terminal portion of the graph of the average serum concentrations. The alpha phase is less readily apparent after oral administration because another process, that of absorption, contributes to the initial portion of the graph. The initial steep part of the rising concentration is affected primarily by processes of absorption, while the less rapid incline represents a combination of absorption and distribution. To obtain a slope reflecting absorptive factors only, the serum concentration in each subject after both oral and intravenous administration must be analysed by special techniques involving curve fitting. By this means a straight line is obtained for the earliest concentration points after oral administration, representing a constant rate of absorption which is depicted by the rate constant K_A. A second and interesting determination that can be made from the

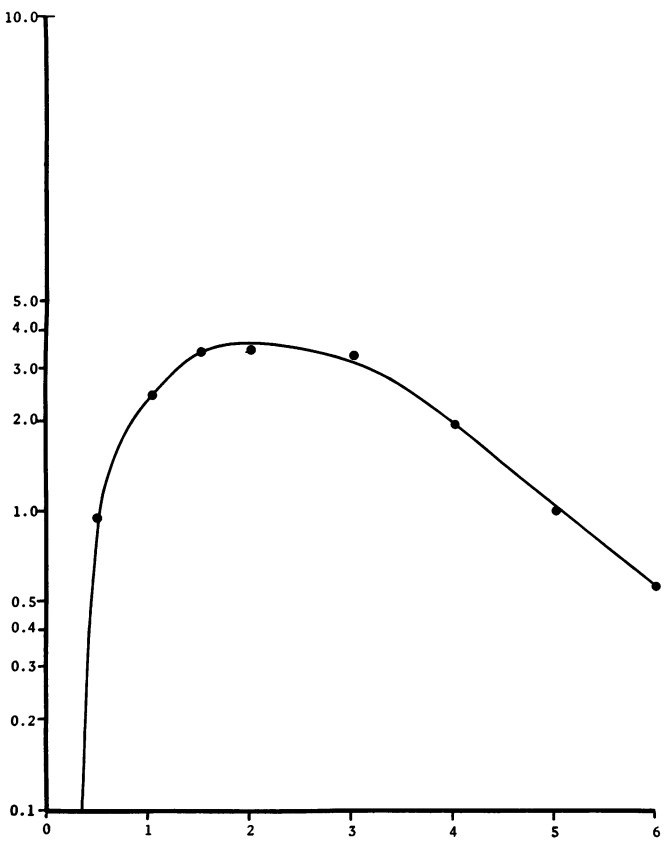

FIG. 3—Mean serum concentrations of ampicillin (μg/ml) in nine men following oral administration of 500 mg on three occasions. Abscissa: time (hours) after administration. Ordinate: ampicillin serum concentration (μg/ml).

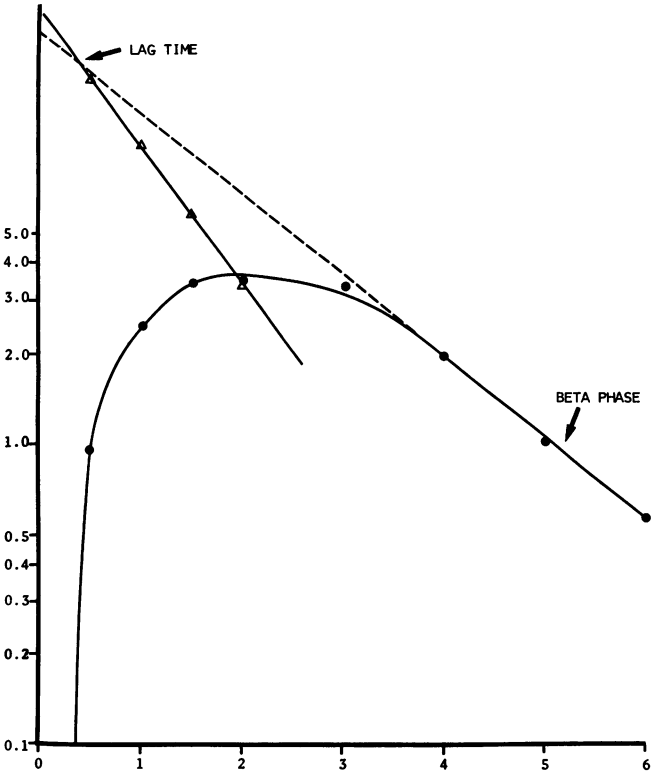

FIG. 4—Pharmacokinetic analysis of mean ampicillin serum concentrations in nine men following oral administration of 500 mg on three occasions. Abscissa: time (hours) after administration. Ordinate: ampicillin serum concentration (μg/ml). Mean serum concentrations (●——●) as in Fig. 3. Refer to text for explanation of extrapolation of the beta phase to the ordinate (- - - -), determination of residual serum concentrations (△——△), and definition of lag time and beta phase.

graph after oral administration is an estimation of the lag time, that is, the time that elapses between the ingestion of the drug and the appearance of the drug in the serum. If the terminal portion of the graph is extrapolated back to time 0 (Fig. 4), residual concentrations may be calculated by subtracting the observed serum concentrations from the concentrations along the extended line for each sample time. When these are replotted a straight line is obtained that intersects the extrapolated beta phase line shortly after time 0. The point of intersection indicates the lag time, and in this study it averaged 0.36 hours. Lag time varies for different drugs.[2] In this study it varied considerably from one individual to another, and the relationship between the lag time and the absorption rate constant K_A was not predictable. Also no correlation was evident between lag time and the total amount of drug absorbed as estimated by the AUC. As with the graph from the intravenous study, the $AUC_{0\rightarrow\infty}$ can be calculated using the trapezoidal rule, and represents the total amount of drug appearing in the serum after oral administration. For a 500-mg dose it averaged 12.2 μg/ml x hr.

Finally, because it is assumed that $AUC_{0\rightarrow\infty}$ after intravenous administration represents total absorption, the fraction of ingested drug absorbed from the gastrointestinal tract can be determined and expressed as the percentage of drug absorbed after oral administration by comparing the $AUC_{0\rightarrow\infty}$ of the orally administered drug with that of the same drug administered intravenously. The average for this study was 47%.

In the present ampicillin study all the above parameters were computed by means of an IBM 360 computer using the ASAAM-23 program provided by the National Institutes of Health, and an Olivetti 101 calculator. This approach provides rapid and accurate calculation of each parameter for each subject and these data are displayed in Table II for the intravenous study and Table III for the oral study.

Discussion

One of the principal features of this ampicillin study was the opportunity to study person-to-person variation in pharmacokinetics. We were surprised to find a twofold range in oral absorption even under controlled conditions. The calculated percentage of ingested drug absorbed varies from 31.8 to 64.1%. This range probably reflects individually determined characteristics of gastrointestinal function and perhaps alteration of the drug prior to its reaching the peripheral venous circulation. An even larger variation of the individual K_A is also observed in these volunteers.

This variation in the extent and rate of absorption between individuals, documenting the often referred to characteristics of good and poor absorbers, is most likely due to gastrointestinal factors including gastric pH, rate of stomach emptying, gastrointestinal motility, transit time and area of mucosa available for absorption. Many of these processes can be modified by autonomic nervous system activity, which may explain day-to-day variations in the same individual. The reason for the difference in lag time from 0 in one individual to almost half an hour in another is not readily explainable because of our lack of knowledge of factors influencing the time needed for the first appearance of drug in the serum. Possible therapeutic implications of this variable factor remain speculative.

While the dose administered was constant, the body size and habit of the volunteers varied considerably. Therefore it is not surprising that the apparent volume of distribution calculated after intravenous administration varies over more than a twofold range. This source of variation is not always suspected upon physical examination of the individual since it depends not only on total body water, but also upon other poorly defined factors including tissue depots, which change depending upon the drug. The volume of distribution is an important factor in the variability of attained serum concentrations.

Few complete pharmacokinetic studies of ampicillin have been reported. Early studies lacked adequate sampling and commented only on peak serum concentrations and areas under curves. More recent studies have been more specific in characterizing the pharmacokinetic pattern of ampicillin. The results obtained after intravenous administration of 250 mg to six normal men[3] were similar to those in this study for beta, half life of the beta phase, AUC (when adjusted for dose) and volume of distribution. However, the values obtained in the present study for the distribution rate constants alpha, K_{12}, K_{21} and K_{el} are lower than those obtained in the previous study. Because the distribution phase constants are obtained from the portion of the curve requiring dilution of serum samples, this potential source of error may account for the differences.

In another study doses comparable to those used here were administered intravenously and orally to four normal men and four normal women.[4] After intravenous administration the mean area under the time serum con-

Table III—Pharmacokinetic parameters of ampicillin in nine men following oral administration of 500 mg on three occasions

Subject	$AUC_{0\rightarrow\infty}$ μg/ml × hr	K_{el} hours^{-1}	K_{12} hours^{-1}	K_{21} hours^{-1}	α hours^{-1}	$t_{\frac{1}{2}\alpha}$ hours	β hours^{-1}	$t_{\frac{1}{2}\beta}$ hours	K_A hours^{-1}	Lag time hours	% absorption
1	9.35	2.129	0.403	1.259	2.851	0.24	0.940	0.74	0.646	0.40	38.6
2	11.32	1.529	0.375	1.147	2.283	0.30	0.768	0.90	0.572	0 40	39.6
3	11.49	1.540	0.380	1.342	2.401	0.29	0.861	0.80	0.657	0.47	41.6
4	11.05	1.414	0.343	1.008	2.080	0.33	0.685	1.01	0.324	0.35	44.5
5	7.28	1.950	0.498	1.379	2.899	0.24	0.927	0.75	0.361	0.30	31.8
6	16.46	1.653	0.686	1.829	3.233	0.21	0.935	0.74	0.720	0.0	49.5
7	12.34	1.191	0.510	1.173	2.254	0.31	0.620	1.12	0.367	0.42	61.9
8	15.78	2.189	1.230	1.563	4.159	0.17	0.823	0.82	0.183	0.45	64.1
9	15.11	1.499	0.705	1.216	2.759	0.25	0.661	1.05	0.440	0.47	52.4
Mean ± SE	12.24 ± 1.02	1.677 ± 0.113	0.570 ± 0.094	1.324 ± 0.082	2.769 ± 0.214	0.26 ± 0.02	0.802 ± 0.041	0.88 ± 0.05	0.474 ± 0.061	0.36 ± 0.05	47.1 ± 3.6

centration curve in that study was 13% smaller than that reported here. The values reported for the distribution rate constants were also somewhat smaller than those found in the present study. When the same subjects were given ampicillin orally the percentage of the dose absorbed varied from 21 to 46% with an average of 32%. In the present study the percentage absorption observed averaged 47%, but the range of variation between individuals was similar (32 to 64%).

Considerable person-to-person variation in the extent and rate of absorption seldom constitutes a critical therapeutic factor for the usual indications of ampicillin as long as the dose used is sufficiently great to give therapeutic blood concentrations. When "overdosing" is sufficient to allow for the unpredictable absorption characteristics of individuals, few therapeutic failures are likely to result. For ampicillin, overdosing is not particularly hazardous and therefore administration of larger than necessary doses is common practice.

Other drugs may also be absorbed to varying extents, but may have greater toxicity. With these drugs it is important to calculate as accurately as possible the dose required to obtain therapeutic, but not toxic, concentrations. If possible, blood samples should be obtained for determination of the serum concentration of the drug in a given individual. It must be noted that the serum concentration is assumed to reflect adequately the concentration of the drug at its tissue site of action only when the major phases of absorption and distribution have been completed.

In addition, disease states can alter absorption from various sites of administration, as exemplified by diarrhea in gastrointestinal administration, or decreased perfusion to the site of subcutaneous or intramuscular injection. The effect of renal disease on excretion of drugs, and liver disease on metabolism of chemicals is obvious. Changes in vascular and extravascular fluid volumes can alter apparent volume of distribution, as can altered protein binding, which is seen in concomitant administration of more than one drug or in diseases that alter the amount and structure of proteins.

For the majority of drugs, information about percentage absorption following oral administration, serum half life and serum concentrations following particular doses are guides to the rational use of the drug. Differences between individuals and possible alterations of normal physiology produced by disease must be considered. When failure of efficacy or toxicity is suspected, one should measure directly the serum concentration of the drug.

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