An increase or a decrease in the apparent partition coefficient of drug B ($PC_{app.}$) when partially complexed with drug A indicates that the ratio $[A\text{-}B_o]/[A\text{-}B_a]$ is greater or less, respectively, than the ratio $[B_o]/[B_a]$, or K_2.[5]

However, the ratio $[A\text{-}B_o]/[A\text{-}B_a]$, the apparent partition coefficient of the complex, reflects only the equilibrium condition and does not give any indication of the relative contribution of each of the several pathways by which this equilibrium can be attained. In fact, it can be shown algebraically that the ratio $[A\text{-}B_o]/[A\text{-}B_a]$ is equal to $K_1K_2K_5/K_3$ whether or not the pathway represented by K_4 actually exists.

It is evident from the above discussion that, unlike the partition coefficient of a pure substance which reflects the ratio of its interfacial transfer rate constants in both directions, the apparent partition coefficient of a complex does not necessarily reflect the rates of transfer of the complex itself from one phase to the other. Yet, it is this direct transfer of the complex itself which would most likely bring about a change in the overall absorption rate of complexed drugs. Herein lies the limitation of the use of partitioning data, which are representative of an equilibrium situation, for correlation with intestinal transfer data, which represent a rate phenomenon. The lack of correlation between the transfer rate constants and partition coefficients of salicylamide, caffeine, and the salicylamide–caffeine complex is consistent with this reasoning.

[5] This statement can be substantiated mathematically, taking as an example the case where complexation results in an increase in the apparent partition coefficient ($PC_{app.} > K_2$). If $PC_{app.} > K_2$, then

$$\frac{[B_o] + [A\text{-}B_o]}{[B_a] + [A\text{-}B_a]} > \frac{[B_o]}{[B_a]}$$

Cross-multiplying yields

$$[B_o][B_a] + [A\text{-}B_o][B_a] > [B_o][B_a] + [B_o][A\text{-}B_a]$$
$$[A\text{-}B_o][B_a] > [B_o][A\text{-}B_a]$$

Therefore,

$$\frac{[A\text{-}B_o]}{[A\text{-}B_a]} > \frac{[B_o]}{[B_a]}$$

It appears that factors which determine the rate of transfer of drug complexes across biologic membranes are likely to be resolved only by a consideration of physicochemical effects at the biologic interface and not on the basis of *in vitro* apparent partition coefficient data or transfer experiments using simple nonbiologic membranes or barriers (10). It is therefore appropriate to direct further attention to the equilibria which exist at the surface of biologic membranes in systems consisting of two interacting drugs. This will be the subject of the following paper.

REFERENCES

(1) Reuning, R. H., and Levy, G., *J. Pharm. Sci.*, in press
(2) Crane, R. K., and Wilson, T. H., *J. Appl. Physiol.*, **12**, 145(1958).
(3) Reuning, R. H., and Levy, G., *J. Pharm. Sci.*, **57**, 1335(1968).
(4) "Evaluation of Drug Activities: Pharmacometrics," vol. 2, Laurence, D. R., and Bacharach, A. L., Eds., Academic Press Inc., New York, N. Y., 1964, p. 892.
(5) Levy, G., and Matsuzawa, T., *J. Pharmacol. Exptl. Therap.*, **156**, 285(1967).
(6) Reuning, R. H., and Levy, G., submitted for publication.
(7) Barr, W. H., personal communication.
(8) Schanker, L. S., Shore, P. A., Brodie, B. B., and Hogben, C. A. M., *J. Pharmacol. Exptl. Therap.*, **120**, 528(1957).
(9) Hogben, C. A. M., Tocco, D. J., Brodie, B. B., and Schanker, L. S., *ibid.*, **125**, 275(1959).
(10) Levy, G., and Mroszczak, E. J., *J. Pharm. Sci.*, **57**, 235(1968).

Keyphrases

Drug absorption—complex formation effect
Salicylamide-caffeine complex—transfer rate
Caffeine retardation—salicylamide transfer
Everted intestine—experimental technique
Partition coefficients-transfer rates—comparison
UV spectrophotometry—analysis

Thiopental Pharmacokinetics

By K. B. BISCHOFF* and R. L. DEDRICK

A mathematical pharmacokinetic model, including flow limitations, lipid solubility, protein binding, and metabolism, is used to make *a priori* predictions of the distribution of thiopental in four body regions. Tissue binding is correlated by means of "effective" protein fractions and bovine serum albumin data. Metabolism is represented by a Michaelis-Menten rate equation. Close agreement with existing experimental data lends confidence in the model as a valuable tool for predictions in a variety of therapeutic situations.

MANY PRIOR studies of thiopental pharmacokinetics have been made, but there remains some question concerning the relative importance of metabolism and lean and adipose tissue uptakes. Some years ago, Brodie and co-workers (1, 2) made quantitative measurements of thiopental concentrations in various body regions and concluded that significant amounts were present

Received January 26, 1968, from the National Institutes of Health, U. S. Public Health Service, Bethesda, MD 20014
Accepted for publication April 2, 1968.
The authors greatly appreciate helpful discussions with Dr. James A. Gillette of the National Heart Institute.
* Present address: Department of Chemical Engineering, University of Maryland, College Park, MD 20740

in adipose tissue. The overall rate of metabolism in man was about 15%/hr. and was believed insufficient to account for the observed ultrashort duration of action. Price *et al.* (3) arrived at similar conclusions from mathematical analysis and an analog computer simulation, except that they attributed more of the drug redistribution effects to the lean body mass. Mark and Brand (4) have reviewed much of this work. A somewhat different viewpoint was taken in a recent analog computer study by Saidman and Eger (5) in that a much more important contribution of metabolism was assumed.

BASIS OF MODEL

The purpose here is to devise a mathematical pharmacokinetic model incorporating as many as possible of the variables known to affect drug distribution. Previous work concerning the dynamics of vascular tracers required detailed examination of blood volume distribution and flow rates (6, 7). Since the time scale of interest in the present paper is on the order of a few minutes to several hours, it is permissible to identify certain gross "lumped" regions over which relevant values of perfusion or physical properties may be considered constant. This, then, leads to the same general breakdown used by some previous workers: (*a*) blood, (*b*) viscera (highly perfused lean tissue), (*c*) poorly perfused lean tissue, and (*d*) poorly perfused adipose tissue.

Values of the regional flow and volume parameters representing the normal adult male were taken from Mapleson's detailed data for several body regions (8). Such detail might be of use in future studies, but sufficient thiopental distribution data are not presently available to justify such a complex pharmacokinetic study at this time. Figure 1 illustrates the four body regions and representative numerical parameters.

A useful mathematical model must explicitly include resistances to mass transfer by membrane permeability and constraints caused by flow limitation. Since thiopental is highly lipid soluble, the authors assume, as have earlier workers, that membrane transport is very rapid and the controlling resistance is flow limitation for all regions. A more detailed analysis is required for other solutes which might have lower lipid solubility. For thiopental, then, only the total tissue volumes, quantities of associated equilibrium blood, and blood flows are required, as shown in Fig. 1.

A possible complication that cannot be accommodated by simple compartmental models has recently been discussed by Rackow, Perl, and co-workers (9, 10), in which direct molecular diffusion occurs between two adjacent tissue regions. This would be most likely to occur between adjacent layers of well perfused lean and poorly perfused adipose tissue where the solute is highly lipid soluble. The initial adipose uptake then could be more a result of direct diffusion from the lean tissue than from its own low perfusion. Perl *et al.* (10) considered this effect for fat-soluble gases and showed that certain discrepancies in the data could thereby be explained. For the longer time intervals of interest here, however, and for more slowly diffusing thiopental, it is felt that this effect can be neglected.

Another complication, until recently neglected in the pharmacology and anesthesiology literature, is the phenomenon of protein binding. A good general discussion has been presented by Gillette (11), who illustrated the significance of binding of several drugs. An extensive consideration of binding as it affects pharmacokinetics analyses is found in a series of papers by Krüger-Thiemer (a few of the most recent are 12–14). For cases such as sulfa drugs, only entire body blood and tissue breakdowns were used, and it was clearly shown how binding can alter the features of the mathematical model. For example, the use of an overall distribution coefficient relating blood and tissue concentrations is not possible, since these ratios are a function of the concentration level when nonlinear binding occurs. Also, since the free (unbound) concentration is presumed to be the proper basis for pharmacological action, the extent of binding must be accounted for in order to produce meaningful predictions. The present work includes binding effects; the details will be discussed later.

MATHEMATICAL MODEL

Transient mass balances can be written for each of the body regions considered, assuming flow-limiting conditions. For the blood compartment:

$$\underset{\text{accumulation of unbound drug}}{f_B V_B \frac{dC_B}{dt}} + \underset{\text{accumulation of bound drug}}{(1 - f_B) V_B \frac{dx_B}{dt}}$$

$$= \underset{\text{outflow of unbound and bound drug}}{- Q_B [f_B C_B + (1 - f_B) x_B]}$$

$$\underset{\text{inflow from other regions of unbound and bound drug}}{+ Q_V [f_{VB} C_V + (1 - f_{VB}) x_{VB}] + Q_L [f_{LB} C_L + (1 - f_{LB}) x_{LB}] + Q_A [f_{AB} C_A + (1 - f_{AB}) x_{AB}]}$$

$$\underset{\text{ingestion term}}{+ Mg(t)} \qquad \text{(Eq. 1)}$$

The physical meaning of each term is indicated below it for clarity, and the particular symbols are defined under *Notation*.

A similar mass balance for the viscera gives:

$$\underset{\text{accumulation of unbound drug in equilibrium blood and tissue}}{(f_{VB} V_{VB} + f_{VT} V_{VT}) \frac{dC_V}{dt}} + \underset{\text{accumulation of bound drug in equilibrium blood}}{(1 - f_{VB}) V_{VB} \frac{dx_{VB}}{dt}}$$

$$\underset{\text{accumulation of bound drug in tissue}}{+ (1 - f_{VT}) V_{VT} \frac{dx_{VT}}{dt}}$$

$$= \underset{\text{net inflow of unbound and bound drug}}{Q_V [f_B C_B + (1 - f_B) x_B - f_{VB} C_V - (1 - f_{VB}) x_{VB}]}$$

$$\underset{\text{rate of metabolism of drug}}{+ r_V(C_V)} \qquad \text{(Eq. 2)}$$

The metabolism rate term is written in the simple Michaelis-Menten form for the calculations:

$$r_V(C_V) = -\frac{k_V C_V}{K_{mV} + C_V} \qquad \text{(Eq. 3)}$$

Since the metabolism mainly occurs in the microsomes of the liver (11), the rate of reaction, r_V, appears in the balance for the visceral region. Similar terms could be added to the other balances if required.

The remaining mass balances have a similar form.

For lean tissue:

$$(f_{LB}V_{LB} + f_{LT}V_{LT})\frac{dC_L}{dt} + (1 - f_{LB})V_{LB}\frac{dx_{LB}}{dt} + (1 - f_{LT})V_{LT}\frac{dx_{LT}}{dt} = Q_L[f_B C_B + (1 - f_B)x_B - f_{LB}C_L - (1 - f_{LB})x_{LB}] \qquad \text{(Eq. 4)}$$

For adipose tissue:

$$(f_{AB}V_{AB} + f_{AT}V_{AT})\frac{dC_A}{dt} + (1 - f_{AB})V_{AB}\frac{dx_{AB}}{dt} + (1 - f_{AT})V_{AT}\frac{dx_{AT}}{dt} = Q_A[f_B C_B + (1 - f_B)x_B - f_{AB}C_A - (1 - f_{AB})x_{AB}] \qquad \text{(Eq. 5)}$$

Explicit relations between the various bound and free concentrations are required to solve Eqs. 1–5. For blood plasma protein binding, the results of Goldbaum and Smith (15) are used. They correlated their bovine serum albumin data with the commonly used (see *Reference 11*) two-term relation,

$$x_B = \frac{B_1K_1C_B}{1 + K_1C_B} + \frac{B_2K_2C_B}{1 + K_2C_B} \qquad \text{(Eq. 6)}$$

Goldbaum and Smith (15) also presented data on rabbit tissue binding of a number of barbiturates. In the absence of more information, it is assumed that the relationship for binding by plasma proteins could also be used for tissue protein binding. "Effective tissue protein fractions" are assigned to produce the measured tissue drug concentrations. This assumption is justified on the basis that consistent "effective tissue protein fractions" are found to result for all the barbiturates studied. Further justification of this method with other classes of compounds would be useful.

With this assumed basis, all of the binding relations take on similar functional forms, each with the appropriate free concentration inserted. For example, the viscera binding relations become:

$$x_{VT} = x_{VB} \equiv x_V = \frac{B_1K_1C_V}{1 + K_1C_V} + \frac{B_2K_2C_V}{1 + K_2C_V} \qquad \text{(Eq. 7)}$$

and the lean tissue values,

$$x_{LT} = x_{LB} \equiv x_L = \frac{B_1K_1C_L}{1 + K_1C_L} + \frac{B_2K_2C_L}{1 + K_2C_L} \qquad \text{(Eq. 8)}$$

Adipose tissue must be handled somewhat differently. The equilibrium blood plasma binding has the usual form,

$$x_{AB} = \frac{B_1K_1C_A}{1 + K_1C_A} + \frac{B_2K_2C_A}{1 + K_2C_A} \qquad \text{(Eq. 9)}$$

but the tissue "binding" here is primarily the lipid solubility for which a simple Henry's law formula is used,

$$x_{AT} = B_A C_A \qquad \text{(Eq. 10)}$$

A final simplification assumes that the fractions of protein in all the various blood regions are the same,

$$f_B = f_{VB} = f_{LB} = f_{AB} \qquad \text{(Eq. 11)}$$

These considerations lead to simplifications in Eqs. 1–5 which can now be solved if the input or ingestion function is specified. An approximation to a normalized impulse function similar to that of Bellman *et al.* (16) is used,

$$g(t) = 30\lambda(\lambda t)^2(1 - \lambda t)^2 \qquad \text{(Eq. 12)}$$

where λ is the reciprocal injection duration. This function simulates a smooth injection and is convenient for computation.

RESULTS AND DISCUSSION

Numerical values for the parameters in the above mass balance equations are provided by the data of Mapleson (8) (see Fig. 1) and Goldbaum and Smith (15), except for the metabolism term. In a comprehensive review paper, Mark (17) presents values for the overall rates of metabolism of barbiturates, which for thiopental is about 15%/hr. The parameters k_V and K_{mV} in Eq. 3 are empirically adjusted to provide values of k_V = 26.5 μmole/min. and K_{mV} = 4 (μmole/l.), which gave the desired rate. The recent data of Mark *et al.* (18) can be correlated by a Michaelis-Menten kinetic form to

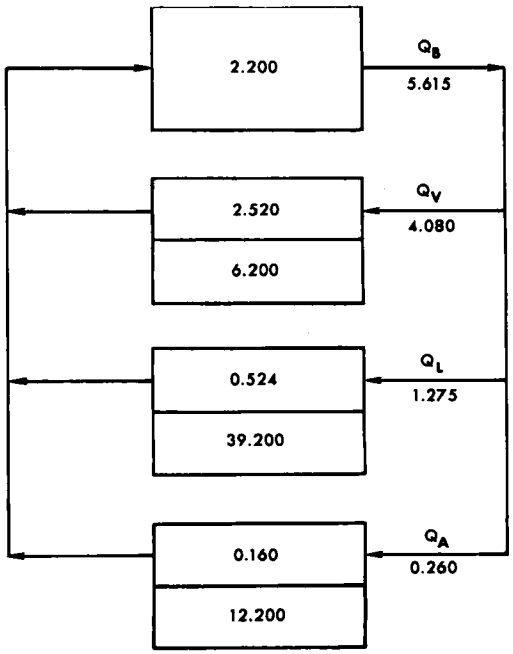

Fig. 1—Body regions and flows. Key: B, blood pool not in equilibrium with tissues; V, viscera; L, lean tissue; A, adipose tissue.

TABLE I—PARAMETER VALUES

Parameter	Numerical Value
Flow distribution, l./min.	$Q_B = 5.615$, $Q_V = 4.080$, $Q_L = 1.275$, $Q_A = 0.260$
Body region size, l.	$V_B = 2.20$, $V_{VB} = 2.52$, $V_{VT} = 6.20$, $V_{LB} = 0.524$, $V_{LT} = 39.20$, $V_{AB} = 0.160$, $V_{AT} = 12.200$
"Effective" fraction water	$f_B = 0.985$, $f_{VT} = 0.963$, $f_{LT} = 0.980$, $f_{AT} = 0.20$
Binding sites, μmole/l.	$B_1 = 18{,}400$, $B_2 = 305{,}000$
Binding equilibrium constant, l./μmole	$K_1 = 0.060$, $K_2 = 0.000625$
Lipid solubility constant	$B_A = 100$

produce parameter values of the same order of magnitude as those required to obtain the 15%/hr. rate. This lends some confidence to the metabolism rate expression, but the need for more quantitative chemical kinetics work remains clear. The lipid solubility constant lies between the value for peanut oil–water from Mark *et al.* (19) and the value from Price *et al.* (3), corrected for protein binding.

Analytical solutions of the differential equations are not feasible because of the nonlinearities caused by the binding terms; the equations have therefore been solved numerically using a digital computer. As a critical test of the pharmacokinetics model, an *a priori* prediction of the experimental results of Brodie *et al.* (1, 2) on thiopental distribution in dogs was attempted. *A priori* here implies that numerical values of all the parameters in the model (except metabolism) were taken from sources other than Brodie *et al.*: human flow distribution and body region size from Mapleson (8); bovine serum albumin and rabbit tissue binding from Goldbaum and Smith (15); lipid solubility from Mark *et al.* (19) and Price *et al.* (3). The numerical values used in the computations are shown in Table I.

In the cited experiments of Brodie *et al.* (1, 2) 25 mg./kg. thiopental was injected i.v. with a 5-min. injection time. Thus, the final two parameters in the model are $M = 6630$ μmoles (for a 70-kg. man) and $\lambda = 0.20$ min.$^{-1}$.

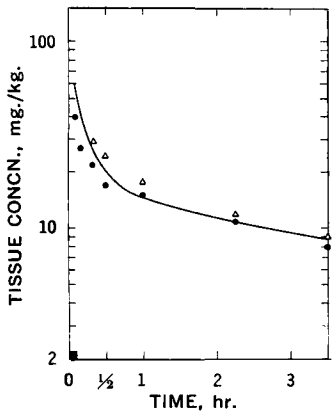

Fig. 2—Comparison of predicted and experimental values for blood. Key: ■, injection duration; ●, dog; Δ, human.

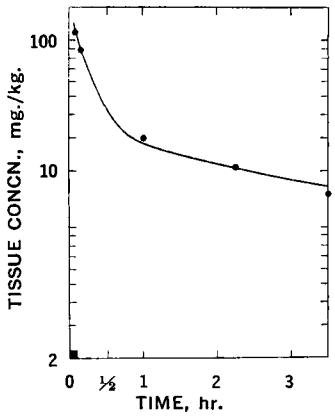

Fig. 3—Comparison of predicted and experimental values for viscera (liver). Key: ■, injection duration; ●, dog.

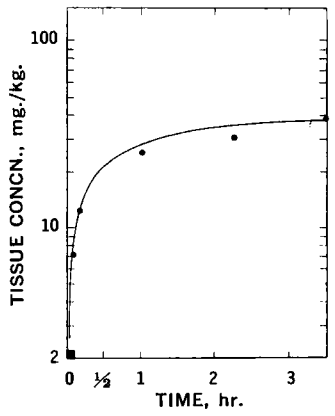

Fig. 4—Comparison of predicted and experimental values for adipose tissue. Key: ■, injection duration; ●, dog.

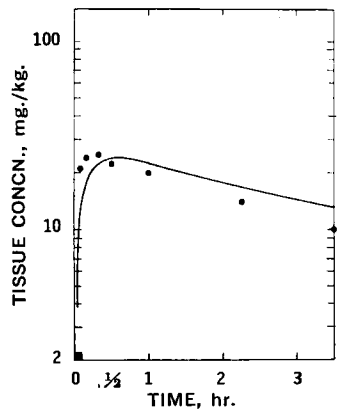

Fig. 5—Comparison of predicted and experimental values for lean tissue. Key: ■, injection duration; ●, dog.

The results of the numerical computations are presented in the graphs. Figure 2 compares the *a priori* predicted and measured values in blood for dogs and for man. The total concentration, bound plus unbound, is plotted. Several features of the graph are worthy of note. First, the pre-

dicted values from the mathematical model agree well with the experimental data. Second, the physical distribution patterns for dog and man are very similar when plotted in a consistent manner. Third, rapid fall in blood thiopental levels is observed for the first half-hour as distribution equilibrium is approached. And finally, the slope of the line at long times principally represents metabolism at the rate of 15%/hr.

Figure 3 compares the computed and experimental (dog) values for the highly perfused or viscera compartment. Again, excellent agreement is obtained. The uptake in adipose tissue is illustrated in Fig. 4, which shows the rapid and continuing solution of the drug in the lipid at the perfusion rate of 2 (ml./min.)/100 g. used (see Table I). The curve for the final compartment, poorly perfused lean tissue, is shown in Fig. 5. The overall predicted trends are correct, but there are some discrepancies between the calculated curve and the experimental data. At short time intervals, the difference can probably be explained by the fact that human flow distribution and perfusion rates are used but that experimental data were obtained on dogs. Since lean tissue of dogs is somewhat more highly perfused than that of humans [value used—3 (ml./min.)/100 g], the measured initial uptake is higher than predicted. Calculations made with a higher lean perfusion rate shift the predicted curves in the correct direction. Since the intent was to see how good an *a priori* prediction could be made, however, these "curve fitting" attempts were not pursued in any detail. The long time portion of the curve also deviates from the data, but slight changes in the lipid solubility produce better agreement; again, a better "curve fit" has not been attempted.

Figure 6 shows the computed behavior over a period of several hours. It is seen that the three nonlipid compartments rapidly approach distribution equilibrium, but that the adipose uptake rises continuously to a maximum at about 3–4 hr. This also corresponds with the observations of Brodie *et al.* (2).

A final very interesting result is exhibited by the plot of computed *free* concentrations in each of the compartments shown in Fig. 7. The blood and viscera free concentrations fall very rapidly and, more important, are essentially equal over the entire time period. This is a good illustration of the concept that blood concentrations are an excellent measure of the viscera concentrations and thus

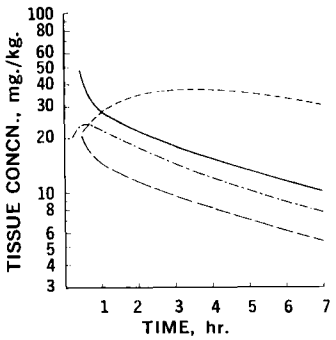

Fig. 6—Long time behavior—total concentration. Key: ———, viscera; – – – –, adipose; — · —, lean; — —, blood.

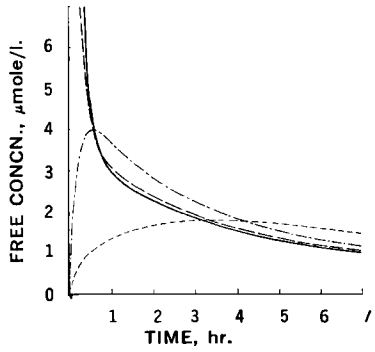

Fig. 7—Long time behavior—free concentration. Key: ———, viscera; – – – –, adipose; — · —, lean; — —, blood.

should provide a basis for correlating pharmacological action. Notice, however, that this is true *only* for the free concentrations (*i.e.*, corrected for binding), since Fig. 6 indicates that the total concentrations in the blood and viscera are not equal.

CONCLUSION

The *a priori* predictions of thiopental distribution resulting from the pharmacokinetic model, including protein binding and lipid solubility, agree closely with published experimental data. Such performance provides confidence in the model as a valuable tool for predictions in a variety of therapeutic situations if it is properly applied and sufficient data are available. One application reported elsewhere (20, 21) considers the physiological solute distributions associated with the extracorporeal treatment of blood with an artificial kidney.

NOTATION

B_A = lipid solubility in adipose tissue (dimensionless)
B_1, B_2 = protein binding sites (μmole/l.)
C = free concentration (μmole/l.)
f = fraction water (dimensionless)
$g(t)$ = injection description function (min.$^{-1}$)
k_V = metabolism rate constant or maximum rate (μmole/min.)
K_{mV} = Michaelis-Menten constant (μmole/l.)
K_1, K_2 = protein binding equilibrium constants (l./μmole)
M = amount of drug injected (μmole)
Q = flow rate (l./min.)
r_V = metabolism rate (μmole/min.)
t = time (min.)
x = bound concentration (μmole/kg.)
V = volume of compartment (l.)
λ = reciprocal injection of time (min.$^{-1}$)

Subscripts

A = adipose
B = blood
L = lean
V = viscera
AB = adipose equilibrium blood
LB = lean equilibrium blood
VB = viscera equilibrium blood
AT = adipose tissue
LT = lean tissue
VT = viscera tissue

REFERENCES

(1) Brodie, B. B., Mark, L. C., Papper, E. M., Lief, P. A., Bernstein, E., and Rovenstine, E. A., *J. Pharmacol. Exptl. Therap.*, **98**, 85(1950).
(2) Brodie, B. B., Bernstein, E., and Mark, L. C., *ibid.*, **105**, 421(1952).
(3) Price, H. L., Kovnat, P. J., Safer, J. N., Conner, E. H., and Price, M. L., *Clin. Pharmacol. Therap.*, **1**, 16(1960).
(4) Mark, L. C., and Brand, L., *Bull. N. Y. Acad. Med.*, **40**, 476(1964).
(5) Saidman, L. J., and Eger, E. I., *Anesthesiology*, **27**, 118(1966).
(6) Bischoff, K. B., and Brown, R. G., *Chem. Eng. Prog. Symp. Ser. No. 66*, **62**, 32(1966).
(7) Bischoff, K. B., in "Chemical Engineering in Medicine and Biology," Hershey, D., ed., Plenum Press, New York, N. Y., 1967, p. 417.
(8) Mapleson, W. W., *J. Appl. Physiol.*, **18**, 197(1963).
(9) Rackow, H., Salanitre, E., Epstein, R. M., Wolf, G. L., and Perl, W., *ibid.*, **20**, 611(1965).
(10) Perl, W., Rackow, H., Salanitre, E., Wolf, G. L., and Epstein, R. M., *ibid.*, **20**, 621(1965).
(11) Gillette, J. R., in "Drugs and Enzymes," *Proc. 2nd Intl. Pharmacol. Mtg.*, 1965, p. 9.
(12) Krüger-Thiemer, E., Diller, W., Dettli, L., Bünger, P., and Seydel, J., *Antibiot. Chemotherap.*, **12**, 171(1964).
(13) Krüger-Thiemer, E., Bünger, P., Dettli, L., Spring, P., and Wempe, E., *Chemotherapia*, **10**, 61, 129, 325(1965–1966).
(14) Krüger-Thiemer, E., Diller, W., and Bünger, P., in "Antimicrobial Agents and Chemotherapy—1965," Hobby, G. L., ed., Am. Soc. Microbiol., Ann Arbor, Mich., 1966.
(15) Goldbaum, L. R., and Smith, P. K., *J. Pharmacol Exptl. Therap.*, **111**, 197(1954).
(16) Bellman, R., Jacquez, J. A., and Kalaba, R., *Bull. Math. Biophys.*, **22**, 181, 309(1960).
(17) Mark, L. C., *Clin. Pharmacol. Therap.*, **4**, 504(1963).
(18) Mark, L. C., Brand, L., Kamvyssi, S., Britton, R. C., Perel, J. M., Landrau, M. A., and Dayton, P. G., *Nature*, **206**, 1117(1965).
(19) Mark, L. C., Burns, J. J., Brand, L., Campomanes, C. I., Trousof, N., Papper, E. M., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **123**, 70(1958).
(20) Bischoff, K. B., and Dedrick, R. L., *Proc. Ann. Conf. Eng. Med. Biol.*, **1967**, p. 8.6.
(21) Dedrick, R. L., and Bischoff, K. B., *Chem. Eng. Prog. Symp. Ser.*, No. 84, **64**, 32(1968).

Keyphrases

Pharmacokinetics—thiopental
Thiopental—distribution
Protein binding—thiopental
Model, mathematical—thiopental distribution

Effect of Chlordan Pretreatment on the Metabolism and Lethality of Cyclophosphamide

By ROBERT L. DIXON

Cyclophosphamide is converted to a cytostatic agent by enzymes present in the hepatic microsomes. Prior treatment of young rats with the insecticide, chlordan, resulted in an increased cyclophosphamide toxicity. Chlordan pretreatment also increased the *in vitro* metabolism of cyclophosphamide and hexobarbital, increased the excretion of the alkylating metabolite of cyclophosphamide *in vivo*, and decreased the sleeping times of animals treated with hexobarbital *in vivo*. It is most probable that increased amounts of cytostatic metabolite occurred as a result of chlordan induced microsomal drug-metabolizing enzyme stimulation.

CYCLOPHOSPHAMIDE[1] [*N,N*-bis(β-chloroethyl)-*N'*, *O*-propylene phosphoric acid ester diamine monohydrate, NSC 26271] is a potent antineoplastic agent of the nitrogen mustard class and has been used in the treatment of patients with many types of neoplastic diseases. Cyclophosphamide is especially valuable in the treatment of patients with malignancies arising from the hematopoietic tissues. The compound can be administered by all routes (1).

In contrast to the nitrogen mustards ordinarily used in cancer chemotherapy, cyclophosphamide is inert when placed in direct contact with bacteria, leukocytes, and most tumor cells in culture. *In vivo* activation occurs in the liver and perhaps in other sites (2). The subcellular site of enzymic activation in the liver is the microsomes. These hepatic microsomal drug-metabolizing systems require oxygen and NADPH (TPNH) and are capable of altering a number of drugs (3). The microsomal enzyme that activates cyclophosphamide is influenced by a number of various drugs (3). For instance, SKF-525a (β-diethylamino-ethyldiphenylpropylacetate), an inhibitor of microsomal drug metabolism, will decrease serum levels of metabolite capable of alkylation after cyclophosphamide (4), while pretreatment of animals with phenobarbital increases the concentration of this metabolic agent three to fourfold by enzyme induction (5).

The fact that the concentration of metabolite capable of alkylation after cyclophosphamide treatment can be increased by enzyme induction

Received November 6, 1967, from the Department of Pharmacology, School of Medicine, University of Washington, Seattle, WA 98105
Accepted for publication April 18, 1968.
Supported by grants from the National Institutes of Health (HD 02350 and CA 08671), Bethesda, Md.
The author wishes to acknowledge the very able technical assistance of Mrs. Valerie Campell during this project.

[1] Marketed as Cytoxan by Mead Johnson & Co., Evansville, Ind.