Thyroid and thyroid tablets ranging in strength from 16 to 325 mg were analyzed for total iodine, liothyronine, and thyroxine by the proposed method. In addition, total iodine was determined by the USP XIX procedure. The results are given in Tables II and III. The iodine results agree with the USP analysis, and the values of liothyronine and thyroxine found in thyroid agree with literature results (1).

The procedures described in this work are relatively straightforward and are specific for the analysis of thyroid since intact liothyronine and thyroxine are determined.

REFERENCES

(1) J. E. Moody, Jr., J. R. Hohmann, Jr., and G. B. Kaplan, *J. Pharm. Sci.*, **57**, 634 (1968).

(2) R. Bilous and J. J. Windheuser, *ibid.*, **62**, 274 (1973).

(3) G. Knapp, H. Spitzy, and H. Leopold, *Anal. Chem.*, **46**, 724 (1974).

(4) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed., vol. II, Interscience, New York, N.Y., 1955, pp. 772–776.

(5) M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy," rev. English ed., Interscience, New York, N.Y., 1958, pp. 216–221.

(6) D. C. M. Adamson, A. P. Domleo, J. F. Jeffries, and W. A. C. Shaw, *J. Pharm. Pharmacol.*, **4**, 760 (1952).

ACKNOWLEDGMENTS

The authors thank Dr. Thomas Medwick, Science Advisor, Food and Drug Administration, Brooklyn, N.Y., and Professor of Pharmaceutical Chemistry, Rutgers University, New Brunswick, N.J., for assistance in the preparation of this paper.

Nonlinear Model for Acetazolamide

ROBERT L. KUNKA * and ALBERT M. MATTOCKS ˣ

Received March 20, 1978, from the *School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication August 31, 1978.

*Present address: School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.

Abstract □ Intravenous bolus injections of ^{14}C-labeled acetazolamide were made in rabbits. Plasma, urine, and washed red blood cell concentrations were measured, the latter indicating bound drug. AUTOAN and NONLIN were used to fit the plasma data to a linear two-compartment model. However, utilization of the urine and red blood cell data suggested that a nonlinear model was more appropriate. The developed nonlinear system uses a one-compartment model with two tissue-binding parameters. The system simultaneously fits three equations describing drug in the plasma, in the body, and bound to red blood cells. Six parameters were estimated. The initial plasma concentration and the maximum amount bound to tissue protein (minus red blood cell protein) correlated with dose. The dissociation constant from this protein fraction suggested that it is composed mainly of the enzyme, carbonic anhydrase. The dissociation constant for the red blood cell fraction suggested that the drug binds to other protein in addition to carbonic anhydrase. The elimination constants were quite similar, indicating little variation from one animal to another. Utilization of the concepts of site and mechanism of action in this model should be of considerable help in relating drug concentration to pharmacological response.

Keyphrases □ Acetazolamide—nonlinear pharmacokinetic model, plasma, urine, and red blood cell data, intravenous bolus injection of ^{14}C-acetazolamide □ Pharmacokinetics—nonlinear model proposed for acetazolamide □ Models—nonlinear pharmacokinetic model proposed for acetazolamide

The efficacy and safety of drug therapy are commonly thought to be functions of the drug concentration at the site of action, but measurement of the drug concentration in remote tissues is usually difficult or impossible. Measurement of drug in the plasma is convenient and relatively easy, however, and plasma drug levels, although recognized to be vaguely related to effect, are used to evaluate therapy.

The common utilization of plasma levels to develop pharmacokinetic models and equations describing drug behavior in the body likewise depends largely on plasma measurements and thus yields equations that describe plasma levels quite accurately but that may not give useful estimates of drug concentrations in other tissues.

Recently, attention has been focused on equations that describe the time course of drug effect and that relate effect to plasma or peripheral compartment concentrations (1, 2). Pharmacokinetics must move in this direction to achieve the goal of enhancing effective and safe drug usage. This paper deals with such research on a well-known drug, acetazolamide.

Acetazolamide was selected since it was reported to be excreted entirely by the kidney in unchanged form (3, 4), its activity being uncomplicated by active metabolites. Furthermore, the mechanism of action is the inhibition of carbonic anhydrase (3, 5). The target organs of primary interest are the kidney, where the drug induces diuresis (3, 6), and the eye, where it causes reduction in intraocular pressure (5, 7–9).

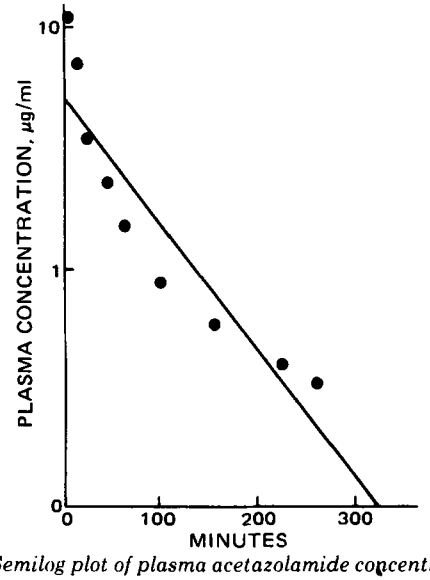
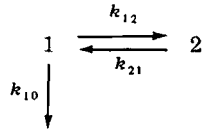


Figure 1—*Semilog plot of plasma acetazolamide concentration* versus *time.*

0022-3549/79/0300-0342$01.00/0



Scheme I

Acetazolamide kinetics have been studied only to describe plasma levels; the plasma data were limited in number, and a one-compartment model was used. These reports (4, 10, 11) reflect the level of interest in kinetics at the time of their publication and are not comparable to a modern detailed pharmacokinetic study.

This paper describes the use of ^{14}C-acetazolamide in rabbits to evaluate its pharmacokinetics, using a model that may be correlated with drug activity. A pharmacokinetic model for acetazolamide was devised using all available data from plasma, urine, and red blood cells. Parameters obtained from this model were later used to relate drug concentration to pharmacological response (12). This paper deals with the development of the nonlinear model.

EXPERIMENTAL

^{14}C-Acetazolamide was synthesized by the deacetylation of acetazolamide[1] and reacetylation with radioactive acetic anhydride[2,3]. TLC was used to test the purity of the radioactive compound. It was demonstrated that essentially all radioactive material was acetazolamide and that the deacetylated intermediate was present in trace amounts. Solutions for injection were prepared by adding appropriate amounts of radioactive and nonradioactive acetazolamide, converting the drug to its sodium salt *via* addition of 0.1 *N* NaOH, and diluting with water to volume. The final pH was 9.0–9.6.

Intravenous bolus acetazolamide doses ranging from 2 to 20 mg/kg were injected into the marginal ear veins of New Zealand White rabbits anesthetized with a 25% urethan[4] solution or sedated with 50 mg of chlorpromazine[5]. Blood and urine samples were collected at intervals up to 6–10 hr after injection; plasma was immediately separated from erythrocytes *via* centrifugation. Plasma samples, 0.5 ml, were mixed with 10 ml of a scintillation cocktail composed of 12 g of 2,5-diphenyloxazole[6] and 300 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene[6] in a mixture of 1 liter of surfactant[7] and 2 liters of toluene. Samples were counted in a liquid scintillation counter[8] and results were corrected for quenching using the channels ratio method (13).

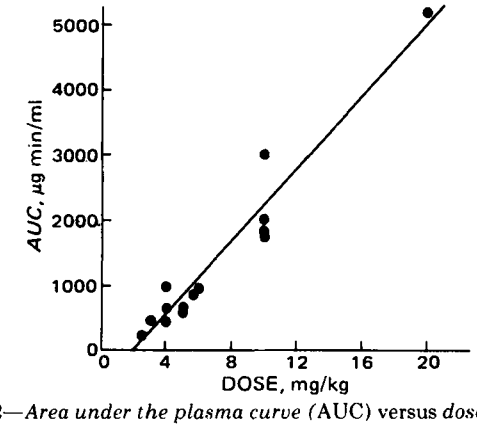


Figure 2—*Area under the plasma curve* (AUC) versus *dose.*

[1] Supplied by Lederle Laboratories, Pearl River, N.Y.
[2] New England Nuclear, Boston, Mass.
[3] R. Kudla, Lederle Laboratories, Pearl River, N.Y., personal communication.
[4] Nutritional Biochemical Co., Cleveland, Ohio.
[5] Thorazine, Smith Kline and French Laboratories, Philadelphia, Pa.
[6] Packard Instruments Co., Downers Grove, Ill.
[7] Triton-X100, Fisher Scientific Co., Raleigh, N.C.
[8] Model 3320 liquid scintillation spectrometer, Packard Instruments Co., Downers Grove, Ill.

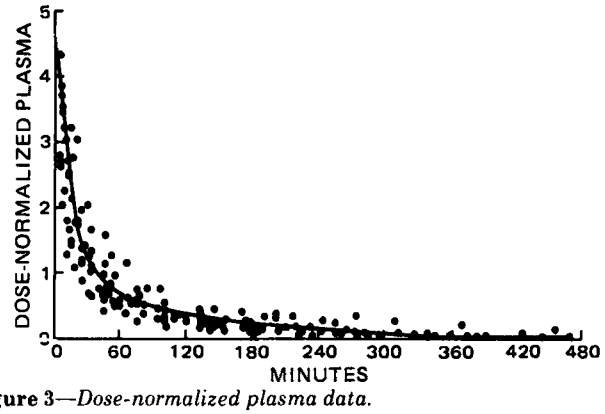


Figure 3—*Dose-normalized plasma data.*

Red blood cells were measured for bound drug by washing approximately 0.5-ml samples with 3 ml of normal saline (4). Accurately pipetted aliquots of packed cells were placed in No. 0 gelatin capsules, dried in a desiccator, and burned in an oxygen flask[9]. A carbon dioxide-capturing cocktail, 10 ml, was used to trap the gas. The solution contained 50 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene and 2 g of 2,5-diphenyloxazole in a mixture of 135 ml of methanol, 135 ml of phenylethylamine, and 230 ml of toluene. These samples were also measured in a scintillation counter.

Although no metabolites have been reported (3, 4), recoveries of 65–103% during the experiment prompted an examination of the urine for possible metabolites. TLC, involving development of urine samples in chloroform–methanol (80:30), indicated that no metabolism had occurred.

RESULTS AND DISCUSSION

Thirteen animals were tested by the described procedure. In all animals, plasma and red cell samples were collected at suitable intervals, but urine collections were not satisfactory in three animals. Curves of plasma, urine, and red cell concentrations were smooth and judged suitable for evaluation of possible kinetic models.

Since previous workers used a simple one-compartment model for acetazolamide, the data were examined as to fit to a monoexponential equation. When plotted as log plasma concentration *versus* time, systematic deviation from linearity was found invariably (Fig. 1). Obviously, a single-compartment model was not suitable.

Possibly, the best means of deciding on the number of exponentials required to describe the data is use of the computer programs AUTOAN and NONLIN. Plasma data from each animal were tested by computer *via* multiple correlation coefficients. The average coefficients obtained were 0.6425 ± 0.0241 for the monoexponential, 0.9959 ± 0.0026 for the biexponential, and 0.9985 ± 0.0013 for the triexponential equations. From these results, it was decided that the biexponential equation was best. The improvement gained by use of a triexponential equation did not justify the increased complexity.

The fit of the biexponential equation indicated that the plasma acetazolamide data fit a linear two-compartment model (Scheme I) where Compartment 1 is the circulating plasma plus other highly perfused

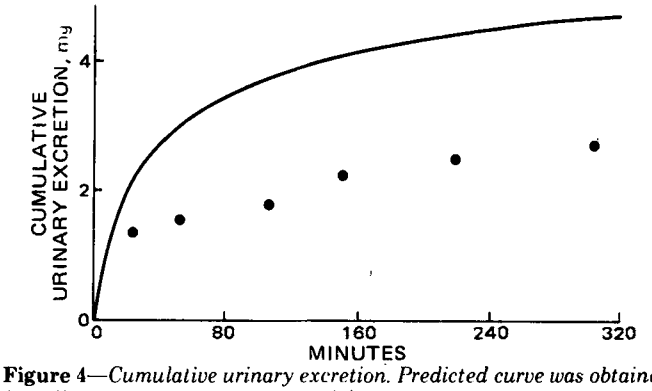


Figure 4—*Cumulative urinary excretion. Predicted curve was obtained from linear two-compartment model.*

[9] Thomas-Ogg model 11, Sargent Instrument Co., Chicago, Ill.

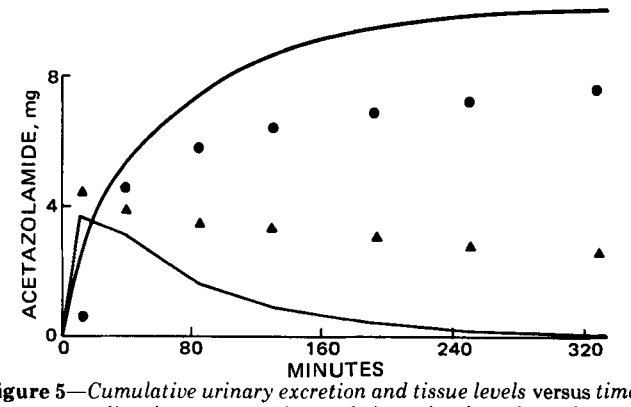


Figure 5—*Cumulative urinary excretion and tissue levels* versus *time. Key: —, predicted curves; ▲, observed tissue level; and ●, observed cumulative urinary excretion.*

tissues, Compartment 2 is less highly perfused tissue, and excretion occurs from Compartment 1 by way of the urine. With NONLIN, the kinetic parameters for this model were obtained for all animals. All plasma curves were fit satisfactorily to the model.

Since the mechanism of action of the drug involves tight binding in a widely distributed enzyme, the model was tested for linearity. Classically, a drug that follows linear kinetics will have a linear relationship between the area under the plasma curve (*AUC*) and dose (14) (Fig. 2). Dose-normalized plasma data obtained from plasma data at various doses should give a satisfactory fit to one superimposable curve if a drug follows linear kinetics. Figure 3 shows excellent agreement from data obtained following doses ranging from 1 to 20 mg/kg. Furthermore, no trends were seen when the individual curves were plotted together. Derived rate constants were also independent of dose and indicated kinetic linearity. In addition to these criteria, acetazolamide excretion was found to follow linear kinetics as judged by the computer program AUTOAN.

While these tests indicated that the linear model was appropriate, further examination utilizing urine and red blood cell data cast doubt on its suitability. Plots of observed and predicted drug elimination showed a consistent and marked overprediction (Fig. 4). Moreover, computed values for plasma concentrations at later time points were consistently below measured values.

The suitability of the linear model was also judged by comparing predicted amounts in the tissue with observed values. These values were determined by:

$$X_2 = D - (CV_1 + X_e) \qquad \text{(Eq. 1)}$$

where X_2 is the observed value, D is the dose, C is the plasma concentration, V_1 is the calculated volume of the central compartment, and X_e is the cumulative amount of acetazolamide measured in the urine. At later times, the value CV_1 becomes very small and the equation effectively reduces to:

$$X_2 = D - X_e \qquad \text{(Eq. 2)}$$

Graphs of the observed and predicted tissue levels showed that the model consistently underpredicted the later points (Fig. 5). While the predicted curve decreased substantially with time, the observed values did not, indicating strong binding to tissue protein. Additional insight was gained

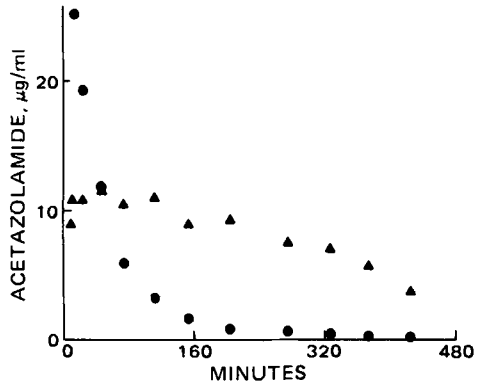


Figure 6—*Acetazolamide concentrations. Key: ●, plasma; and ▲, washed red blood cells.*

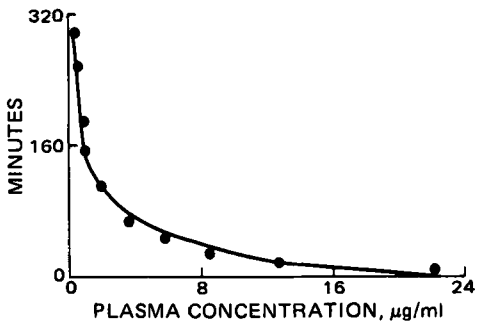


Figure 7—*Plasma curve obtained from nonlinear model with two tissue binding parameters.*

by obtaining the difference between the actual and predicted values of X_2 at later times. A fairly consistent difference of approximately 2.2 mg was found, indicating a nearly constant fraction of drug not accounted for by the model.

Washed red blood cells were also examined for the drug. Following bolus injection, prompt saturation of the cells was attained (Fig. 6). Plateau drug levels in the red cells were consistently above plasma concentrations and were maintained for varying times, again indicating tight binding to protein.

While several classical tests for linearity indicated that the model was satisfactory, utilization of the urine and red blood cell data showed that the linear model did not accurately describe all data. The strong binding suggested that a nonlinear model would be more appropriate than a more complicated linear model.

A nonlinear model system presented by Wagner (15) incorporates the concept of tissue binding. While a linear model gives a linear plot of *AUC versus* dose, a nonlinear model can also give this relationship. Wagner showed that data following the tissue-binding model can also be fitted to a biexponential equation (15). The simplest model in this system, a one-compartment model with one type of tissue binding, was tested first. The model makes two assumptions: that maximum binding occurs instantaneously on injection since the plasma concentration is highest at this time (data from red blood cells indicated that uptake of the drug is sufficiently rapid to make this assumption) and that different types of protein–drug interactions are similar enough to be represented by one binding equation.

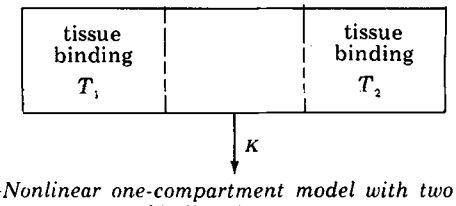
The data were fitted to the nonlinear model, and satisfactory fits to plasma and amount of drug in the body were obtained. The tissue curve, however, did not describe red blood cell concentrations well. Therefore, it was decided to expand the model to contain two tissue-binding parameters, one a generalized tissue and the other the red blood cells. The equations for this model (Scheme II) are:

$$\frac{dC}{dt} = \frac{-KC}{1 + \dfrac{A_1B_1}{(B_1 + C)^2} + \dfrac{A_2B_2}{(B_2 + C)^2}} \qquad \text{(Eq. 3)}$$

$$t = \frac{1}{K}\left[\left(1 + \frac{A_1}{B_1} + \frac{A_2}{B_2}\right)\ln\left(\frac{C_0}{C}\right) + \frac{A_1}{B_1}\ln\left(\frac{B_1 + C}{B_1 + C_0}\right) + \frac{A_2}{B_2}\ln\left(\frac{B_2 + C}{B_2 + C_0}\right) + A_1\left(\frac{C - C_0}{(B_1 + C_0)(B_1 + C)}\right) + A_2\left(\frac{C - C_0}{(B_2 + C_0)(B_2 + C)}\right)\right] \qquad \text{(Eq. 4)}$$

$$T_1 + T_2 = \frac{A_1C}{B_1 + C} + \frac{A_2C}{B_2 + C} \qquad \text{(Eq. 5)}$$

where C is the plasma concentration with C_0 its initial value; t is time; K is the elimination constant; A_1 is the maximum concentration of drug bound to the generalized binding tissue, T_1; B_1 is the dissociation constant for the drug–tissue complex; and A_2 and B_2 are corresponding values for red blood cells, T_2.



Scheme II—Nonlinear one-compartment model with two types of binding sites.

Table I—Summary of Pharmacokinetic Parameters for Nonlinear Model with Two Binding Parameters

Rabbit	C_0, μg/ml	A_1, μg/ml	B_1, μg/ml	A_2, μg/ml	B_2, μg/ml	K, μg/ml	Volume, ml/kg	R^2 RBC[a]	R^2 X_b	R^2 Plasma	R^2 Total
14	39.38 ± 0.83	11.27 ± 0.49	0.00011 ± 0.00001	4.00 ± 0.47	1.3777 ± 0.4628	0.0165 ± 0.0156	182.98	0.980	0.997	0.963	0.997
15	8.33 ± 0.37	3.22 ± 0.43	0.0137 ± 0.0150	0.77 ± 0.25	0.8331 ± 0.6383	0.0126 ± 0.0062	405.84	0.994	0.996	0.966	0.996
16	15.10 ± 0.46	1.72 ± 0.14	0.00011 ± 0.00004	2.45 ± 0.12	0.0708 ± 0.00094	0.0173 ± 0.0088	259.47	0.994	0.995	0.977	0.995
22	9.23 ± 0.19	1.50 ± 0.09	0.00014 ± 0.00003	1.80 ± 0.0857	0.00014 ± 0.00004	0.0145 ± 0.0091	399.04	0.980	0.999	0.972	0.997
23	5.43 ± 0.02	4.20 ± 0.23	0.0373 ± 0.0040	2.94 ± 0.11	0.00012 ± 0.00001	0.0290 ± 0.0126	198.89	0.981	0.998	0.983	0.994
25	13.58 ± 1.04	6.07 ± 0.83	0.0021 ± 0.0014	4.05 ± 0.63	0.3377 ± 0.1293	0.0251 ± 0.0089	168.78	0.980	0.989	0.998	0.988
26	4.88 ± 0.15	1.30 ± 0.15	0.00011 ± 0.00003	1.10 ± 0.07	0.00022 ± 0.00006	0.0113 ± 0.0058	412.09	0.993	0.998	0.934	0.997
27	5.66 ± 0.09	1.49 ± 0.10	0.00033 ± 0.00013	0.98 ± 0.06	0.1095 ± 0.0397	0.0153 ± 0.0073	492.00	0.987	0.999	0.949	0.999
28	19.74 ± 0.54	9.44 ± 0.47	0.00063 ± 0.00008	4.84 ± 0.28	0.2540 ± 0.0447	0.0222 ± 0.0056	176.37	0.995	0.999	0.997	0.999
29	29.16 ± 0.32	16.29 ± 0.29	0.00016 ± 0.000004	2.84 ± 0.10	0.0826 ± 0.0080	0.0155 ± 0.0446	207.08	0.971	1.000	0.992	0.999
		$\bar{x}$	0.00547 ± 0.0119	2.58 ± 1.42	0.3066 ± 0.4539	0.0179 ± 0.0057	290.25 ± 123.01	Rabbits 18, 19, and 21 were fit using estimates from above data. No urine was taken.			
18	8.39 ± 1.42	31.69 ± 8.72	0.0057 ± 0.0011	3.95 ± 0.76	0.2613 ± 0.0517	0.0151 ± 0.0071	129.00	0.998	—	0.979	0.998
19	121.11 ± 21.04	34.69 ± 15.79	0.0058 ± 0.0026	6.29 ± 0.93	0.3975 ± 0.0924	0.0156 ± 0.0106	123.39	0.996	—	0.970	0.996
21	30.23 ± 2.09	7.28 ± 1.73	0.0025 ± 0.0007	3.20 ± 0.25	0.0583 ± 0.0250	0.0116 ± 0.0031	245.64	1.000	—	0.926	0.999

[a] Red blood cells.

The data were fitted by computer to three equations simultaneously. The first (Eq. 4) is the integrated plasma equation. The equation describing the amount of drug in the body, X_b, as a function of plasma concentration is:

$$X_b = \frac{\text{dose}}{C_0 + A_1 + A_2}\left(C + \frac{A_1 C}{B_1 + C} + \frac{A_2 C}{B_2 + C}\right) \qquad \text{(Eq. 6)}$$

where dose/$(C_0 + A_1 + A_2)$ is the volume of distribution. The equation for the quantity of drug bound to the red cells, T'_2, is:

$$T'_2 = \frac{\text{dose}}{C_0 + A_1 + A_2}\left(\frac{A_2 C}{B_2 + C}\right) \qquad \text{(Eq. 7)}$$

Data for the first equation were observed plasma concentrations, data for the second equation were calculated amounts remaining in the body (dose $- X_e$), and data for the third equation were observed amounts in the red cells. While good initial estimates of C_0 could be obtained from the biexponential fit, estimates of the other parameters proved hard to obtain. The value of A_2 was obtained from a nonlinear fit of the bound red blood cell data, and A_1 was assumed to be larger than A_2. The value of B_2 was also estimated from individual fitting of red blood cell data while the value of B_1 was assumed to be much smaller than B_2. An unsatisfactory estimate resulted in an improbable parameter value, and the fitting procedure was rerun with a new estimate. Excellent fits were obtained for all but one data set with regression coefficients of about 0.99. Figures 7–9 are examples of the types of curves obtained. Table I lists the estimated parameters, calculated volumes, and multiple regression coefficients for the data sets.

The initial plasma concentration, C_0, increased proportionally with dose, as might be expected. The maximum concentration of drug bound to red cells, A_2, was not correlated with dose but was highly variable from one animal to another. The erythrocytes, being readily accessible to drug in plasma and limited in number, might be expected to be saturated readily, but individual variation appeared to mask this effect.

The maximum concentration of drug bound to the undefined binding tissue, T_1, was found to be a function of dose with no evidence of saturation. Since carbonic anhydrase, which is thought to be the major binding component of this tissue, is present in practically all body cells, saturation would not be expected except at extremely large doses. The effect of increasing dose in the normal range probably is the result of the penetration of the drug to less accessible regions.

Insight into the nature of the binding in T_1 is obtained from values of B_1; B_1 represents the dissociation constant of the complex in micrograms per milliliter. Conversion of the average value for B_1, 0.00547 μg/ml, into molar units yields 2.5×10^{-8} M. If acetazolamide binding to plasma

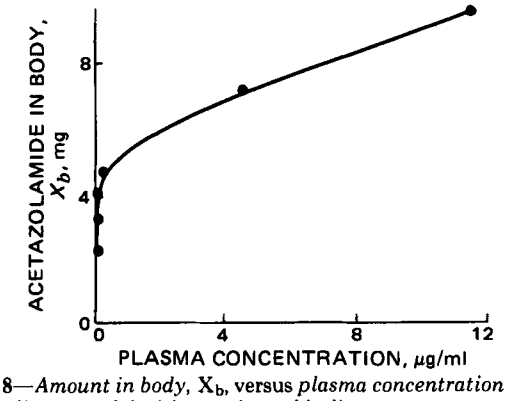


Figure 8—*Amount in body,* X_b, versus *plasma concentration obtained from nonlinear model with two tissue-binding parameters.*

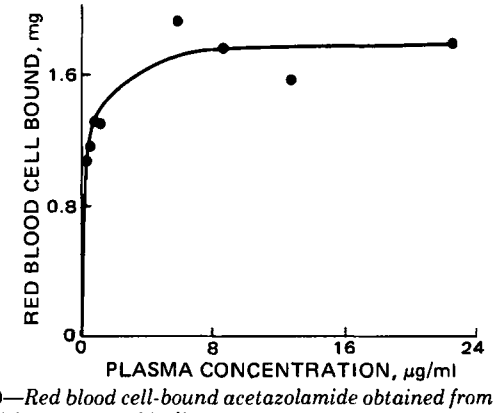


Figure 9—*Red blood cell-bound acetazolamide obtained from nonlinear model with two tissue-binding parameters.*

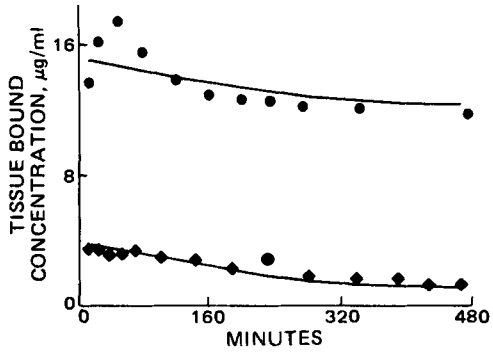


Figure 10—*Tissue-bound acetazolamide from nonlinear model with two tissue-binding parameters. Key:* ●, *observed total bound* ($T_1 + T_2$); *and* ♦, *observed red blood cells bound.*

protein (90% bound at this range of concentration) is considered, this value would be somewhat smaller but still within the limits expected for carbonic anhydrase, $(0.8–8.0) \times 10^{-8} M$ (16). This value agrees with the concept that carbonic anhydrase probably is the major component of binding tissue, T_1. Likewise, the highest value of B_2 probably reflects the larger proportion of binding proteins other than carbonic anhydrase in the red blood cells.

Values obtained for the various data sets for the excretion constant, K, were quite similar, indicating little variation in the excretion rate from one animal to another. The average K, 0.0179 min^{-1}, when multiplied by the average V, 290.25 ml/kg, gives a value of 5.20 ml/min kg as the computed clearance of acetazolamide. Comparison of this value to the average creatinine clearance for rabbits, 3.23 ml/min kg (17), indicates that acetazolamide is partially excreted by an active process.

Although values of R^2 indicate good fit for these data sets, the equation predicts lower values for the early part of the plasma curve than were observed. This finding is reflected in estimates of C_0, some of which are lower than the earliest observed points. This discrepancy is the result of the assumption of instantaneous maximal binding when actual data indicate that maximal binding may not occur for as long as 20 min after injection. This error occurs only in the early portion of the curve, the rest being predicted within experimental error.

An excellent way of determining how well the model fits the data is to compare predicted and observed tissue values. Observed tissue concentration values, T_{obs}, are obtained using the equation:

$$T_{obs} = \frac{X_b - CV}{V} \qquad \text{(Eq. 8)}$$

while predicted values are the sums of $T_i + T_2$ for the same points in time. Figure 10 is a typical plot of predicted and observed values. Predicted and observed values for the red blood cell concentrations, T_2, are also shown. Excellent agreement was seen with all data.

As a result of this work, it is concluded that the nonlinear tissue model is superior to a linear model in describing acetazolamide kinetics. Since it describes the binding of the drug to particular tissues, it appears to be especially suitable for correlation of kinetics of distribution with physiological activity. Such a study is the subject of a subsequent report (12).

REFERENCES

(1) J. G. Wagner, *J. Mond. Pharm.*, **4**, 14 (1971).

(2) J. G. Wagner, *J. Theoret. Biol.*, **20**, 173 (1968).

(3) T. H. Maren, E. Mayer, and B. C. Wadsworth, *Bull. Johns Hopkins Hosp.*, **95**, 199 (1954).

(4) P. Wistrand, *Acta Pharmacol. Toxicol.*, **16**, 171 (1959).

(5) B. Lehmann, E. Linner, and P. J. Wistrand, *Adv. Biosci.*, **5**, 197 (1969).

(6) T. H. Maren, *Physiol. Rev.*, **47**, 595 (1967).

(7) O. I. Nissen, *Acta Ophthalmol.*, **53**, 537 (1957).

(8) B. Becker, *Am. J. Ophthalmol.*, **47**, 342 (1959).

(9) R. P. Thomas and M. W. Riley, *ibid.*, **60**, 240 (1965).

(10) P. Wistrand, J. A. Rawls, Jr., and T. H. Maren, *Acta Pharmacol. Toxicol.*, **17**, 337 (1960).

(11) T. H. Maren and B. Robinson, *Bull. Johns Hopkins Hosp.*, **106**, 1 (1960).

(12) R. L. Kunka and A. M. Mattocks, *J. Pharm. Sci.*, **68**, 347 (1979).

(13) G. A. Brujo and J. E. Christian, *Anal. Chem.*, **33**, 650 (1961).

(14) J. G. Wagner, "Fundamentals of Clinical Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1975, pp. 271–283.

(15) J. G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence, Hamilton, Ill., 1971, pp. 302–317.

(16) H. Green, C. A. Bocher, A. F. Calnan, and I. H. Leopold, *Arch. Ophthalmol.*, **53**, 463 (1955).

(17) H. W. Smith, "The Kidney: Structure and Function in Health and Disease," Oxford, New York, N.Y., 1958, p. 536.

ACKNOWLEDGMENTS

Supported in part by a grant from Lederle Laboratories.

R. L. Kunka is a Fellow, American Foundation for Pharmaceutical Education.