ammonium sulfate/liter of the reaction mixture. The solutions were centrifuged at 8000 rpm for 20 min at 4°. A brown precipitate was obtained from the reaction mixture with the enzyme suspension, while a yellow precipitate was obtained from the control reaction mixture. Both precipitates were dissolved in 25 ml of water separately, and 2.0 ml of 5 *N* HCl was added to each solution. These solutions were centrifuged at 8000 rpm for 20 min at 4°, and the precipitates were treated according to the same procedure again.

At the end, a bright-orange precipitate was obtained from the reaction mixture containing the enzyme suspension, while a yellow precipitate was obtained from the control reaction. The orange precipitate was identified as 8,8′-dioxo-6,6′-azopurine by comparing the R_f values in three different solvent systems (Table I) and the UV characteristics with authentic samples of II. The yellow precipitate was identified as I. Compound I was unchanged under the same conditions even after 6 hr in the absence of enzyme.

Compound I is the first example of a purine dimer that can react with rabbit liver aldehyde oxidase. This finding is significant because it has been suggested that controlled inhibition of aldehyde oxidase will reduce the cytotoxic effects of the immunosuppressive agent azathioprine and modify its chemotherapeutic effects in order to develop more effective treatment schedules (3). Compound I can be considered as a sequential inhibitor of two enzymes involved in azathioprine metabolism. It should inhibit aldehyde oxidase and be converted to II, which is a potent inhibitor of another enzyme in the metabolic pathway, xanthine oxidase. Thus, investigation of the inhibition of mammalian aldehyde oxidase by I may provide such an agent.

Because of the significance of aldehyde oxidase in the metabolism of various biologically active *N*-heterocyclic compounds, I can be used in the investigation of the mechanism of the action of these compounds.

(1) K. V. Rajagopalan and P. Handler, *J. Biol. Chem.*, **239**, 2027 (1964).

(2) T. L. Loo, C. Lim, and D. G. Johns, *Biochim. Biophys. Acta*, **134**, 467 (1967).

(3) A. H. Chalmers, P. R. Knight, and M. R. Atkins, *Aust. J. Exp. Biol. Med. Sci.*, **47**, 263 (1969).

(4) D. G. Johns, D. Farquhar, M. K. Wolpert, B. A. Chabner, and T. L. Loo, *Drug Metab. Dispos.*, **1**, 580 (1973).

(5) I. Tsukada, T. Kunimoto, M. Hori, and T. Komi, *J. Antibiot.*, **22**, 36 (1969).

(6) M. R. Sheen, H. R. Martin, and R. E. Parks, Jr., *Mol. Pharmacol.*, **6**, 225 (1970).

(7) D. L. Hill, W. R. Lester, Jr., and R. F. Struck, *Cancer Res.*, **32**, 658 (1972).

(8) D. L. Hill, W. R. Lester, Jr., M. C. Kirk, S. E. Dareer, and R. F. Struck, *ibid.*, **33**, 1016 (1973).

(9) J. R. Davis, A. L. Jadhav, and J. Fareed, *J. Med. Chem.*, **17**, 639 (1974).

(10) A. L. Jadhav, Ph.D. dissertation, Loyola University of Chicago, Maywood, Ill., 1976.

Arun L. Jadhav ˣ
Kanti G. Bhansali
School of Pharmacy
Texas Southern University
Houston, TX 77004
Joseph R. Davis
Department of Pharmacology
Loyola University of Chicago
Maywood, IL 60153

Received February 23, 1979.
Accepted for publication June 22, 1979.
The authors thank Dr. Alfred Giner-Sorolla for the gift of 6,6′-azopurine disodium salt.

Effect of Altered Plasma Protein Binding on Apparent Volume of Distribution

Keyphrases ◻ Volume of distribution—effect of altered plasma protein binding, pharmacokinetics ◻ Drug binding—plasma, volume of distribution, effect of altered plasma protein binding ◻ Plasma protein binding—effect of alterations on apparent drug volume of distribution ◻ Models, pharmacokinetic—apparent volume of distribution, effect of altered plasma protein binding

To the Editor:

Changes in the apparent volume of distribution occur with age, disease, and displacement (drug interaction) and in the presence of saturable binding. Relationships have been developed (1–6) to explain these changes based on alterations in plasma and/or tissue binding. Our discussion is restricted to measurement of drug concentrations in plasma. Gillette (1) showed that the volume of distribution, V, can be expressed by:

$$V = \alpha(V_f + XV_T) + (1 - \alpha)V_p \qquad \text{(Eq. 1)}$$

where α is the fraction unbound in plasma, V_f is the volume into which the unbound drug is distributed, X is the ratio of tissue drug concentration to unbound plasma drug concentration, V_T is the tissue volume, and V_p is the apparent volume of distribution of the plasma protein to which the drug binds. A simplified relationship, based on the physiological concepts of Gillette (1, 4–6), was proposed (2, 3) as follows:

$$V = V_P + (V_T)\left(\frac{\alpha}{\alpha_T}\right) \qquad \text{(Eq. 2)}$$

where V_P is the plasma volume, V_T is the volume outside plasma into which the drug distributes, and α and α_T are the fractions unbound in these two compartments.

The relationship of Eq. 2 does not take into account that plasma proteins are distributed throughout the extracellular fluids. When the binding to proteins in plasma is altered, similar changes are expected in the binding to these proteins located in other extracellular fluids. Furthermore, this relationship cannot distinguish between binding to these proteins and binding elsewhere in the body. This distinction is important for anticipating changes in the volume of distribution on altering drug binding and the converse.

The following derivation provides a method for making this distinction. From mass balance considerations:

$$A = A_P + A_E + A_R \qquad \text{(Eq. 3)}$$

where:

A = total amount of drug in the body
A_P = total amount of drug in plasma
A_E = total amount of drug in the extracellular fluid outside plasma

0022-3549/79/0900-1203$01.00/0

A_R = total amount of drug in the remainder of the body

The amount in plasma is equal to:

$$A_P = (V_P)(C) \quad \text{(Eq. 4)}$$

where V_P is the plasma volume and C is the total plasma concentration. The total amount of drug in the extracellular fluid outside the plasma is equal to:

$$A_E = (V_E)(C_E) \quad \text{(Eq. 5)}$$

where V_E is the extracellular space minus the plasma volume and C_E is the average total concentration in this fluid. The amount of drug in the remainder of the body is equal to:

$$A_R = (V_R)(C_R) \quad \text{(Eq. 6)}$$

where V_R is the physical volume into which the drug distributes minus the extracellular space and C_R is the average concentration in this space.

Substituting Eqs. 4–6 into Eq. 3, dividing by C, and knowing that A/C is, by definition, the apparent volume of distribution, we obtain:

$$V = V_P + (V_E)\left(\frac{C_E}{C}\right) + (V_R)\left(\frac{C_R}{C}\right) \quad \text{(Eq. 7)}$$

Unless active transport or other complications occur, at distribution equilibrium the unbound drug concentration, Cu, is the same in all tissues into which it distributes. Defining α_R as Cu/C_R, α as Cu/C, and Cb_E as the average concentration of bound drug in the extracellular space outside plasma, we obtain:

$$V = V_P + (V_E)(\alpha)\left(\frac{Cu + Cb_E}{Cu}\right) + (V_R)\left(\frac{\alpha}{\alpha_R}\right) \quad \text{(Eq. 8)}$$

For a given protein with one class of binding sites, the law of mass action gives:

$$K_a = \frac{Cb_P}{(Cu)(P)_P} = \frac{Cb_E}{(Cu)(P)_E} \quad \text{(Eq. 9)}$$

where Cb_P and Cb_E are the bound drug concentrations and $(P)_P$ and $(P)_E$ are the concentrations of unoccupied protein binding sites in the plasma and in the other extracellular fluids, respectively, and K_a is the association or affinity constant. Since the unbound drug concentration is identical in both fluids:

$$\frac{Cb_P}{(P)_P} = \frac{Cb_E}{(P)_E} \quad \text{(Eq. 10)}$$

Moreover, since:

$$(P)_P + Cb_P = (Pt)_P \quad \text{(Eq. 11)}$$

and:

$$(P)_E + Cb_E = (Pt)_E \quad \text{(Eq. 12)}$$

where $(Pt)_P$ and $(Pt)_E$ are the average total concentrations of binding sites in the plasma and in the other extracellular fluids, respectively, it follows that:

$$Cb_E = (Cb_P)\left[\frac{(Pt)_E}{(Pt)_P}\right] \quad \text{(Eq. 13)}$$

or:

$$Cb_E = (Cb_P)(R_{E/I})\left(\frac{V_P}{V_E}\right) \quad \text{(Eq. 14)}$$

where $R_{E/I}$ is the ratio of the total number of binding sites or the amount of protein in extracellular fluids outside the plasma to that in the plasma. Equation 14 can be shown to be valid also when there is more than one class of binding sites on a protein, when saturation is approached, and when several proteins are present, provided the ratio $R_{E/I}$ is the same for each protein. Substituting Eq. 14 into Eq. 8 gives:

$$V = V_P + \alpha\left[\frac{V_E Cu + Cb_P V_P R_{E/I}}{Cu}\right] + \frac{V_R \alpha}{\alpha_R} \quad \text{(Eq. 15)}$$

However, since:

$$Cb_P/Cu = (1 - \alpha)/\alpha \quad \text{(Eq. 16)}$$

then:

$$V = V_P(1 + R_{E/I}) + \alpha V_P(V_E/V_P - R_{E/I}) + \frac{V_R \alpha}{\alpha_R} \quad \text{(Eq. 17)}$$

This relationship is similar to that proposed by Gillette (1) (Eq. 1) but includes terms for the intravascular–extravascular distribution of the binding protein as well as the actual volumes of these extracellular fluids.

The extracellular fluid outside the plasma is usually considered to be 12 liters and the plasma volume to be 3 liters in a normal 70-kg man (7). Furthermore, 55–60% of the total extracellular albumin is usually found outside the plasma (8); with the assumption that the plasma proteins to which the drug binds are distributed like albumin, the extravascular to intravascular ratio, $R_{E/I}$, is ~1.4. With these normal values, Eq. 17 becomes:

$$V = 7.2 + \alpha(7.8) + (V_R)\left(\frac{\alpha}{\alpha_R}\right) \quad \text{(Eq. 18)}$$

or approximately:

$$V = 7 + 8(\alpha) + V_R\left(\frac{\alpha}{\alpha_R}\right) \quad \text{(Eq. 19)}$$

This equation states that when a drug is only distributed to the extracellular fluid and cannot enter the cells (V_R = 0), the smallest apparent volume of distribution a drug can have is:

$$V = 7 + 8(\alpha) \quad \text{(Eq. 20)}$$

Thus, at distribution equilibrium, the observed apparent volume of distribution of any drug cannot be less than 7 liters, no matter how tightly bound the drug is to albumin. For a drug restricted to the extracellular fluid only (V_R = 0) and not plasma protein bound (α = 1), the apparent volume of distribution is limited to the value of the total extravascular fluid volume, 15 liters.

Equations 17 and 19 are particularly useful for drugs with low apparent volumes of distribution (<15 liters or <0.2 liter/kg). For example, the volume of distribution of tolbutamide was shown to be essentially unchanged in hepatitis patients, even though the plasma albumin binding was altered in the acute phase of the disease (9). With Eq. 19 and the assumption of no change in α_R, the volume of distribution is calculated to increase from 0.15 to 0.164 liter/kg when the fraction unbound in plasma is increased from 0.068 to 0.087 in hepatitis (9). Thus, a 28% increase in α only results in a 9.5% increase in the apparent volume of distribution.

The observation of little or no change in the volume of distribution when α is increased is consistent with decreased binding to albumin throughout the extracellular fluids, and no alteration in α_R is indicated. However, if Eq. 2 is used, a change in α_T is necessary to explain this ob-

servation, as stated by Gibaldi and McNamara (3), even though only a change in albumin binding may have occurred. When the apparent drug volume of distribution is large (>50–100 liters), the sum $V_P(1 + R_{E/I}) + \alpha(V_P)(V_E/V_P - R_{E/I})$ in Eq. 17 can be neglected since its largest possible value is 15 liters. The apparent volume of distribution is then:

$$V \cong (V_R)\left(\frac{\alpha}{\alpha_R}\right) \qquad \text{(Eq. 21)}$$

which is also predicted by Eq. 2.

The relationship presented in Eq. 17 should be helpful in analyzing and predicting alterations in the apparent volume of distribution of any drug when there is an alteration in the unbound fraction in plasma, in the unbound fraction outside the extracellular fluids, in the volumes of the extracellular fluids, or in the extravascular to intravascular plasma protein ratio, as occurs, for example, in prolonged bed rest and in severe burns. It will also be useful to identify where the alteration occurs.

(1) J. R. Gillette, *Ann. N.Y. Acad. Sci.*, **281**, 136 (1976).

(2) G. R. Wilkinson and D. G. Shand, *Clin. Pharmacol. Ther.*, **18**, 377 (1975).

(3) M. Gibaldi and P. J. McNamara, *Eur. J. Clin. Pharmacol.*, **13**, 373 (1978).

(4) J. R. Gillette, in "Importance of Fundamental Principles in Drug Evaluation," D. H. Tedeschi and R. E. Tedeschi, Eds., Raven, New York, N.Y., 1968, p. 69.

(5) J. R. Gillette, *Ann. N.Y. Acad. Sci.*, **179**, 43 (1971).

(6) J. R. Gillette and K. S. Pang, *Clin. Pharmacol. Ther.*, **22**, 623 (1977).

(7) A. C. Guyton, "Textbook of Medical Physiology," Saunders, Philadelphia, Pa., 1976, p. 424.

(8) W. J. Jusko and M. Gretch, *Drug Metab. Rev.*, **5**, 43 (1976).

(9) R. L. Williams, T. F. Blaschke, P. J. Meffin, K. L. Melmon, and M. Rowland, *Clin. Pharmacol. Ther.*, **21**, 301 (1977).

Svein Øie ˣ
Thomas N. Tozer
School of Pharmacy
University of California
San Francisco, CA 94143

Received February 2, 1979.
Accepted for publication June 7, 1979.
Supported in part by Grant 1R01 GM 25807-01 from the National Institutes of Health.

BOOKS

REVIEWS

Pharmazeutische Technologie. Edited by HEINZ SUCKER, PETER FUCHS, and PETER SPEISER. George Thieme Verlag, Herdweg 63, Postfach 732, D-7000 Stuttgart 1, West Germany. 1978. 906 pp. 17 × 24 cm. Price DM 235.

Industrial pharmacy as a scientific and technological branch of the profession of pharmacy covers a wide spectrum of operations involving a multidisciplinary body of fundamental knowledge and a diversified technology constrained by a specific set of governmental regulations intended to serve as controls over the laboratory and manufacturing environment as well as the quality of the drug preparation itself. An area of such diversity and complexity remains dynamic, and advances in basic pharmaceutics and pharmaceutical technology have accelerated during the past three decades. These advances have come about as a consequence of the pharmaceutical industry's expanding research and development investment and through the acquisition of scientific knowledge and technology from external sources such as universities and other industries.

An early effort by the Pharmaceutical Institute (ETH) in Zurich to reduce the high level of empiricism in the teaching of pharmacy students through the introduction of "physical pharmacy" principles resulted in the publication of a textbook, "Galenisches Praktikum"[1], in which the authors attempted to relate theoretical concepts to practice by means of explanatory text, laboratory exercises, and pertinent literature references. A decade later, the publication of "The Theory and Practice of Industrial Pharmacy"[2] provided a text much closer to the reality of industrial operations and constraints in the development and production of drug preparations.

The latest textbook that endeavors to portray accurately industrial pharmacy is "Pharmazeutische Technologie," a collaborative effort involving 25 authors, most of whom are associated with Swiss or German pharmaceutical companies and each of whom has been selected as a working specialist in his field. By the judicious decision to organize the material as an applied science based on current theoretical concepts of unit operations, only seven chapters were needed. The book begins with a thorough and excellently organized chapter, which develops the mathematical concepts of practical importance to research and development pharmacists engaged in dosage form design. The statistical section is particularly noteworthy for the manner in which research design and optimization techniques and scale-up theory are presented. Along similar lines, the second chapter reviews the theoretical basis for most of the unit operations involved in pharmaceutical dosage form development and production. Among these are the flow properties of gases and liquids, heat transfer, dissolution, comminution, dispersion, mixing, granulating, compressing, and antimicrobial treatment.

In this period of high interest in biopharmaceutics, since a textbook on technology cannot overlook the biological aspects of pharmaceutical product development, a short third chapter covers pharmacokinetic modeling, methodology, and specific applications to bioavailability, sustained-release formulations, and new delivery systems. The fourth chapter treats the important and often neglected subject of pharmaceutical excipients in accordance with the functional role of the excipient in a dosage form. A series of tables listing most of the commonly used excipients conveniently provides incomplete but useful technical information, including average concentration range. Standards for excipients and other forms of regulatory control are described, but deficiencies in existing standards and variability in controls receive minimal attention.

In covering the key subject of dosage forms, the fifth chapter requires over 40% of the total number of pages in the book. The subdivision of topics is based on physical form, route of administration, and sequence of unit operations. This approach proves to be an effective means of organizing a large mass of technical material which, in general, is representative of the current state of pharmaceutics and process technology. The section on parenteral dosage forms and especially the discussions of production control methods and the organization and technological aspects of parenteral production operations are outstanding.

In the sixth chapter, attention is directed to the protective role of packaging, the types of packaging materials used in pharmaceutical containers, and the various chemical, physical, and microbiological tests used to control their quality. A second section includes a brief review of packaging line operations. The last chapter deals with quality control assurance and begins with a rarely seen section on quality of design as studied during preformulation. This discussion is followed by material covering the subsequent formulation studies, which involve drug release characteristics, bioavailability and tissue tolerance of various dosage