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Scatchard Plots with a Positive Slope and Role of Albumin Concentration

Keyphrases □ Scatchard plots—with positive slopes, various drugs, effect of albumin concentration □ Binding, protein—various drugs, Scatchard plots with positive slopes, effect of albumin concentration □ Albumin—effect on drug–protein binding, Scatchard plots with positive slopes

To the Editor:

A recent publication (1) reported Scatchard plots (2) with a positive slope for the binding of three narcotic analgesics and other basic drugs to human albumin. It was implied (1) that such Scatchard plots may be characteristic of organic bases and alkaloids. Positive Scatchard plots have been obtained for hydrocortisone (3), thiopental (4), phenytoin (5, 6), L-tryptophan (5, 6), and 2-(4′-hydroxybenzeneazo)benzoic acid[1] when the protein concentration was varied. Shen and Gibaldi (4) considered some aspects of this problem, but it was not mentioned in a recent review (7). Our own work and the reports listed in Table I suggest that this phenomenon is more common than hitherto supposed and requires explanation.

Two main experimental approaches are used to obtain drug binding data for Scatchard or similar analysis: A, vary the total ligand concentration and use one albumin concentration; and B, vary the albumin concentration and use a single total ligand concentration. Method A is more commonly used and was used in the study of narcotic analgesics (1). The apparent association constant, K, and the number of binding sites, n, derived from data obtained experimentally by Method A are tacitly assumed to be independent of protein concentration.

The positive Scatchard plots obtained for hydrocortisone, thiopental, phenytoin, L-tryptophan, and 2-(4′-hydroxybenzeneazo)benzoic acid indicate that n and/or K (*i.e.*, nK) decrease as the protein concentration increases. Method B was used for ultrafiltration or equilibrium dialysis with these five ligands, so the possibility arises that the results were an artifact of the method. However, this result is unlikely because identical Scatchard plots having the usual negative slopes were obtained experimentally by Methods A and B for both *o*-methyl red (6) and methyl orange[1], using experimental conditions similar to those for phenytoin and L-tryptophan. Previous workers (15, 16) found that nK for methyl orange may be dependent upon protein concentration with bovine albumin, but we have been unable to confirm this finding with human albumin.

Table I lists ligands for which there is an indication that nK may decrease when the protein concentration increases, although many of the data were not reported in a form suitable for Scatchard analysis. Several explanations for these findings are possible.

Commercial albumin preparations contain variable quantities of contaminants, *e.g.*, *N*-acetyl-L-tryptophan and other indoles (23) and fatty acids that may inhibit drug binding. At a constant drug concentration (Method B), the contaminant to ligand ratio increases as the albumin concentration increases and thereby decreases nK. Results with hydrocortisone (3) and 1-dodecanol (12) were attributed to contaminants. The sequential changes of buffer used in the dynamic dialysis technique may remove inhibitors of binding but seem unlikely to account for the results with the narcotic analgesics (1). Scholtan (20) found that drug binding in whole serum with pathologically lowered albumin concentration did not decrease as much as anticipated. Therefore, an increase in nK with lower albumin concentrations cannot be fully explained by dilution of endogenous inhibitory substances since no such dilution would have occurred in whole serum.

Cooperativity was implicated in several unusual ligand–protein interactions (4, 24, 25) and could explain Scatchard plots with positive slopes. However, these cooperative effects all were observed at one protein concentration or, at most, with only a small range of protein concentrations. If cooperativity is responsible, it should be manifest when the albumin concentration, as opposed to the ligand concentration, is varied. However, no such effects were apparent in studies where the ligand concentration was also varied.

[1] Unpublished results.

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Table I—Studies Where *nK* Appears to Decrease with an Increase in Plasma Protein Concentration

Drug	Protein[a]	Method[b]	References
Acid Violet 6B	BSA	DS	8[c]
Alprenolol	HSA	ED	9[c]
Bromosulfophthalein	HSA	DF	10
2-Chromonecarboxylic acid	HSA	UF	11[c]
1-Dodecanol	BSA	ED	12
Dodecyl sulfate	BSA	ED	12
Hydrocortisone	HSA	ED	3[c]
2-(4′-Hydroxybenzeneazo)benzoic acid	BSA	DS	13[c]
Lorazepam	BSA	GF, CD	14
Methyl orange	BSA	ED, P	15, 16
Oxazepam	BSA	GF, CD	14
Phenylbutazone	HSA	CD, FP	17[c]
Phenytoin	HSA	ED	5,[c] 6[c]
Progesterone	HSA	ED	18[c]
Thiopental	BSA	UF	4[c]
Salicylate	HSA	CD, FP	17[c]
Sulfadiazine	HSA	ED, CD, FP	17,[c] 19[c]
Sulfamethoxydiazine	HSA, human serum	ED	19,[c] 20
3-Sulfonamido-4-chlorobenzoic acid	HSA	ED	21
Testosterone	BSA	FQ	22
L-Tryptophan	BSA	ED	5,[c] 6[c]

[a] HSA = human serum albumin, and BSA = bovine serum albumin. [b] ED = equilibrium dialysis, DF = diafiltration, UF = ultrafiltration, DS = difference spectrophotometry, GF = gel filtration, CD = circular dichroism, P = polarography, FP = fluorescence polarization, and FQ = fluorescence quenching. [c] Scatchard analysis used.

Protein–protein interaction may decrease *nK* as the albumin concentration increases, and molecular aggregation (4) or some more specific polymerization process may be involved. Several workers (10, 11, 14, 21) viewed albumin polymerization as an explanation for their results but offered no experimental evidence. Commercial preparations of albumin used *in vitro* contain polymers, and the percent of dimers and other oligomers may increase during storage (26). Removal of bovine albumin aggregates was necessary before reliable results could be obtained for 8-anilino-1-naphthalenesulfonate (27).

Westphal and Harding (18) observed that the binding affinity of progesterone was 33% less to the trimeric–polymeric components of human albumin than to the monomer or dimer fractions. Similarly, digitoxin was bound less to the human albumin dimer than to the monomer (28), and excessive concentrations of digitoxin did not influence the monomer to dimer ratio. However, bendazac and phenylbutazone inhibited the heat-induced aggregation of bovine albumin *in vitro* (29).

Several other proteins besides albumin have an affinity for various ligands that is dependent upon protein concentration (30). Hemerythrin monomer has a higher affinity for thiocyanate than does the octamer, and Scatchard plots indicated that this affinity increased with a decreasing protein concentration (30). Studies with the plasma protein apolipoprotein A-1 suggested that the state of association of this protein in aqueous solution largely determines its capacity to bind lipids or other ligands and that only the monomeric apolipoprotein binds lipids readily (31). When a globular protein interacts to form a square planar tetramer, each monomer unit loses 28.6% of accessible surface (32). An ellipsoid molecule like albumin may lose an even greater percent of accessible surface, together with its binding sites, in this way.

The dependence of *nK* upon protein concentration has several implications. Experimentally, the use of a single albumin concentration may be insufficient to characterize a drug–albumin interaction adequately, and the albumin preparation may need to be checked for aggregate content. The dye binding methods used to measure plasma albumin concentrations give erroneously high values at low concentrations (33), and this result is at least partly explicable in terms of the results discussed here.

More *in vivo* information is required on the state of albumin aggregation in health and disease. When the albumin concentration falls because of disease or trauma, an increase in *nK* for some drugs may homeostatically influence the potential increase in the concentration of unbound drug.

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BOOKS

REVIEWS

Advances in General and Cellular Pharmacology. Vol. 1. Edited by TOSHIO NARAHASHI and C. PAUL BIANCHI. Plenum, 227 W. 17th St., New York, NY 10011. 1977. 252 pp. 16 × 23 cm. Price $24.50.

This book is divided into five sections with the titles and authors as follows: Cardiac Cellular Pharmacology, Automaticity in Cardiac Muscle: Its Alteration by Physical and Chemical Imbalances, by Frances M. Wald and J. Thomas Bigger, Jr.; Actions of Opiates and their Antagonists on Cholinergic Transmission in the Guinea Pig Ileum, by Seymour Ehrenpreis; Pharmacology of Heart Cells During Ontogenesis, by Achilles J. Pappano; Analysis of Dose–Response Relationships, by Douglas R. Waud; and Cellular Pharmacology of Ganglionic Transmission, by Syogoro Nishi.

Characteristics of the automaticity in the myocardium after spontaneous diastolic depolarization are included in the first section. The subsection describing depolarization modification by physical and chemical factors contains especially comprehensive information on alterations induced by several cardiac drugs including β-adrenergic blocking agents, lidocaine, phenytoin (diphenylhydantoin), quinidine, procainamide, and digitalis.

Ehrenpreis describes experiments utilizing the electrically stimulated guinea pig ileum. A table listing 12 opiates or their antagonists, relating a correlation between effects on the ileum and analgesic potency, is of interest. A postulated relationship of the prostaglandin system to cholinergic transmission is given special treatment.

A major contribution of the section on ontogenesis is information regarding the effects of autonomic drugs on the physical and mechanical properties of chick embryo hearts. Nicotine and tyramine, thought to act by neurotransmitter release, tetrodotoxin, and the digitalis glycosides were also studied on this test object. In addition, limited experiments on heart cells in culture were described, giving data on a nerve free system.

The section entitled Analysis of Dose–Response Relationships has its forte in the comprehensive presentation of the kinetic approach. Pertinent information on experimental design and statistical evaluation is especially valuable.

Nishi concludes the volume with a section that thoroughly considers postsynaptic muscarinic and nicotinic sites, as well as receptors described as excitatory noncholinoreceptive and inhibitory adrenoceptive sites. A review of presynaptic receptor sites and transmitter liberation is a highlight of this section. Physiopharmacologic characteristics of the postsynaptic membrane are also discussed.

This book is a definite contribution to the pharmacologic literature. The authors are prolific in the reference portion of their sections. The well-documented reviews in each section and the concise presentation of the author's original research are uniformly exceptional throughout this publication. This book is valuable primarily as a reference text across interdisciplinary lines in the biological and physical science areas, and one anticipates future volumes of this quality.

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Biopharmaceutics and Clinical Pharmacokinetics. By MILO GIBALDI. Lea & Febiger, Washington Square, Philadelphia, PA 19106. 1977. ix + 181 pp. 17.5 × 25.5 cm. Price $8.50.

This book began as a chapter in "Theory and Practice of Industrial Pharmacy" edited by Lachman, Lieberman, and Kanig (Lea & Febiger, 1970). The first edition was a reprint of the chapter in paperback. The second edition retains the advantages of an inexpensive paperback, but it has been considerably expanded and updated. The most useful changes are: division of the book into chapters, addition of many valuable new references, and addition of a significant amount of clinical pharmacokinetic material.

Chapter 1 is a very brief introduction to pharmacokinetics, in which the discussion is restricted to the one-compartment body model. The concepts of drug accumulation and repetitive dosing are also introduced. This chapter could serve as a concise review for a recently graduated pharmacist, but it is not sufficiently detailed to be used alone for the teaching of pharmacokinetics on the undergraduate level.

The next three chapters deal with the GI absorption of drugs. The discussion progresses clearly and logically from biological factors, such as membrane structure, to the role of the dosage form. Chapter 2 reviews membrane structure and function and GI physiology in humans. Chapter 3 discusses such physiochemical factors as pH-partitioning, solubility, and rate of dissolution. Chapter 4 discusses dosage form factors that influence drug dissolution in the GI tract. This chapter contains a survey of several drugs found to present bioavailability problems in humans, with a brief discussion of each drug. This chapter is particularly effective in giving the reader a perspective of the importance of bioavailability testing and control of drug products.

Chapter 5 deals with routes of administration other than oral and discusses relatively recent observations concerning the variability and unreliability of the intramuscular route.

Chapter 6 discusses drug disposition, including tissue distribution, renal excretion, and drug metabolism. Although this chapter is not thorough enough to be used as a sole resource for teaching purposes, it is a suitable review in preparation for the subsequent chapters on the clinical utility of plasma drug concentrations.

Chapter 7, Intersubject Differences in Drug Concentration in Plasma, is an excellent introduction to the complexities one faces in trying to control and adjust drug dosages in individual patients in the real clinical world. Some topics covered are: body weight; sex; age, particularly the newborn; genetic factors; renal, hepatic, and other diseases; and drug interactions. In this chapter, as in Chapter 4, many examples of specific drugs and specific clinical conditions are presented, and the discussion is generously referenced.

The final chapter continues in the drug-by-drug style, with the emphasis on the significance of plasma drug concentrations as guides to efficacy and toxicity. Some of the drugs discussed are warfarin, digoxin, gentamicin, phenytoin, theophylline, salicylate, lidocaine, propranolol, lithium, and nortriptyline. The presentation is clear and to the point, with the added advantage of having practical value in identifying drugs for which monitoring of plasma concentrations may be helpful in guiding therapy.

The book is well written in a very understandable style, with many examples and ample references in each chapter. Although certain chapters would require considerable amplification in the classroom, this book could serve as an inexpensive text for an undergraduate course in bio-