90° in many metal complexes of tetraphenylporphine (4, 8), although angles as small as 69 (9) and 76° (10) were found in certain metal complexes. Steric interactions between pyrrole hydrogen and phenyl hydrogen atoms have been estimated to require a dihedral angle of 60 (10) or 70° (4).

The sequence of spectral changes given in Fig. 1 is nearly identical to those reported for tetrakis[(*p*-isopropylphenyl)porphinato]ruthenium carbonyl complexes (8, 11). The averaging process for the indium complex occurs at slightly lower temperatures than for the ruthenium complex.

Phenyl ring rotation has been observed in tetra-*o*-hydroxylphenylporphine and its copper complex (12). The rate constant for rotation of the free porphine at 23° in methanol was 1.5×10^{-5}/sec. ($\Delta G^{\neq}$ = 24 kcal./mole); rotation was about 10 times slower for the copper complex. Isomers of tetra-*o*-tolylporphine and its nickel complex have also been studied (13). The NMR signals for the methyl resonances of this compound did not appreciably broaden up to 180°, indicating that $\Delta G^{\neq}$ for rotation exceeds 26 kcal./mole (13). The present studies of chloro(tetra-*p*-methylphenylporphinato)indium (III) confirm earlier observations on ruthenium complexes (II); there appears to be a more rapid phenyl group rotation when the *ortho*-substituent is hydrogen than when it is hydroxyl or methyl.

Alternative interpretations of the NMR spectrum for chloro(tetra-*p*-methylphenylporphinato)indium (III) appear less plausible. Since AA' and BB' coupling was not resolved for indium (III) tetraphenylporphine, it is possible to attribute the room temperature spectra to rapidly rotating phenyl groups in nonequivalent positions with accidental degeneracy of nonequivalent pyrrole and methyl resonances. The temperature dependence of such a spectrum would result from the interchange of nonequivalent phenyl sites. However, it has been demonstrated that phenyl rotation is slow in the ruthenium complexes at room temperature (8, 11); it is not likely that rotation would be fast in the chloro(tetra-*p*-methylphenylporphinato)indium (III) complex at the same temperature. If the chloride in the indium complex were dissociated, ion migration or inversion of a "dished" ring conformation could explain the NMR results; however, there are no data to substantiate such an interpretation.

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Specificity of Binding to Human Serum Albumin

Keyphrases □ Drug-human serum albumin binding—specificity, lipophilic dependency, equations □ Binding, drug-human serum albumin—specificity, lipophilic dependency, equations

Sir:

Whitehouse *et al.* (1) suggested the need for reevaluation of the widely held concept (2, 3) that the fraction of certain acidic drugs bound to plasma protein is pharmacologically inactive. In support of this suggestion, data were presented indicating that a correlation exists between the ability of certain anti-inflammatory agents and uricosuric agents to displace uric acid from its primary binding site on human serum albumin *in vitro* (1) and their ability to reduce uric acid binding capacity *in vivo*. The results of other studies were cited to support this thesis (4, 5). One cited study showed that fatty acids displace warfarin and phenylbutazone from their binding sites on human serum albumin *in vitro* (5).

A relationship was recently shown (6) between the ability of certain acidic drugs to displace albumin-bound uric acid and their affinity for the primary binding site of 5-dimethylaminonaphthalene-1-sulfonamide on human serum albumin. Analysis of these affinity data revealed that for certain members of two classes of nonsteroid anti-inflammatory and uricosuric agents—*viz.*, (*a*) carboxylic acids, and (*b*) benzenesulfonamides and

benzenesulfonylureas, a highly significant relationship exists between the displacing ability of the two structure types and the lipophilicity of these agents as measured by their partition coefficients in the 1-octanol–water system (7). These relationships are given in Eqs. 1 and 2 (7).

50% Displacement of 5-Dimethylaminonaphthalene-1-sulfonamide from Human Serum Albumin by Carboxylic Acids—

$$\log 1/C = 1.06\ (\pm 0.67) + 1.26\ (\pm 0.42) \log P - 0.12\ (\pm 0.06) \log^2 P \quad \text{(Eq. 1)}$$
$$n = 29 \quad R = 0.91 \quad SD = 0.36$$
$$\log P_0 = 5.48\ (4.69\text{–}7.80)$$

50% Displacement of 5-Dimethylaminonaphthalene-1-sulfonamide from Human Serum Albumin by Benzenesulfonamides and Benzenesulfonylureas—

$$\log 1/C = 3.64\ (\pm 0.21) + 0.74\ (\pm 0.28) \log P - 0.34\ (\pm 0.10) \log^2 P \quad \text{(Eq. 2)}$$
$$n = 13 \quad R = 0.91 \quad SD = 0.21$$
$$\log P_0 = 1.10\ (0.89\text{–}1.29)$$

In these equations, C is the concentration (moles/liter) required to displace 50% of 5-dimethylaminonaphthalene-1-sulfonamide from its binding site on human serum albumin, P is the 1-octanol–water partition coefficient, n represents the number of agents in the set, R is the correlation coefficient, SD is the standard deviation of the regression, and P_0 is the partition coefficient associated with optimum affinity for the 5-dimethylaminonaphthalene-1-sulfonamide binding site. The terms in parentheses are the 95% confidence intervals for the appropriate regression coefficients. The analysis of additional data (8) for the displacement of 5-dimethylaminonaphthalene-1-sulfonamide from its primary binding site on human serum albumin by various sulfonamides substantiates the nonlinear relationship between the displacing ability and lipophilicity (Eq. 4).

Percent Decrease in Fluorescence of 5-Dimethylaminonaphthalene-1-sulfonamide–Albumin Complex by Various Sulfonamides—

$$\log \%D = 1.56\ (\pm 0.20) + 0.18\ (\pm 0.14) \log P \quad \text{(Eq. 3)}$$
$$n = 13 \quad SD = 0.27 \quad R = 0.63$$

$$\log \%D = 1.62\ (+0.09) + 0.42\ (\pm 0.10) \log P - 0.13\ (\pm 0.04) \log^2 P \quad \text{(Eq. 4)}$$
$$n = 13 \quad R = 0.94 \quad SD = 0.13$$
$$\log P_0 = 1.60\ (1.32\text{–}2.05)$$

Including the nonlinear term in Eq. 3 is significant at the 99% level of confidence, $F_{1,13} = 43.76$, $F_{1,13\ \alpha\ 0.01} = 9.07$.

On comparing the analyses of the data for the sulfa drugs (Eqs. 2 and 4), it is significant to note the similarities in $\log P_0$'s from the two relationships. The data used to derive Eq. 4 are given in Table I. The agreement is very good considering that the data were obtained from different laboratories. The difference in the two values may be due to the fact that the data used to derive Eq. 2 were obtained at 37° while those used for Eq. 4 were obtained at 24°. The dependence of $\log P_0$ on temperature is not understood, however. The difference in intercepts of Eqs. 2 and 4 may be due to the fact that the dependent variable in the former case is a standard response while that of the latter case is a relative biological response.

Table I—Influence of Various Sulfa Drugs on the Fluorescence of 5-Dimethylaminonaphthalene-1-sulfonamide–Human Serum Albumin Complex

Sulfa Drug	log P	log Percent Decrease in Fluorescence: Observed	log Percent Decrease in Fluorescence: Calculated[a]	$\|\Delta \log \%D\|$
Sulfadimethoxine	1.56[b]	1.99	1.96	0.03
Sulfamethoxypyridazine	0.40[b]	1.98	1.77	0.21
Sulfamethazine	0.27[b]	1.91	1.73	0.18
Sulfaphenazole	1.57[b]	1.90	1.96	0.06
Sulfisoxazole	1.15[b]	1.90	1.94	0.04
Sulfadoxine	1.56[c]	1.89	1.96	0.07
Sulfamerazine	0.13[b]	1.71	1.68	0.03
Sulfathiazole	0.35[b]	1.65	1.76	0.11
Sulfamethoxazole	0.54[b]	1.61	1.81	0.20
Sulfanilamide	−0.83[b]	1.22	1.18	0.04
Sulfaguanidine	−1.22[b]	0.84	0.91	0.07
Probenecid	3.21[d]	1.63	1.62	0.01
Tolbutamide	2.34[d]	1.96	1.89	0.07

[a] Equation 4. [b] *Reference 9.* [c] log P estimated to be equivalent to that for sulfadimethoxine. [d] Experimental value, this laboratory.

The parabolic nature of Eqs. 1, 2, and 4 is unusual in that recent studies (10–12) of the dependence of the binding of drugs to serum albumin on lipophilicity led to linear relationships between $\log P$ and an appropriate binding parameter. This report is at variance with these studies in that the dependent variable in this case is a measure of the affinity of the displacing agent, relative to that of 5-dimethylaminonaphthalene-1-sulfonamide, for a specific site on human serum albumin. In the cases where linear relationships were found between binding and log P, the binding parameter was an equilibrium binding constant or a related parameter which could give no information about binding specificity.

The nonlinearity found in this and the prior study (7) can be explained by proposing a secondary binding site with an affinity for the displacing agents. As the lipophilicity becomes greater than $\log P_0$ for the series of the displacing agents, affinity for this secondary site increases and the effective concentration of the drug decreases.

There is theoretical justification for the nonlinearity we have found and this lies in the pseudoequilibrium model developed by Higuchi and Davis (13). They found that for systems in equilibrium or quasiequilibrium, nonlinear relationships can result between relative affinities for binding to specific sites or for receptor sites and lipophilicity.

In summary, the findings of this report indicate that drug–human serum albumin interactions can be highly specific and dependent on lipophilicity. It may be possible to take advantage of this specificity and, using Eqs. 1, 2, and 4 as guides in design, synthesize more effective uricosuric agents.

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BOOKS

REVIEWS

International Encyclopedia of Pharmacology and Therapeutics—Neuromuscular Blocking and Stimulating Agents, Volumes I and II. Edited by J. CHEYMOL. Pergamon Press, Inc., Maxwell House, Fairview Park, Elmsford, NY 10523, 1973. 654 pp. 14.5 × 23 cm. Price $52.50 (two-volume set).

These two comprehensive volumes reviewing the literature on agents which modify neuromuscular transmission represent the joint effort of the following authors: F. Bourillet, D. Bovet, W. C. Bowman, A. S. V. Burgen, C. Chagas, J. Cheymol, R. Couteaux, J. Debecker, J. E. Desmedt, D. Duncalf, Lise Engbaek, F. F. Foldes, G. Genkins, H. Grundfest, J. I. Hubbard, A. R. McIntyre, I. G. Marshall, K. E. Osserman, L. Sollero, G. Suarez-Kurtz, O. Vital-Brazil, G. Vourc'h, S. N. Webb, and P. G. Waser. Both volumes were edited by Professor J. Cheymol.

These two volumes are probably the most comprehensive review of agents that modify neuromuscular transmission that is available. The style of writing and arrangements of the manuscripts are of the highest quality and it is truly a pleasure to read the various chapters. As Professor Cheymol indicates in the Introduction, these volumes are comprehensive and include information that any researcher or clinician must have to understand the basic physiology and pharmacology of transmission. The various sections in the book include an excellent discussion of the anatomy that is involved with the end plate and skeletal muscle. At the physiological level, extensive discussions are included concerning pertinent enzymes, acetylcholine, and the influence of ions on transmission. Most of the volumes are devoted to the pharmacological level of discussion and comprehensive reviews are included covering comparative, chemical, and theoretical aspects of pharmacological actions of neuromuscular blocking agents and stimulants. Detailed discussions of the theoretical receptor as well as basic function of proteins are included. This is followed by discussion of pathological problems involved in neuromuscular transmission and finally the volumes conclude by comprehensive analysis of therapeutic considerations and treatments of various neuromuscular diseases.

Another reason that these are such delightful volumes is that the authors have exercised extreme care in searching the literature. The authors review the printed word all the way from the history of curare to the more recent research on the nerve terminal actions of drugs.

It is a pleasure for this reviewer to recommend these comprehensive volumes and assure any reader that they will be reviewing the most recent published work as well as our important historical heritage that leads to present knowledge. Volume I contains 423 pages and Volume II has 231 pages. Any scientist or clinician who is interested in neuromuscular transmission should have these volumes available as a necessary reference.

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Foreign Compound Metabolism in Mammals, Volume 2. By D. E. HATHWAY. The Chemical Society, Burlington House, London, WIV OBN, England, 1972. xv + 513 pp. 14.5 × 22 cm. Price £ 11.00.

The second volume of this series covers the two-year period 1970–1971. It consists of an introduction section, seven chapters, and an author index and a compound index, the latter being a new feature added with this volume.

In the introduction, the editor D. E. Hathway discusses some broad points relative to the book, such as validation in man, biological availability, selection of animals, implications of metabolic studies on determining "no effect" levels, and perspectives in toxicology.

The first chapter on tracers for metabolism includes general considerations, syntheses of various radioisotopes, reactions, and stable isotopes.

The transference of radioactively labeled foreign compounds is covered in the second chapter; it is subdivided into drugs, pesticides, food additives, and other compounds.

Biotransformations, which comprise the third chapter, are discussed by the subdivisions drugs, food additives, carcinogens, lathyrogens, silicon and boron compounds, toxins, rodenticides, insecticides, herbicides, fumigants and fungicides, and other compounds.

Chapter Four includes the mechanisms of biotransformation such as oxidation, reduction, hydrolysis, and conjugation.

The last three chapters cover species, sex, and strain differences in metabolism; drug kinetics; and interactions of drugs and foreign compounds.

Staff Review ■

The Molecular Basis of Antibiotic Action. By E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, and M. J. Waring. Wiley, New York, N.Y., 1972. xviii + 456 pp. 14.5 × 23 cm.

The authors have chosen a topic for this book that is so potentially overwhelming in scope and detail one may justifiably wonder whether a single volume could adequately develop a molecular basis for antibiotic action. Their intent, however, is remarkably well approached. The format established is to classify the antibiotics according to whether they are inhibitors of bacterial cell wall synthesis, nucleic acid synthesis, or ribosome function or whether they affect the function of the cytoplasmic membrane. A clear effort is made to include under each classification examples of each major antibiotic known to function in the specified manner. The unifying thread is a lucid and critical discussion of biochemical or intact cell experiments which point to a particular molecular mechanism of action. Included in certain of the surveys, as well as in a separate chapter, is a fine introduction to the biochemical basis for bacterial resistance to antibiotics.