Binding Study of *p*-Hydroxybenzoic Acid Esters to Bovine Serum Albumin by Fluorescent Probe Technique

H. W. JUN, R. T. MAYER*, C. M. HIMEL†, and L. A. LUZZI

Abstract □ *N-n*-Butyl[(5-dimethylamino)-1-naphthalene]sulfonamide is a fluorescent probe which has minimal fluorescence in water but fluoresces strongly when bound to protein. The spectral property of this probe was used for an indirect measurement of the binding of *p*-hydroxybenzoic acid esters (parabens) to bovine serum albumin. The method is capable of providing simple, sensitive, and rapid determination of binding and the nature of binding. The data indicate that the mechanism leading to the binding of parabens to bovine serum albumin could be of a hydrophobic nature. The aromatic ring rather than the aliphatic side chain appears to be the primary binding site of the parabens when competing with this probe. Quantum yield information for the sulfonamide is discussed.

Keyphrases □ Drug–plasma protein binding—determination, fluorescent probe technique □ *N-n*-Butyl[(5-dimethylamino)-1-naphthalene]sulfonamide—as a fluorescent probe, parabens–bovine serum albumin binding □ Parabens–bovine serum albumin binding—determination, fluorescent probe technique □ Bovine serum albumin–parabens binding—determination, fluorescent probe technique □ Fluorescent probe technique—parabens–bovine serum albumin binding determination □ NMR spectroscopy—determination, parabens–bovine serum albumin binding

The binding of drugs by plasma proteins has been recognized as an important factor in drug availability, drug efficacy, and drug transport for many years (1). Various experimental techniques have been employed to study drug–protein interactions (2), with the majority of them directed at the elucidation of the extent of binding. In spite of extensive studies in this area, little information has been generated concerning the nature of binding sites and the mechanisms responsible for binding. One technique recently employed to examine the conformational nature of drug–protein interactions is nuclear magnetic relaxation measurements (3, 4). Fluorescence spectroscopy also has been employed to study binding phenomena, but it has been used primarily to examine enzyme–substrate interactions (5, 6).

The fluorescence techniques described in this study make use of a fluorescent probe as an indicator of binding. Fluorescent probes are defined (7) as fluorescent compounds whose spectral properties (such as fluorescence excitation, emission, and quantum yield) are affected by the physical and chemical environment of the probe molecules. Since the concept of a fluorescent probe was first introduced by Weber (8) in 1952, various experimenters (5, 6, 9) studied the applications of this technique in biochemical research related to binding.

Fluorescent probe techniques are capable of providing data similar to what could be obtained through dialysis studies without the relevant problems of membrane binding, Donnan effects, and time consumption. In addition, it may be possible to estimate the nature of binding and binding sites of the complex from the chemical structure and spectral properties of fluorescent probes.

The purpose of the present study was to examine the potential of the fluorescent probe techniques in the area of drug–protein interactions. Specifically, an attempt was made to establish the usefulness of the techniques for elucidating the nature of binding of parabens and bovine serum albumin. It was also thought that NMR data would be helpful in substantiating fluorescence findings.

EXPERIMENTAL

Apparatus—All absorption and fluorescence spectra were run on a Spectro 210[1]. This instrument was fully described in a previous publication (10). The pH measurements were obtained with a pH meter[2] calibrated using standard buffer solutions.

Chemicals and Solvents—The fluorescence probe, *N-n*-butyl-[(5-dimethylamino)-1-naphthalene]sulfonamide, was prepared in this laboratory[3]. Synthesis of this compound and other pertinent data concerning it will be presented elsewhere. Methyl-*p*-hydroxybenzoate[4] and ethyl-, propyl-, and *n*-butyl-*p*-hydroxybenzoates[5] were used. Water was double distilled from glass. Methanol was spectroscopic grade, and all other chemicals were reagent grade or of special purity. Bovine serum albumin was purchased[6]. Concentrations of the protein were determined by measuring the absorbance at 280 nm. and using $a = 0.66$ ($E^{1\%}_{1\,cm.} = 6.6$); the molecular weight of 66,000 was used to determine molar concentrations (11).

Methods—Fluorescence intensities of the bound sulfonamide were measured at two protein concentrations (4.0×10^{-6} and 1.0×10^{-4} *M*) in appropriate buffers. Two milliliters of each protein concentration was titrated with successive additions of 2 μl. of 1×10^{-3} *M* probe in methanol. Methanol did not affect the binding of the probe in the concentration range used. Binding of the parabens was determined by titrating a mixture of the drug and protein with the probe solution, and calculations were based on comparison to albumin–sulfonamide data.

Fluorescence titrations were performed manually with Hamilton microsyringes. All binding data were determined at an emission wavelength of 515 nm. Excitation and emission bandwidths were set at 100 Å. Temperatures in the fluorescence well of the fluorometer were controlled to 25 ± 0.1°, except in those experiments where temperature was a variable. The inner filter effect was minimized by keeping absorbance low at excitation wavelengths (less than 0.05 *A*). Irradiation periods for protein solutions were maintained for less than 5 min. to minimize photodecomposition of the tryptophan and histidine residues within the protein. Quantum yield determinations were made using the following equation given by Turner (12):

$$\phi_u = \phi_s \frac{a_u A_s d_s \lambda_s}{a_s A_u d_u \lambda_u} \qquad \text{(Eq. 1)}$$

where:

a = emission peak area corrected for solvent blank
A = absorbance at excitation wavelength
d = dilution factor in dilution of sample used for optical density measurements to concentration used in fluorescence measurements

[1] G. K. Turner and Associates, Palo Alto, Calif.
[2] Radiometer model 26, The London Co., Westlake, Ohio.
[3] By the method described by C. M. Himel *et al.* in data as yet unpublished from these laboratories.
[4] Fisher Scientific Co.
[5] Eastman Organic Chemicals.
[6] Nutritional Biochemicals Corp.

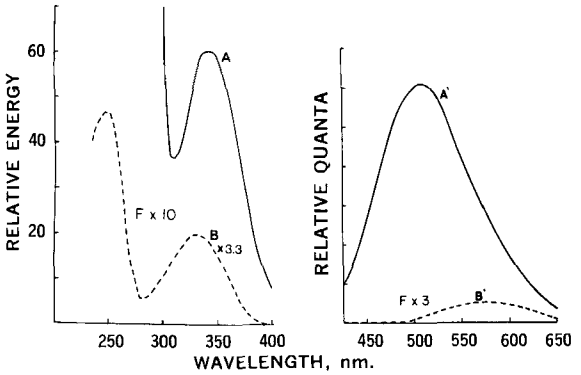

Figure 1—*Excitation and emission spectra of 1 × 10⁻⁶ M probe in the presence and absence of bovine serum albumin. Curves A and A' are the excitation and emission curves of probe in pH 7.2 tromethamine buffer solution of 8 × 10⁻⁵ M albumin. Curves B and B' are the excitation and emission curves of probe in the absence of albumin. Curve B is at a sensitivity about 3.3 times greater than B', A, and A'.*

λ = excitation wavelength
ϕ = quantum yield
s, u = subscripts denoting standard (s) and unknown (u)

Although quantum yield can be obtained by absolute methods (13), it is more convenient to use comparative methods in which compounds of known quantum yield are used as reference standards. Fluorescence measurements for quantum yield studies were made in 3-mm. quartz microcells[7] with special holders to limit inner filter effects. 5-Dimethylamino-1-naphthalenesulfonic acid was used as the reference standard, taking its quantum yield to be 0.36 at an excitation wavelength of 320 nm. (14). NMR data were obtained as described in a previous report (4). For the NMR studies, solutions were prepared by dissolving the parabens in 50% v/v polyethylene glycol 400 and water.

TREATMENT OF DATA

The fluorescence enhancement of the probe, upon addition to bovine serum albumin, was determined, and these data were used to calculate the binding constants for the albumin–probe complex. The fraction of bound probe was calculated from the following equation (5):

$$X = \frac{(I_0/I_f) - 1}{(I_b/I_f) - 1} \qquad \text{(Eq. 2)}$$

where I_0 and I_f refer to the fluorescence intensities of a given concentration of probe in solutions of low protein concentration and in solutions without protein, respectively. I_b refers to the fluorescence intensity of the same concentration of probe in solutions of high protein concentration; I_b then gives the fluorescence intensity of the probe in the presence of excess binding sites. After the value X is found for each point along the titration curve, the Scatchard equation (15) may be applied to determine the association constant of the probe:

$$\bar{V}/A = nK_a - \bar{V}K_a \qquad \text{(Eq. 3)}$$

where $\bar{V}$ is the number of moles of bound probe per mole of protein, A is the concentration of free probe, n is the number of binding sites on the protein, and K_a is the association constant of probe. The $\bar{V}$ is determined by multiplying the value for X by the ratio of the total probe concentration to the total protein concentration in solution. When $\bar{V}/A$ is plotted against $\bar{V}$, a straight line is obtained with a slope equal to $-K_a$. The ordinate and abscissa intercepts of this line give nK_a and n, respectively. The Scatchard equation assumes that the intrinsic association constant for all binding sites are equal.

When compounds such as the parabens were added to albumin solutions prior to titrations with probe, a decrease in fluorescence of the probe–albumin complex was observed due to competition for binding sites. When Scatchard plots were made from the binding data taken for the sulfonamide in the presence of the parabens, straight lines were obtained with decreased slopes but with identical abscissa intercepts, indicating competition between the probe and parabens for the same binding sites. The decrease in probe binding and the subsequent decrease in fluorescence of the probe–albumin complex can be used to calculate the binding constant of the parabens. Klotz *et al.* (16) derived equations describing simple competition between two ligand molecules for identical protein binding sites:

$$K_b = \frac{n(P_t)K_a(A) - K_a(A)(PA) - (PA)}{(B_t)K_a(A) - (P_t)K_a(A) + K_a(A)(PA) + (PA)} \times \frac{K_a(A)}{(PA)} \qquad \text{(Eq. 4)}$$

where:

K_b = association constant for competitor
K_a = association constant for probe
A = concentration of free probe
PA = concentration of bound probe
n = number of binding sites
P_t = total concentration of protein
B_t = total concentration of competitor

RESULTS AND DISCUSSION

Examination of the binding of parabens to bovine serum albumin was carried out with the use of fluorescent probe techniques. Since the fluorescent probe undergoes changes in fluorescence properties as a result of binding to the albumin, it was useful as an indication of interactions. Figure 1 shows fluorescence excitation and emission spectra of the probe in the presence and absence of bovine serum albumin. The fluorescence intensity of the probe (1×10^{-6} M) in aqueous solution was not significant (Curve B'); but when the albumin was added, the intensity was greatly enhanced, with a 60-nm. hypsochromic shift in wavelength of the emission maximum (Curve A'). The emission wavelength for Curve A was 515 nm., and the excitation wavelength for Curve A' was 340 nm. The emission wavelength for Curve B was 575 nm., and the excitation wavelength for B' was 335 nm. Excitation and emission bandwidths were fixed at 2.5 and 10.0 nm., respectively, for all curves. The areas under the emission curves are proportional to the quantum yields.

The quantum yield (0.057) of probe in aqueous solution increased approximately 12 times to 0.7 in the presence of albumin. These changes were due to alterations in the environment of the sulfonamide (17, 18). This is strong evidence of the binding between probe and protein. The high quantum yield of the probe may also be taken as an indication of a hydrophobic interaction, since quantum yield of similar molecules varies with the polarity of the environment[8]

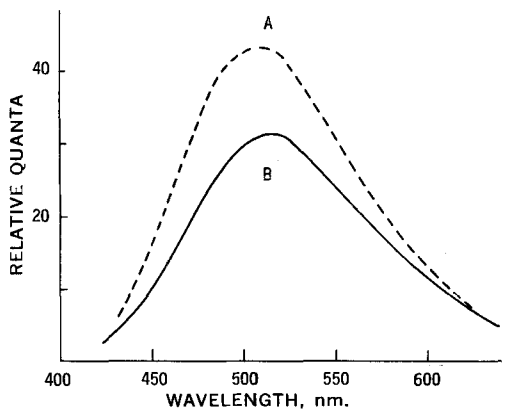

Figure 2—*Corrected emission spectra of the probe–albumin complex in the absence of a binding site competitor (- - -) and in the presence of 1 × 10⁻⁴ M methylparaben (——). Albumin concentration is 1.62 × 10⁻⁵ M in pH 7.2 tromethamine buffer. Excitation wavelength is at 335 nm.; emission and excitation bandwidths are set at 10.0 and 2.5 nm., respectively.*

[7] American Instrument Co.

[8] R. T. Mayer and C. M. Himel, *Biochemistry* (1971), in press.

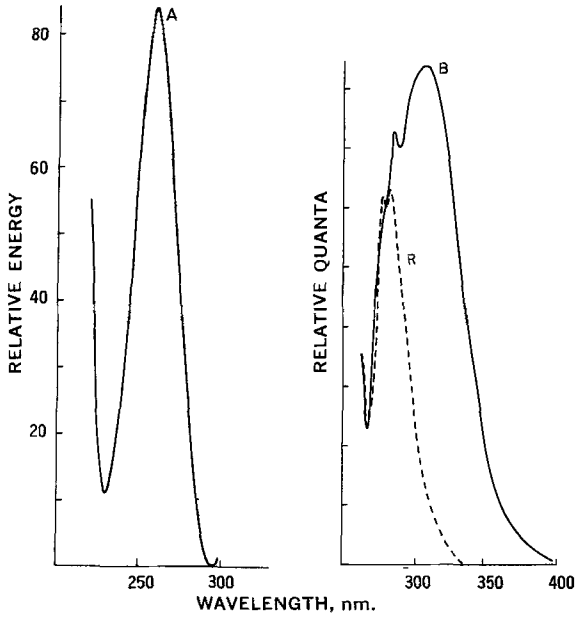

Figure 3—*Corrected excitation (A) and emission (B) spectra of a 6.6 × 10⁻⁶ M solution of* n*-butylparaben. Curve R is the Raman scatter peak for methanol at an excitation wavelength of 260 nm. Curve A excitation is at 260 nm. Curve B emission is at 305 nm. Excitation and emission bandwidths are fixed at 10.0 and 2.5 nm., respectively, at 25°.*

(9, 17). As an example, wavelength shifts and increases in quantum yield are seen when this probe is dissolved in less polar solvents such as ethanol and *n*-butyl alcohol; consequently, its emission properties can be equated to the polarity of the environment of the molecule. The large increase in quantum yield in the presence of albumin makes this probe useful to the study because interference from unbound probe material is low. In addition to these observations, the increase in binding constant for the probe from 1.618×10^5 M^{-1} at 15° to 1.810×10^5 M^{-1} at 25° to 2.115×10^5 M^{-1} at 35° and the increase in binding sites from one at 0.05 *M* KCl to two at 1.0 *M* KCl concentrations are further evidence that the nature of the complex is indeed hydrophobic. Structurally similar probes were reported to bind at hydrophobic binding sites of proteins (9).

Figure 2 shows the fluorescence emission spectra of the complex in the absence (Curve A) and in the presence (Curve B) of competitively binding methylparaben. A decrease in fluorescence of the complex was due to competition between the probe and methylparaben for the same or adjacent binding sites. The difference in fluorescence intensities with and without paraben is related to the binding of the drug and is used to calculate the binding constants for the paraben–protein complex (see *Treatment of Data*).

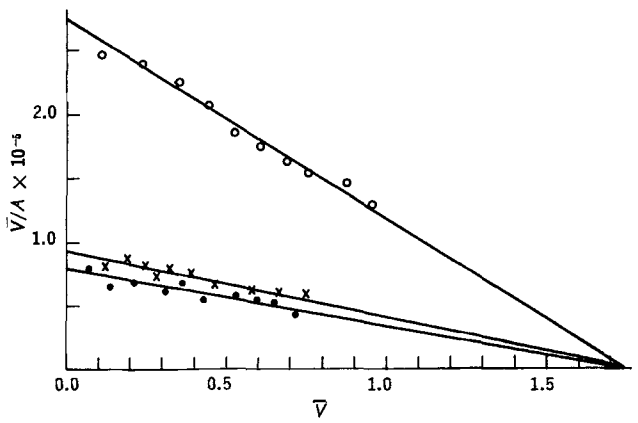

Figure 4—*Scatchard plots for probe–albumin complex (○) in the presence of methylparaben (●) and in the presence of ethylparaben (×) at 15°.*

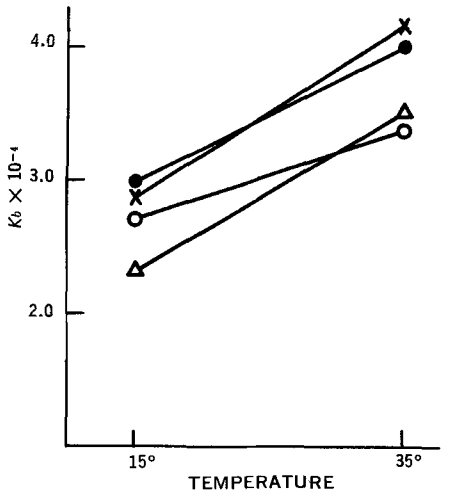

Figure 5—*Effect of temperature on the binding of parabens by bovine serum albumin. Key: ×, methylparaben; △, ethylparaben; ○, propylparaben; and ●,* n*-butylparaben.*

Figure 3 shows the excitation and emission spectra of *n*-butylparaben. The Raman scatter peak (*R*) for methanol at an excitation wavelength of 260 nm. is also shown. This figure is included since the fluorescence of paraben in methanol may prove to be useful for quantitative determination of parabens. Methyl-, ethyl-, and propylparabens also fluoresce in methanol, but their spectra are not shown because of the great similarity to that of *n*-butylparaben. The excitation and emission maxima are 260 and 300 nm. for methylparaben and 260 and 302.5 nm. for ethyl- and propylparabens, respectively.

Figure 4 consists of Scatchard plots utilized to calculate the binding constant of the probe and to show the decrease in the binding of this compound when in competition with methyl- and ethylparabens. Identical abscissa intercepts and decreased slopes indicated competition between the probe and methyl- and ethylparabens for the binding sites.

From the finding that the sulfonamide binds at the hydrophobic binding site of the albumin, and since the parabens were shown to compete with the sulfonamide for the binding sites, it is possible to rationalize that the binding of parabens to the albumin is also of a hydrophobic nature. Furthermore, a competition for the binding site between paraben and probe would not be observed if parabens did not bind at the same binding sites as did the probe. A hydrophobic interaction is considered to exist when two or more nonpolar groups come into contact with each other, thereby decreasing the extent of interaction with the surrounding water. Higuchi and Lach (19) indicated a possible role of hydrophobic interactions for binding of these types of drugs to macromolecules, and various other authors (20–22) discussed the contribution of hydrophobic interactions to drug–protein complex formation.

Corollary experiments were carried out to obtain additional information concerning the nature of binding in the paraben–albumin complex. Figure 5 shows the effect of temperature on paraben–albumin binding. The binding appeared to increase with an in-

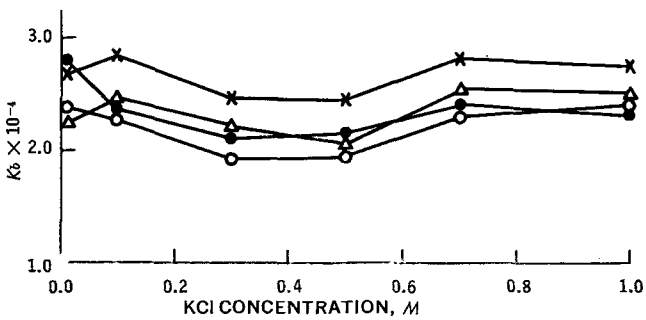

Figure 6—*Effect of ionic strength on the binding of parabens by bovine serum albumin at 25°. Key: ×, methylparaben; △, ethylparaben; ○, propylparaben; and ●,* n*-butylparaben.*

Table I—Thermodynamic Parameters for Bovine Serum Albumin–Paraben Binding at pH 7.2

	Temperature	Binding Constant, $M^{-1} \times 10^{-4}$	$\Delta H°$, kcal./mole	$\Delta G°$, kcal./mole	$\Delta S°$, e.u.	$T\Delta S°$, kcal./mole
Sulfonamide–albumin	15°	16.18	2.362	−0.275	9.16	2.637
	35°	21.15	2.362	−0.458	9.16	2.820
Methylparaben	15°	2.881	3.322	−0.605	13.6	3.917
	35°	4.199	3.322	−0.878	13.6	4.189
Ethylparaben	15°	2.330	3.639	−0.685	14.3	4.118
	35°	3.521	3.639	−0.771	14.3	4.404
Propylparaben	15°	2.745	2.182	−0.577	9.58	2.765
	35°	3.513	2.182	−0.769	9.58	2.957
n-Butylparaben	15°	2.978	2.679	−0.625	11.5	3.312
	35°	4.037	2.679	−0.854	11.5	3.542

Table II—Comparison of the Relaxation Rate[a] Ratios of Bound[b] over Free Drug[c] at Various pH Values at Constant Temperature

	Methylparaben		Ethylparaben		Propylparaben		*n*-Butylparaben	
pH	Phenyl Proton	Side-Chain[d] Methyl Proton	Phenyl Proton	Side-Chain Methyl Proton	Phenyl Proton	Side-Chain Methyl Proton	Phenyl Proton	Side-Chain Methyl Proton
4.5	1.75	—	1.94	1.22	1.87	1.18	1.93	1.31
7.6	2.38	—	2.36	1.68	2.30	1.52	2.41	1.56
8.8	2.06	—	2.22	1.77	2.18	1.40	2.28	1.88
9.5	2.02	—	2.13	1.85	2.04	1.32	1.96	1.74

[a] NMR spectra were obtained with Varian Associates HA-100 (see *References 4* and *5*). [b] With 1% bovine serum albumin. [c] In the absence of bovine serum albumin. [d] Was not able to measure due to overlap.

crease in temperature from 15 to 35° for all parabens. It is known that hydrophobic interactions tend to increase as temperature increases within the range studied (23). This endothermic nature of the binding suggests that hydrophobic interactions play a major role in the formation of the paraben–albumin complex.

Thermodynamic data obtained using the probe technique are shown in Table I. Since the enthalpy change is smaller than the term $T\Delta S$ for the binding of paraben to albumin, the binding may be due largely to the entropy effect, which is a typical thermodynamic observation for hydrophobic interactions (23, 24). In other words, the paraben–albumin binding was enhanced with a positive entropy term through the destruction of the "iceberg" surrounding paraben molecules. The term iceberg was introduced by Frank and Evans (25) to describe the icelike structure of water surrounding hydrophobic molecules.

Figure 6 shows the effect of ionic strength on paraben–albumin binding. Binding was not influenced by ionic strength resulting from concentrations of 0.05 to 1.0 *M* KCl. Since the energy of electrostatic interactions varies inversely with the dielectric constant for the solution (26), an electrostatic interaction between paraben and albumin would have been decreased by addition of salt. However, no influence of salt concentration on the binding was found, thus limiting the possibility of electrostatic interactions as a primary binding mechanism.

Hydrophobic binding between parabens and albumin can take place by virtue of interaction between the hydrocarbon groups on the side chain (27) of the paraben with hydrophobic sites of bovine suerm albumin. On the other hand, the aromatic ring system (28) of the parabens might also be involved in hydrophobic binding. Since NMR techniques for the elucidation of binding sites of drug molecules are well established (3, 4), this method was used to find the preferential binding sites of the small molecules. The NMR results, as shown in Table II, indicate that the aromatic portion of the parabens rather than the aliphatic side chain was primarily involved in the binding. It can be seen from this table that the NMR peaks of the phenyl protons of parabens were broadened to a greater extent as a result of the interaction with bovine serum albumin than were those for the side-chain protons under the identical experimental conditions.

The finding that the binding constants for methyl-, ethyl-, propyl-, and *n*-butylparabens are of the same order of magnitude (Table I) indicates that the contribution from the side chain of parabens may not be significant for the paraben–albumin interaction. This, however, is not in agreement with a previous study (29) using dialysis techniques, in which the binding constants of parabens were found to differ. The discrepancies in the binding constants determined in the two studies may be due to the fact that dialysis techniques measure more binding sites for the parabens, whereas the fluorescent probe techniques detect only those sites to which the probe molecules may bind. It was found that the probe binds to only one or two sites and, consequently, the competition could be observed only for these sites. In this case, the aromatic ring system of the paraben was involved in the binding, thus partially explaining the different binding constants obtained from the experimentation reported here as compared with dialysis studies. Continuing studies are in progress to clarify this point.

SUMMARY

Although recent studies (30, 31) indicated that probably more than one mechanism is responsible for a particular complex, this study showed, through several avenues, that hydrophobic interactions are primarily responsible for the paraben–albumin complex. These studies included: (*a*) the demonstration of competition between the parabens and *N*-*n*-butyl[(5-dimethylamino)-1-naphthalene]sulfonamide, which were found to bind at hydrophobic binding sites of the protein; (*b*) thermodynamic data indicating that the binding process between parabens and albumin was in concert with those described for hydrophobic interactions; (*c*) the paraben–albumin interaction which was not weakened by an increase in salt concentration; and (*d*) NMR data showing that the aromatic ring was primarily involved in binding.

REFERENCES

(1) B. B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345(1957).

(2) M. C. Meyer and D. E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).

(3) J. J. Fischer and O. Jardetzky, *J. Amer. Chem. Soc.*, **87**, 3237(1965).

(4) H. Zia, R. H. Cox, and L. A. Luzzi, *J. Pharm. Sci.*, **60**, 89 (1971).

(5) L. Brand, J. R. Gohlke, and D. S. Rao, *Biochemistry*, **6**, 3510(1967).

(6) G. Weber and L. B. Young, *J. Biol. Chem.*, **239**, 1415(1964).

(7) R. F. Chen, in "Fluorescence Theory, Instrumentation and Practice," G. C. Guilbault, Ed., Dekker, New York, N. Y., 1967, p. 443.

(8) G. Weber, *Biochem. J.*, **51,** 155(1952).
(9) W. O. McClure and G. M. Edelman, *Biochemistry*, **5,** 1908 (1966).
(10) G. K. Turner, *Science*, **146,** 183(1964).
(11) E. Daniel and G. Weber, *Biochemistry*, **5,** 1893(1966).
(12) G. K. Turner and Associates, Bull. 8885, Palo Alto, Calif., 1966.
(13) W. H. Melhuish, *J. Phys. Chem.*, **65,** 229(1961).
(14) C. M. Himel and R. T. Mayer, *Anal. Chem.*, **42,** 130(1970).
(15) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51,** 660(1949).
(16) I. M. Klotz, H. Triwush, and F. M. Walker, *J. Amer. Chem. Soc.*, **70,** 2935(1948).
(17) G. Weber and D. J. R. Laurenee, *Biochem. J.*, **51,** 31(1954).
(18) S. Udenfriend, "Fluorescence Assay in Biology and Medicine," vol. 2, Academic, New York, N. Y., 1969, p. 276.
(19) T. Higuchi and J. L. Lach, *J. Amer. Pharm. Ass., Sci Ed.*, **43,** 465(1954).
(20) H. J. Weder and M. H. Bickel, *J. Pharm. Sci.*, **59,** 1563 (1970).
(21) J. Clausen, *J. Pharmacol. Exp. Ther.*, **153,** 167(1966).
(22) M. Sahyun, *Nature*, **209,** 613(1966).
(23) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66,** 1773 (1962).
(24) H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.*, **16,** 2248(1968).
(25) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13,** 507(1945).
(26) A. N. Martin, "Physical Pharmacy," Lea & Febiger, Philadelphia, Pa., 1965, p. 248.
(27) W. Kauzmann, *Advan. Protein Chem.*, **65,** 1071(1961).
(28) O. Jardetzky and N. G. Wade-Jardetzky, *Mol. Pharmacol.*, **1,** 214(1965).
(29) N. K. Patel, P. C. Sheen, and K. E. Taylor, *J. Pharm. Sci.*, **57,** 1370(1968).
(30) W. H. Johns and T. R. Bates, *ibid.*, **59,** 329(1970).
(31) T. Higuchi and H. Kristiansen, *ibid.*, **59,** 1601(1970).

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Microbiologic Determination of Drug Partitioning I: Gelatin–Acacia Complex Coacervate System

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Abstract □ An experimental method was developed for the determination of the extent of the partitioning of phenylmercuric nitrate in coacervate systems, as measured by the effect of the antimicrobial drug on the growth of *Escherichia coli*. Bacterial growth is utilized to investigate changes in the coacervate partition coefficient as a function of drug concentration. The method is sensitive for microgram quantities of this antimicrobial agent and is useful in drug partitioning at levels found in physiologic systems.

Keyphrases □ Drug partitioning—microbiological determination, gelatin–acacia coacervate system □ Phenylmercuric nitrate—microbiological determination of partitioning in coacervate systems □ Antimicrobial agents—determination of drug partitioning using coacervate systems □ Coacervates, complex—drug partitioning of antimicrobial agents □ Gelatin–acacia complex coacervate system—microbiological determination of drug partitioning

The term coacervation has been used to describe the salting out of certain types of lyophilic colloids into liquid droplets (1). Kruyt and Bundenberg de Jong (2) introduced the word in 1929 to characterize the flocculation or separation of liquids from solution when at least one separated phase contained a colloidal solute. The term has since been subdivided into simple coacervation and complex coacervation; complex coacervation is mainly dependent on the charges on the molecules, while simple coacervation results from phase separation due to the interaction of nonionized entities of macromolecules (1, 3). The mechanism for the coacervation process was studied by Kruyt (4), Bundenberg de Jong (5, 6), Voorn (7), Cohen and Vassiliades (8), Takruri (1), and others.

Coacervates generally contain a large amount of water; when these systems separate, the more dense coacervate settles to the bottom and contains about 99% of the colloidal solute. The upper layer is termed the "equilibrium liquid" or "dissolved coacervate layer" (2) and is in equilibrium with the coacervated phase. Water in the coacervate layer is structurally different from regular (liquid) water or from water in equilibrium with the coacervate phase. The state of aggregation of water molecules in coacervates was compared to the state of water in living systems. It was postulated (1) that the difference in aggregation states between water in coacervates and in the equilibrium layer is an important determinant in the distribution of electrolytes in these systems.

Takruri (1) studied the high uptake of organic molecules in complex coacervate systems and compared them to similar systems existing in nature. Several researchers (1, 9–12) suggested that these systems may serve as models for the study of absorption and distribution characteristics of drugs.

The purpose of this study was to utilize complex coacervates containing gelatin and acacia as a model system whereby drug partitioning of antimicrobial agents could be determined. Concentrations of phenylmercuric nitrate below the minimum inhibitory con-