(19) G. W. Peng, M. A. F. Gadalla, and W. L. Chiou, *Res. Commun. Chem. Pathol. Pharmacol.*, **18**, 233 (1977).
(20) J. Overbach, N. F. Johnson, T. R. Bates, H. J. Pieniaszek, Jr., and W. J. Jusko, *J. Pharm. Sci.*, **67**, 1250 (1978).
(21) K. Harzer, *J. Chromatogr.*, **155**, 399 (1978).
(22) T. B. Vree, Y. A. Hekster, A. M. Baars, J. E. Damsma and E. van der Kleijn, *J. Chromatogr.*, **146**, 103 (1978).
(23) R. W. Bury and M. L. Mashford, *ibid.*, **163**, 114 (1979).
(24) D. Jung and S. Øie, *Clin. Chem.*, **26**, 51 (1980).
(25) P. A. Bristow, P. N. Brittain, C. M. Riley, and B. F. Williamson, *J. Chromatogr.*, **131**, 57 (1977).
(26) I. Jane, *ibid.*, **111**, 227 (1975).
(27) B. B. Wheals, *ibid.*, **122**, 85 (1976).
(28) B. Emanuelsson and R. G. Moore, *ibid.*, **146**, 113 (1978).
(29) J. Crommen, in "Advances in Chromatography," A. Zlatkis, ed., 1979, p. 783.
(30) D. Westerlund, A. Theodorsen and Y. Jaksch, *J. Liq. Chromatogr.*, **2**, 969 (1979).
(31) D. Westerlund and E. Erixson, *J. Chromatogr.*, **185**, 593 (1979).

Binding of Selected Phenol Derivatives to Human Serum Proteins

JOSEPH JUDIS

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Abstract □ The binding of phenol and four of its derivatives to whole human serum and several human serum proteins was investigated. ^{14}C-labeled derivatives were utilized and binding was studied by either equilibrium or dynamic dialysis. Phenol itself was bound least to most of the serum proteins as compared to the derivatives and albumin, and whole human serum exhibited the highest percent binding of the proteins used. Percent binding to albumin and serum paralleled molecular weights of the derivatives, but no definite pattern was observed in ranking the percent binding of the other derivatives to the other serum proteins. Binding constants (K_1, K_2, n_1 and n_2) were determined from Scatchard plots for all the derivatives except *p*-chloro-*m*-xylenol. Phenol was found to have the highest association constant (K_1) and *p-tert*-amylphenol, the lowest. For the entire group of five derivatives and albumin as the protein, a direct, statistically significant correlation was found between percent binding and Hansch π values. No correlation could be found with Hammett σ values. It is concluded that binding of the phenol derivatives to albumin involves primarily hydrophobic bonds.

Keyphrases □ Binding—selected phenol derivatives, human serum proteins ■ Phenol—selected derivatives, binding, selected human serum proteins □ Serum—human, binding of selected phenol derivatives to proteins ■ Derivatives—phenol, selected, binding to human serum proteins

Since the discovery of phenol in 1834 and its introduction to antiseptic surgery by Lister in 1867 (1), both phenol and many of its derivatives have become firmly established as germicidal agents (2, 3). Phenol derivatives, unlike many other germicides, have been shown to be less active in the presence of organic matter (2–4). Blood is a common organic contaminant in materials to be sterilized. Preliminary investigations of the binding of phenol derivatives to human serum proteins were carried out as part of the work in this study on the mechanism of action of phenol derivatives (5, 6). These preliminary studies were expanded to include individual major human serum proteins, percent binding to each, and binding parameters for human serum albumin for a selected group of phenol derivatives.

EXPERIMENTAL

Materials—The phenol derivatives used were obtained with carbon-14 labels[1]. The compounds used and specific activities were as follows: [^{14}C]phenol, 1.57 mCi/mmole; [2,4-^{14}C]dichlorophenol, 0.68 mCi/mmole; [2,4,6-^{14}C]trichlorophenol, 0.68 mCi/mmole; [^{14}C]*p-tert*-amylphenol, 0.27 mCi/mmole; [^{14}C]*p*-chloro-*m*-xylenol, 0.0027 mCi/mmole. Phenol stock solutions were made in distilled water and those of the other derivatives were made with 0.1% NaOH as the solvent. Crystalline human serum albumin and the other human serum proteins were obtained commercially[2] and the whole human serum was of tissue culture quality[3]. All other chemicals used were of reagent grade.

Methods—Dialysis methods (equilibrium and dynamic) were carried out as previously described (7). Radioactivity was determined in a liquid scintillation system using techniques previously described (7, 8). Estimates of binding parameters were calculated using the method of Sandberg (8) and standard Scatchard techniques. All averages were based on a minimum of three replicates.

Table I—Binding of [^{14}C]Phenol and [^{14}C]-*p-tert*-Amylphenol to Human Serum Proteins [a]

Serum Proteins		Ligands Percent Bound[b]	
Fraction	Concentration[c], mg/ml	Phenol	*p-tert*-Amylphenol
Albumin	40.0	48.7 (0.68)	89.1 (0.74)
α Globulin IV-1	1.0	7.9 (0.47)	28.5 (1.92)
α Globulin IV-4	5.0	23.0 (0.63)	46.5 (1.60)
β Globulin III	7.0	3.13 (0.14)	35.2 (0.60)
γ-Globulin II	11.0	8.21 (0.63)	13.0 (0.69)
Human serum	—	52.7 (1.16)	95.3 (0.18)

[a] Each system contained a total of 1.00×10^{-7} mole of [^{14}C]phenol or 1.86×10^{-7} mole of [^{14}C]*p-tert*-amylphenol. [b] The values in parentheses are standard deviations. Data obtained using equilibrium dialysis method. [c] Concentrations used approximate those normally found in human serum.

RESULTS AND DISCUSSION

The values obtained for percent binding to whole human serum and the several serum proteins for the five derivatives studied are listed in Tables I and II. Weakest binding was found with phenol and the latter was bound primarily to albumin. Although phenol was bound to the other serum proteins, the extent was <10% except for α-globulin IV-4. The addition of alkyl groups or halogens to the phenol molecule has been found to increase the latter's germicidal activity (9). It would appear from the results in Tables I and II that alkylation and/or halogenation of phenol also increases the latter's binding affinity for serum proteins. The addition of a tertiary amyl group to phenol increased percent binding of phenol almost twofold (Table I) for albumin and whole human serum and

[1] New England Nuclear Corp.

[2] Nutritional Biochemicals Division of ICN Life Sciences Group.
[3] Difco Laboratories (desiccated TC human serum).

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Table II—Binding of [2,4-^{14}C]Dichlorophenol, [2,4,6-^{14}C]Trichlorophenol, and [^{14}C]-*p*-Chloro-*m*-Xylenol to Human Serum Proteins [a]

Serum Proteins		Ligands Percent Bound [b]		
Fraction	Concentration, mg/ml	2,4-Dichlorophenol	2,4,6-Trichlorophenol	*p*-Chloro-*m*-xylenol
Albumin	40.0	87.7 (0.45)	94.1 (0.36)	85.2 (2.32)
α-Globulin IV-1	1.0	17.3 (0.30)	8.09 (0.139)	23.8 (0.20)
α-Globulin IV-4	5.0	57.5 (0.28)	55.2 (0.93)	10.9 (0.88)
β-Globulin III	7.0	20.5 (0.53)	13.0 (1.66)	23.8 (2.57)
γ-Globulin II	11.0	8.0 (0.51)	6.4 (0.55)	14.9 (1.12)
Human serum	—	94.6 (0.40)	95.8 (0.15)	89.8 (2.99)

[a] Each system contained a total of 1.56×10^{-7} mole of [2,4-^{14}C]dichlorophenol, or 2.21×10^{-7} mole of [2,4,6-^{14}C]trichlorophenol or 2.04×10^{-7} mole of [^{14}C]*p*-chloro-*m*-xylenol. [b] The values in parentheses are standard deviations. Data obtained using equilibium dialysis method.

Table III—Binding Parameters [a] (Albumin) of Phenol, 2,4-Dichlorophenol, 2,4,6-Trichlorophenol, and *p-tert*-Amylphenol

Ligand [b]	Percent Binding	K_1 [c]	n_1	K_2	n_2
Phenol	48.7	67,200,000	0.0487	16,020	0.186
2,4-Dichlorophenol	87.7	1,010,000	0.1930	71,900	0.345
p-tert-Amylphenol	89.1	64,600	0.3670	21,500	0.714
2,4,6-Trichlorophenol	94.1	30,200,000	0.2940	1,470,000	0.323

[a] Data obtained by dynamic dialysis method. [b] Initial number of moles added to each system: phenol, 1.03×10^{-6}; *p-tert*-amylphenol, 9×10^{-7}; 2,4-dichlorophenol, 9.2×10^{-7}; 2,4,6-trichlorophenol, 9.95×10^{-7}. Dynamic dialysis method was used to obtain data. [c] Liters per mole.

Table IV—Ranking of Phenol Derivatives [a] in Order of Percent Binding to Various Serum Proteins

Serum Protein	Ranking by Percent Binding
Whole human serum	III > IV > II > V > I
Human serum albumin	III > IV > II > V > I
α-Globulin IV-1	IV > V > II > III > I
α-Globulin IV-4	II > III > IV > I > V
β-Globulin III	IV > V > II > III > I
γ-Globulin II	V > IV > I > II > III
Ranking on basis of molecular weight	III > IV > II > V > I

[a] Phenol derivatives above are: phenol, I; 2,4-dichlorophenol, II; 2,4,6-trichlorophenol, III; *p-tert*-amylphenol, IV; and *p*-chloro-*m*-xylenol, V.

also substantially for the other serum proteins. Again, the serum protein showing the highest percent binding was albumin, and α-globulin IV-4 also exhibited substantial binding (46.5%).

Halogenation of phenol also caused a great increase in binding with whole serum and the serum proteins. 2,4,6-Trichlorophenol was found to be bound to a higher percent to whole serum and albumin than 2,4-dichlorophenol ($p < 0.05$), but binding to the other serum proteins did not show a similar pattern. The derivative that was both a halogenated and alkylated derivative of phenol, *p*-chloro-*m*-xylenol, exhibited a higher percent binding to whole serum and most of the serum proteins than phenol itself, but not higher than the two halogenated derivatives for albumin and human serum.

The Scatchard plot for *p-tert*-amylphenol and albumin is shown in Fig. 1. Data for Scatchard plots were limited, in this study, to albumin as the protein, because all of the derivatives showed the highest percent binding to albumin. The plots for phenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol were similar, differing only in values for the axes. It can be seen that the curve is not a straight line as would be expected for a single binding system. It appears that the curve has two distinct segments indicative of two binding systems. The binding parameters calculated are given in Table III[4]. The two derivatives exhibiting the highest percent binding to whole serum and albumin, did not yield the largest K_1 association constants. Phenol itself had the highest calculated association constant (K_1) and *p-tert*-amylphenol's constant (K_1) was the lowest of all. All of the *n* values, the number of binding sites, were fractional. Values for $n < 1$ have been reported in other studies (10–12) and present difficulties in interpretation in terms of a physical model.

The ranking by percent binding of the several phenol derivatives to the protein fractions studied is illustrated in Table IV. The only obvious pattern is that ranking of the phenols with respect to binding to albumin or whole human serum is exactly the same as ranking by molecular weight. No clearcut pattern is apparent for the other proteins. Hansch (13–15), in his studies of the relationships between structure and biological activity, found correlation of the latter with partition coefficients expressed as the constant, π. An additional constant, σ, was developed by Hammett (16) to express the electronic activity of the substituents on an organic molecule. Thus, π is an expression of the degree of lipophilicity of a compound and σ is the electronic (polar) nature of a compound. The Hansch equation attempts to take into account the contribution of both lipophilic and electronic properties of a compound with respect to its biological activity. The latter is usually represented as the log of the reciprocal of the measure of biological activity.

The π and σ values for the phenol derivatives were determined, either from literature values reported (15) or calculated on the basis of the sum of the values of substituent groups (14–17). The log of the reciprocal of percent binding to each protein was analyzed by regression analysis with the π and σ values to search for possible correlations. The results obtained are listed in Table V. For those values (π or σ values and log of 1/percent bound) yielding a correlation coefficient of at least 0.900 ($p \leq 0.05$), correlation coefficients are listed. A direct relationship with π values would indicate that the percent binding increased with an increase in lipophilic properties. Significant correlation with σ values would suggest increased binding with more polar properties if the relationship was direct

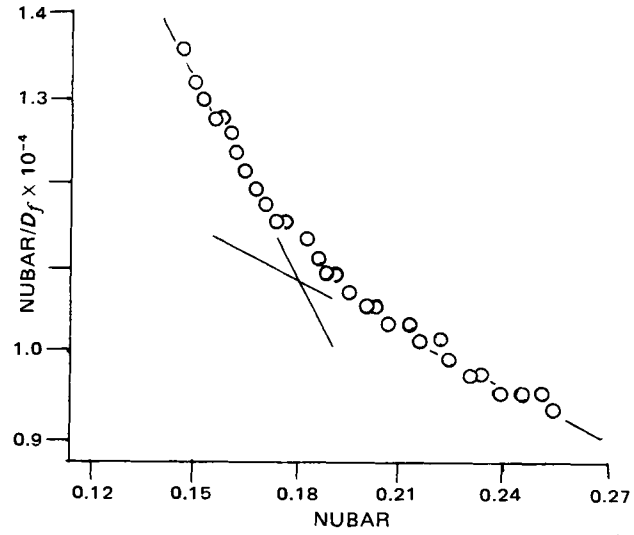

Figure 1—*Scatchard plot of data for binding of* p-tert-*amylphenol to human serum albumin, determined by dynamic dialysis. A total of 9.3* $\times 10^{-7}$ *moles of* p-tert-*amylphenol were added at the beginning of the run and albumin concentration in the bag (5 ml volume) was* 5.797×10^{-4} *M (40 mg/ml). Nubar is equal to total bound moles of ligand divided by moles of albumin in the system and* D_f *is the molar concentration of unbound ligand.*

[4] It was not possible to obtain binding parameters for *p*-chloro-*m*-xylenol because a sufficient quantity of the labeled compound was not available.

Table V—Relationships Between Percent Binding of Phenol Derivatives to Various Serum Proteins and π or σ Values or Molecular Weights of Phenol Derivatives

Phenol Derivative[a] Groups	Serum Protein	Relationship[b] π[c] Values	σ Values	Molecular Weight
I–V	Albumin	Direct (0.963)	—	Direct (0.964)
II–V	α-Globulin IV-1	—	Inverse (−0.964)	—
	β-Globulin III	—	Inverse (−0.954)	—
I–III	Albumin	Direct (0.98)	Direct (0.979)	Direct (0.98)
	α-Globulin IV-4	Direct (0.924)	Direct (0.920)	Direct (0.924)
I, IV, V	Albumin	Direct (0.979)	—	Direct (1.0)
	α-Globulin IV-1	Direct (.997)	—	Direct (1.0)
	β-Globulin III	Direct (0.998)	—	Direct (0.966)
	γ-Globulin II			Direct (0.931)

[a] Phenol derivatives above are: I, phenol; II, 2,4-dichlorophenol; III, 2,4,6-trichlorophenol; IV, *p*-chloro-*m*-xylenol; V, *p*-*tert*-amylphenol. [b] Figures in parentheses are coefficients of correlation. [c] π Values for I–V, respectively, were 1.46, 3.0, 3.69, 3.27, and 3.59. σ Values, respectively, were −0.36, 0.10, 0.33, −0.47, and 0.85.

and the reverse, if inverse. For the entire group of five phenol derivatives, correlation was direct with increasing molecular weight (as pointed out previously) and with increasing π values. The relationship with σ values was not statistically significant.

It would appear that for the entire group of five derivatives, the more lipophilic the molecule, the higher the binding to albumin. Other statistically significant relationships were found when the phenol derivatives were divided into other groups. Namely, either the halogenated phenols plus phenol or the alkylated phenols (including *p*-chloro-*m*-xylenol) and phenol. The data in Table V also suggest something about the hydrophilic–hydrophobic properties of several of the proteins. Albumin appears to have both hydrophobic and polar binding sites in that there was a direct relationship between binding and π values and also with σ values for the more polar derivatives (Group 3). On the other hand, α-globulin IV-1 had a direct relationship with π values and an inverse relationship with σ values for the more polar derivatives (Group 2.) suggesting capability for a polar binding primarily. Like albumin, α-globulin IV-4 appears to have both nonpolar and polar binding sites as indicated by the direct relationship with both π and σ values for the more polar derivatives.

In one form of the Hansch equation (17):

$$\log 1/C = a\pi + b\sigma + c \qquad \text{(Eq. 1)}$$

where C represents the measure of biological activity. The values a, b, and c are constants characteristic of the system, while π is the Hansch constant and σ is the Hammett constant. It was possible to fit this equation with statistical significance ($p = 0.05$) only in the cases of binding of the five phenols to either albumin or human serum. The constants obtained were:

For albumin:

$$\log 1/\text{percent binding} = -0.13\pi + 0.016\sigma - 1.51 \qquad \text{(Eq. 2)}$$

For human serum:

$$\log 1/\text{percent binding} = -0.12\pi + 0.0091\sigma - 1.56 \qquad \text{(Eq. 3)}$$

In studies of the toxicity of phenol derivatives to two microorganisms (18) the Hansch equations calculated suggested that the σ value was of little consequence. This would also appear to be true in the equations describing the binding of the phenol derivatives to either albumin or human serum in that the coefficient for the σ term is relatively small.

In conclusion, it would appear from the data obtained that for the derivatives of phenol studied, serum protein binding is significant, especially to whole human serum and albumin and that binding is related to molecular weight and Hansch π values. Binding to albumin apparently involves primarily hydrophobic bonds rather than polar bonding. This conclusion would be consistent with the findings of Teresi (19) that treatment of bovine serum albumin to remove epsilon-amino positive charges had little effect on binding of 2,4-dichlorophenolate (pH 7.6). In addition, Scholtan (20) concluded that nonspecific hydrophobic binding to human serum albumin is the major mechanism for a wide variety of organic molecules.

REFERENCES

(1) J. Lister, *Lancet*, **2**, 353 (1867).

(2) F. Putnam, *Adv. Protein Chem.*, **IV**, 79 (1948).

(3) G. Sykes, "Disinfection and Sterilization," D. Van Nostrand Co., Princeton, N.J., 1958, p. 244.

(4) E. Bennett, "Advances in Applied Microbiology," vol. 1, Academic, New York, N.Y., 1958, p. 123.

(5) J. Judis, *J. Pharm. Sci.*, **55**, 803 (1966).

(6) *Ibid.*, **57**, 768 (1968).

(7) *Ibid.*, **66**, 802 (1977).

(8) A. Sandberg, H. Rosenthal, S. Schneider, and M. Slaunwhite, in "Steroid Dynamics," T. Nkro, G. Pincus, and J. Tait, Eds., Academic, New York, N.Y. 1966, p. 33.

(9) S. Block, "Disinfection, Sterilization and Preservation," Lea & Febiger, Philadelphia, Pa., 1977, p. 227.

(10) J. Vallner, *J. Pharm. Sci.*, **66**, 447 (1977).

(11) C. Bowmer and W. Lindup, *Biochim. Biophys. Acta*, **624** 260 (1980).

(12) G. Powis, *J. Pharm. Pharmacol.*, **26**, 113 (1974).

(13) C. Hansch, P. Maloney, T. Fujita, and R. Muir, *Nature (London)*, **194**, 178 (1962).

(14) T. Fujita, J. Iwasa, and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).

(15) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).

(16) H. Jaffe, *ibid.*, **53**, 191 (1953).

(17) C. Hansch, R. Muir, T. Fujita, P. Maloney, F. Geiger, and M. Streich, *J. Am. Chem. Soc.*, **85**, 2817 (1963).

(18) C. Hansch and T. Fijita, *ibid.*, **86**, 1616 (1964).

(19) J. Teresi, *ibid.*, **72**, 3972 (1950).

(20) V. Scholtan, *Arzneim.-Forsch.*, **18**, 505 (1968).

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