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Protein Concentration Effects on Binding of ^{14}C-Codeine, ^{14}C-Morphine, and ^{3}H-Methadone to Human Serum Albumin

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Abstract □ The binding of ^{14}C-codeine, ^{14}C-morphine, and ^{3}H-methadone to human serum albumin was studied at a constant ligand concentration and varying albumin concentrations. Scatchard plots of the data were linear and had positive slopes. The curves for the three ligands had similar data values and slopes. With increasing albumin concentrations, β (fraction bound) values increased. The β change per albumin concentration change $[d(\beta)/d(\text{albumin})]$ dropped most markedly at lower albumin concentrations and changed less as the albumin concentrations approached those normally observed in human blood. Values of nK, the number of binding sites or systems on the protein times the association constant, calculated from β and the albumin concentrations decreased with an increase in albumin concentration increase. The curves were similar for codeine and morphine, while the methadone curve indicated less of a decrease in nK with an increasing albumin concentration. For data published previously on the binding of various ligands at different albumin concentrations, plots of β values or calculated nK values *versus* albumin concentration revealed that β generally increased and nK decreased with an increasing albumin concentration. Desipramine, propoxyphene, prednisone, and phenylbutazone were exceptions to these relationships.

Keyphrases □ Codeine—binding to human serum albumin, varying albumin concentration □ Morphine—binding to human serum albumin, varying albumin concentration □ Methadone—binding to human serum albumin, varying albumin concentration □ Binding, protein—codeine, morphine, and methadone to human serum albumin, varying albumin concentration

In most studies of drug binding to serum proteins, a single protein concentration was used and the ligand concentration was varied (1–3). Scatchard plots of the data gave either a straight line or curve and almost invariably had a negative slope. However, studies of cortisol binding to serum albumin were conducted in which the cortisol concentration was constant and the albumin concentration was varied (4). The resulting Scatchard plot was a straight line with a positive slope. The results could not be explained on the basis of microheterogeneity or by variations of the albumin activity coefficient. This observation was not investigated further.

Several subsequent studies involved the binding of various ligands to serum proteins using a constant ligand concentration and varying protein concentrations. Scatchard plots with a positive slope were observed with thiopental (5), phenytoin (6, 7), and L-tryptophan (6, 7). Bowmer and Lindup (8) recently commented on the several published studies involving data yielding Scatchard plots with positive slopes and the significance of obtaining binding data when the protein concentration is varied and the ligand concentration is constant.

Previous studies involving the binding of ^{14}C-codeine, ^{14}C-morphine, and ^{3}H-methadone to human serum albumin yielded Scatchard plots with positive slopes when the albumin concentration was constant and the ligand concentration was varied (9). No explanation for the positive slopes (rather than the usually observed negative slopes) could be offered. This report is an extension of the earlier studies and involves variation of the albumin concentration and constant ligand concentrations.

EXPERIMENTAL

Methods—All protein binding determinations were performed by the equilibrium dialysis method (9) using multiple-cell blocks. Each cell had 1 ml on each side of the cellulose membrane. Radioactivity was determined in a liquid scintillation system as described previously (9).

Materials—^{14}C-Codeine[1], ^{14}C-morphine[1], ^{3}H-methadone[2], and crystalline human serum albumin[3] were obtained commercially. All chemicals were reagent grade.

RESULTS AND DISCUSSION

The human serum albumin concentration was varied between 0.217×10^{-4} and 0.87×10^{-3} *M*. The ligand concentration was kept constant (^{14}C-morphine, 0.00356; ^{14}C-codeine, 0.00339; and ^{3}H-methadone, 0.00332 *M*). Scatchard plots of the data for the three ligands are shown in Fig. 1. The albumin concentrations for each ligand fell in the same sequence on the curve as found in studies (4, 5, 7) involving a constant ligand concentration and varying albumin concentrations. The slopes

[1] Amersham Corp.
[2] New England Nuclear Corp.
[3] ICN Life Sciences Group.

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Table I—Relationship between β and Human Serum Albumin Concentration in the Binding of ^{14}C-Codeine, ^{14}C-Morphine, and ^{3}H-Methadone

Albumin Concentration, M	Ligand					
	^{14}C-Codeine		^{14}C-Morphine		^{3}H-Methadone	
	β	$d(\beta)/d$(albumin)	β	$d(\beta)/d$(albumin)	β	$d(\beta)/d$(albumin)
0.217×10^{-4}	—	—	—	—	0.0158	636.6
0.870×10^{-4}	0.0438	273.2	0.0512	329.7	—	—
0.145×10^{-3}	0.05	222.4	0.07	263.3	0.09	446.3
0.29×10^{-3}	0.074	168.2	0.095	194.1	0.145	391.9
0.58×10^{-3}	0.132	127.3	0.154	143.1	0.252	344.2
0.870×10^{-3}	0.16	108.1	0.184	119.8	0.305	319.0

Table II—Literature Data for Drug Binding at Varying Human Serum Albumin Concentrations

Reference	Ligand	Plot of Human Serum Albumin Concentrations, M, *versus*			
		β		nK	
		r[a]	Slope	r	Slope
10	Prednisone	0.8	+621	0.176	$+0.999 \times 10^{6}$
11	Methadone	0.945	+490	0.912	-0.904×10^{6}
12	Phenylbutazone	0.947	+53.8	0.986	$+0.104 \times 10^{9}$
13	Diphenylhydantoin	0.496	+148	0.383	-0.937×10^{7}
13	Desipramine	0.212	−22.2	0.826	-0.337×10^{8}
14	Morphine	0.373	+116	0.482	-0.549×10^{6}
14	Diphenylhydantoin	0.496	+197	0.158	-0.277×10^{8}
15	Furosemide	0.873	+88.2	0.91	-0.101×10^{9}
16	Propoxyphene	0.43	−244	0.712	-0.22×10^{8}

[a] The r value is the correlation coefficient for the best fit of the data points to a straight line. The slope given is that calculated for the best fit straight line.

for the curves for all three ligands were on the same order of magnitude.

When the extent of binding *versus* albumin concentration was examined, β (fraction of ligand bound) increased with albumin concentration (Fig. 2). The slopes of the curves were relatively close for codeine and morphine and slightly higher for methadone. Since it generally is recognized that drug binding to serum proteins may affect pharmacological activity because the active drug form is the free or unbound drug, serum albumin concentration changes could be important. Serum albumin concentrations vary among normal individuals to a limited extent and may be reduced in certain pathological circumstances.

To understand the relative effects of differences in the serum albumin concentration and the fraction of ligand bound, $d(\beta)/d$(albumin) was calculated for each albumin concentration and its corresponding β value. This parameter is the rate of change in β with respect to a change in albumin concentration. The value was calculated by fitting albumin concentrations and corresponding β values to a curve for which a statistically significant fit could be obtained by a computer. The first derivative of the curve then was taken at each albumin concentration.

The $d(\beta)/d$(albumin) values dropped rapidly as the albumin concentration increased from the low values and then dropped less as the higher albumin concentrations were reached (Table I). These values are represented in Fig. 3. At the higher albumin concentrations, β changed less per albumin concentration change. Since the higher albumin concentrations are those generally encountered, even in individuals with albumin concentrations considered somewhat below normal, β would not be reduced drastically, and only moderate modifications (if any) of the dose level would be indicated with slightly decreased albumin concentrations. The large β change per change in albumin concentration would exist at the much lower albumin concentrations, which generally are not encountered clinically.

Numerous studies were identified (8) in the literature in which binding was affected by albumin concentration changes. A protein concentration increase corresponding to an nK decrease was common to these studies[4]. The data obtained in this study were analyzed similarly, and nK was calculated using an equation (2) that permitted calculation of nK from β and the protein concentration. Figure 4 shows the curves obtained when the calculated nK values were plotted *versus* albumin concentration. For all three ligands, an albumin concentration increase was accompanied by an nK decrease.

Numerous investigators (10–16) have published data for drug binding

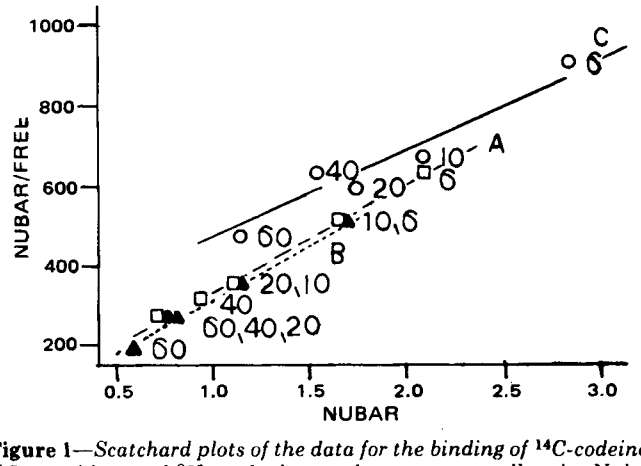

Figure 1—*Scatchard plots of the data for the binding of ^{14}C-codeine, ^{14}C-morphine, and ^{3}H-methadone to human serum albumin. Nubar refers to the moles of ligand bound per mole of albumin in the system. Numbers near the data points represent the milligrams of albumin per dialysis cell (1-ml cell). Key: A, □, ^{14}C-morphine; B, ▲, ^{14}C-codeine; and C, ○, ^{3}H-methadone.*

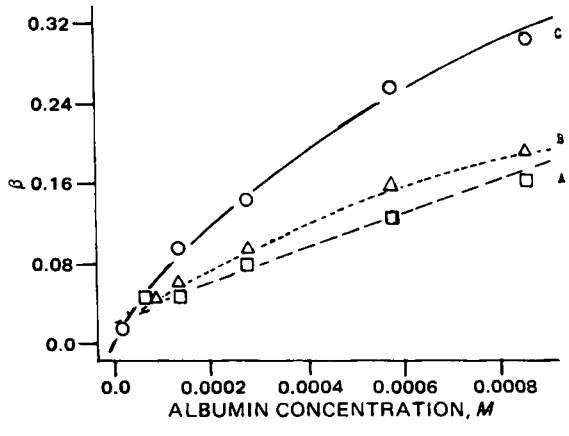

Figure 2—*Relationship between β and the human serum albumin concentration for the binding of ^{14}C-codeine, ^{14}C-morphine, and ^{3}H-methadone to human serum albumin. Key: A, □, ^{14}C-codeine; B, △, ^{14}C-morphine; and C, ○, ^{3}H-methadone.*

[4] The n refers to the number of binding sites or systems on the protein, and K refers to the association constant.

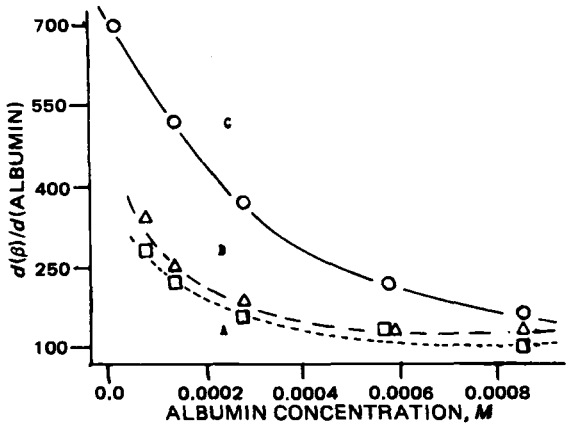

Figure 3—*Change in β per human serum albumin concentration change* [d(β)/d(*albumin*)] versus *albumin concentration for the binding of* ^{14}*C-codeine,* ^{14}*C-morphine, and* ^{3}*H-methadone to human serum albumin. Key: A,* □, ^{14}*C-codeine; B,* △, ^{14}*C-morphine; and C,* ○, ^{3}*H-methadone.*

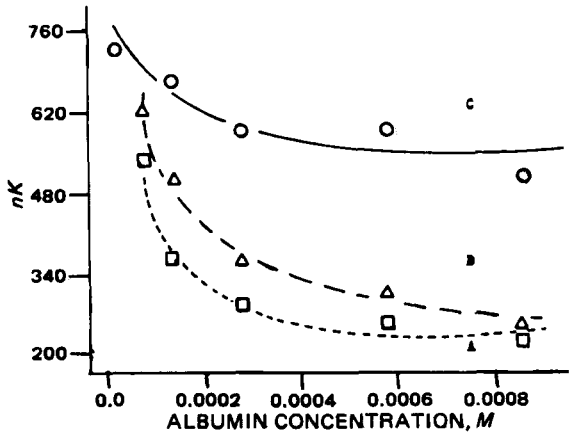

Figure 4—*Relationship between* nK *and human serum albumin concentration for the binding of* ^{14}*C-codeine,* ^{14}*C-morphine, and* ^{3}*H-methadone to human serum albumin. Key: A,* □, ^{14}*C-codeine; B,* △, ^{14}*C-morphine; and C,* ○, ^{3}*H-methadone.*

(β) at varying human serum albumin concentrations. The data in these reports were plotted as β *versus* albumin concentration (molar) and calculated *nK* values (calculated as described) *versus* albumin concentration.

The results are summarized in Table II. In many cases, the plots did not follow closely a straight line, as evidenced by statistically insignificant correlation coefficients (*i.e.*, <0.9); the slopes calculated thus represent a trend of the scattered points rather than a precise slope. In general, β increased with an albumin concentration increase except for desipramine and propoxyphene. The *nK* values decreased with an albumin concentration increase, as found by Bowmer and Lindup (8) in their study of literature for binding data. Table II reveals two other exceptions, prednisone and phenylbutazone. Thus, *nK* values apparently do decrease with an albumin concentration increase but with some exceptions. These exceptions merit further study for other possible unique albumin binding characteristics.

Bowmer and Lindup (8) suggested that positive-slope Scatchard curves might be explained by cooperativity if the positive-slope curves are obtained from data where the protein concentration was constant and the ligand concentration was varied, as well as from data for the same system where the ligand concentration was constant and the protein concentration was varied. This cooperativity seems to apply to the binding of codeine, morphine, and methadone to human serum albumin. Positive-slope Scatchard plots were obtained in a previous study (9) where the albumin concentration was constant and the ligand concentration was varied. In the present study in which the ligand concentration was constant and the albumin concentration was varied, Scatchard plots with positive slopes also were obtained.

More ligand–protein systems should be reinvestigated by keeping the ligand concentration constant and varying the protein concentration, not only for more complete characterization of these systems but also for the determination of the relative effects on the fraction bound with protein concentration changes, especially where blood proteins are involved. For drugs that are highly bound and very potent, the dosage may have to be adjusted for a reduction in serum protein concentration when the latter deviates significantly from normal.

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