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Distribution Coefficients and *In Vitro* Human Serum Protein Binding of Spironolactone and Its 7α-Carboxymethyl Analog

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Abstract □ The distribution coefficients of spironolactone (I) and its 7α-carboxymethyl analog (II) were determined at 22–25° in systems of *n*-octanol or chloroform and 0.1 *M* phosphate buffer at pH 7.4. The respective values for I in the two systems were 153.9 and 15.1, and those for II were 15.9 and 3.1. Protein binding studies of I and II were conducted with human serum albumin and human γ-globulin *via* equilibrium dialysis at 37°. The I fractions bound to 4% (w/v) albumin and to 1.16% (w/v) γ-globulin were 66 and 18%, respectively. The corresponding II fractions bound to the two proteins were 46 and 12%. The greater protein binding of I agrees with its superior lipophilicity to that of II. The binding of both I and II to albumin increased with increasing albumin concentration, whereas the binding of I and II to albumin did not change significantly as the concentrations of I or II were varied from 50 to 1300 ng/ml. Cooperativity and/or multiple classes of binding sites appear to be associated with the binding of I and II to albumin.

Keyphrases □ Spironolactone—distribution coefficients, *in vitro* human serum protein binding, 7α-carboxymethyl analog □ Protein binding—spironolactone, 7α-carboxymethyl analog, *in vitro* human serum □ Distribution coefficients—spironolactone, 7α-carboxymethyl analog, phosphate buffer with *n*-octanol or chloroform

The spirolactones are steroidal aldosterone antagonists clinically used to produce potassium-sparing diuresis. The spirolactones have been studied extensively because of their considerable biotransformation (1), potential carcinogenicity (2), and ability to induce hepatic detoxification of chemicals (3–5). The pharmacodynamic properties of spirolactones vary because of their differing structure–activity relationships and physicochemical properties (6). At least 19 spirolactones have been investigated (6, 7).

The purpose of this study was to determine the lipid–aqueous distribution coefficients and the extent of plasma protein binding of spironolactone and its 7α-carboxymethyl derivative.

EXPERIMENTAL

Materials—Spironolactone[1] (I) was tritiated[2] randomly and used without further purification. Tritiated 7α-carboxymethyl spirolactone[3] (II), human serum albumin[4], and human γ-globulin[5] were used as received. Other solvents and reagents were of analytical grade of purity.

Procedures—Ten solutions of I and II in the 50–500-ng/ml range were prepared in 0.1 *M* phosphate buffer at pH 7.4 and saturated previously with *n*-octanol or chloroform.

Three milliliters each of the sample solution and of the *n*-octanol or chloroform saturated previously with the phosphate buffer were placed in a glass tube with a polytef-lined screw cap, and the mixture was agitated on a horizontal shaker[6] for 30 min at room temperature (22–25°). The mixtures were allowed to stand for 2 hr, during which time the phases separated. An accurate volume from each phase then was mixed with 10 ml of Bray's solution (8), and the samples were analyzed for I or II by counting on a liquid scintillation spectrometer[7] for 5 min. Each experiment was conducted in triplicate. Distribution coefficient (*P*) values were calculated from (9):

$$P = (\text{counts in } n\text{-octanol phase}/\text{counts in aqueous phase}) \quad \text{(Eq. 1)}$$

$$\log P_{\text{chloroform}} = 1.12 \log P_{n\text{-octanol}} - 1.343 \quad \text{(Eq. 2)}$$

Eight solutions of I and II in the 50–1300-ng/ml range were prepared in 0.1 *M* phosphate buffer at pH 7.4. Human serum albumin solutions (1, 2, 3, 4, and 5% w/v) and a human γ-globulin solution (1.16% w/v) also were prepared in the phosphate buffer. Exactly 1.0 ml of the sample solution was introduced into one side of a dialysis cell[8] separated by a cellulose membrane[9], and 1.0 ml of protein solution was placed into the opposite half. After the cells were incubated for 24 hr at 37°, measured volumes of the sample and protein solutions were mixed with 10 ml of Bray's solution, and the mixtures were counted on a liquid scintillation spectrometer for 5 min. The percent of I or II bound to albumin or γ-globulin was determined from:

$$\text{percent bound} = \left[\frac{(\text{counts in protein}) - (\text{counts in sample solution})}{\text{counts in protein}}\right] \times 100 \quad \text{(Eq. 3)}$$

DISCUSSION

The distribution coefficients of I and II are reported in Table I. There is a considerable discrepancy between the values for I and II in this study and those determined previously (7). The I value determined in chloroform was 17% greater than the theoretical I value in chloroform calculated from Eq. 2 using the experimental value for the distribution coefficient of I in *n*-octanol. However, the reported value (7) for the distribution coefficient of I in *n*-octanol is 289% larger than the corresponding present study value. Similarly, the experimental value for the distribution coefficient of II in chloroform was 210% greater than the corresponding theoretical value, whereas the reported value (7) for the distribution coefficient of II in *n*-octanol is 449% greater than the value determined from this study.

The disagreement between the reported (7) and present study distribution coefficients of I and II in *n*-octanol is not readily discernible. The

[1] SC-9420, lot 308, mol. wt. 416.57, assay 100.44%, G. D. Searle & Co., Chicago, Ill.
[2] Catalytic exchange method, 95.5% assay by radiochromatogram, specific activity 4.27 mCi/mg, New England Nuclear, Boston, Mass.
[3] SC-25152, mol. wt. 400.53, tritiated in the 1- and 2-positions, 95.76% assay by radiochromatogram, specific activity 83.20 mCi/mg, G. D. Searle & Co., Chicago, Ill.
[4] Lot 34G-8120, mol. wt. 69,000, Sigma Chemical Co., St. Louis, Mo.
[5] Lot 113C-1010, Sigma Chemical Co., St. Louis, Mo.
[6] Precision Scientific Co., Ann Arbor, Mich.
[7] Model 3320, Packard Instrument Co., Downers Grove, Ill.
[8] Constructed of Plexiglas, Technilab Instrument Inc., Pequannock, N.J.
[9] Nominal pore size of 4.8 nm, impermeable to molecules with mol. wt. > 6000, Technilab Instrument Inc., Pequannock, N.J.

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Table I—Distribution Coefficients of I and II Determined in 0.1 *M* Aqueous Phosphate Buffer at pH 7.4 *versus* *n*-Octanol and Chloroform

Compound	Experimental Values, mean ± *SEM*, %		Reported Values for	Calculated Values[a] for
	n-Octanol	Chloroform	*n*-Octanol	Chloroform
I	153.9 ± 15.3	15.1 ± 1.7	598.4[b]	12.9[c]
II	15.9 ± 0.7	3.1 ± 0.3	87.3[d]	1.0[e]

[a] From Eq. 2. [b] Antilog of log P = 2.777; Ref. 7. [c] Antilog of log P = 1.11, calculated from Eq. 2. [d] Antilog of log P = 1.941; Ref. 7. [e] Antilog of log P = 0.001, calculated from Eq. 2.

Table II—Binding of I and II to Human Serum Albumin and Human γ-Globulin.

Compound	Protein Binding, mean ± *SEM*, %			Binding Difference, %[a]		
	Albumin, 4%	Globulin, 1.16%	Total	Albumin, 4%	Globulin, 1.16%	Total
I	66.5 ± 0.4	18.3 ± 0.5	84.7 ± 0.4	—	—	—
II	45.7 ± 0.5	11.8 ± 0.5	57.5 ± 0.5	−31.2	−35.4	−32.1

[a] [(percent II − percent I)/percent I] × 100.

Table III—Comparison of Protein Binding Values for I and II

Compound	Present Study[a]	Protein Binding, %	
		Reference 7[b]	Reference 10[c]
I	84.7	94.2	91.1, 98.2
II	57.5	73.5	—

[a] Based on the sum of binding by 4% human serum albumin and 1.16% human γ-globulin. [b] Ultrafiltration method. [c] Lower value was obtained *via* equilibrium dialysis, and higher value was obtained *via* ultrafiltration; both were determined in human plasma at 550 ng of I/ml.

experimental distribution coefficients for I and II reported here are in closer agreement with the values predicted by Eq. 2. All distribution coefficient data determined in *n*-octanol indicate I to be 7–10 times more lipophilic than II. This difference may be attributed to the presence of a sulfur atom in the acetylthio substituent at the 7-position on I. The dominating contribution of the sulfur atom to lipophilicity is emphasized often *via* reference to the exemplary ninefold greater distribution coefficient of thiopental *versus* that of pentobarbital (9).

The values for percent protein binding determined in this study and those reported earlier (7, 10) are listed in Tables II and III. No binding of I or II to the dialysis membrane was demonstrated. The significant disagreement of the present results with those reported earlier may be attributed to at least the following factors:

1. The experimental conditions of Ref. 7 included using 20% (v/v) 1,3-butylene glycol and 4% (v/v) methanol to maintain drug solubility in the phosphate buffer as well as sample ultrafiltration *via* centrifugation at 4°. This low temperature could have influenced binding, resulting in values different from those found at 37°. The concentrations of human serum albumin utilized (7) are typical of clinical hypoalbuminemia (11). The previous investigators (7) did not study the binding of I or II to human γ-globulin.

2. The authors of Ref. 10 used fresh human plasma for I binding studies; however, serum protein concentrations and temperature conditions were not reported.

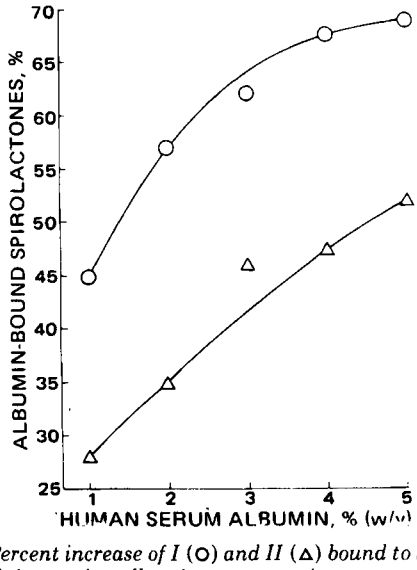

Figure 1—*Percent increase of I (○) and II (△) bound to human serum albumin with increasing albumin concentration.*

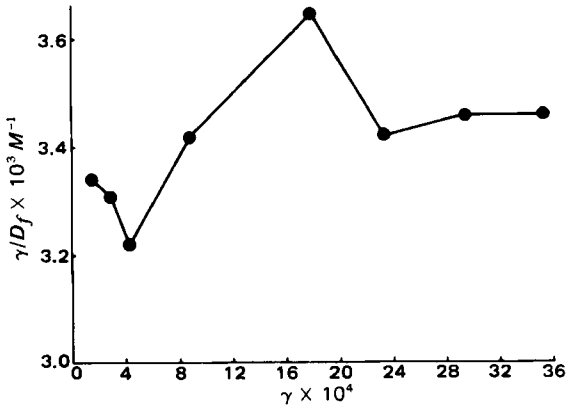

Figure 2—*Scatchard plot of* γ/D_f *versus* γ *for I over the 53.3–1278.0-ng/ml range (1.3–30.1 × 10⁻⁷ M) in 4.0% (w/v) (5.8 × 10⁻⁴ M) human serum albumin at pH 7.4 and 37°.*

Although several steroidal compounds, *e.g.*, corticosteroids, are bound extensively to human γ-globulins, albumin accounts for the greatest proportion of binding of these drugs to serum or plasma proteins (11–13). Furthermore, some steroids also are highly bound to serum lipoproteins (14). The present study results indicate 18% binding of I to human γ-globulin. Therefore, the comparative data in Table III suggest that the percent protein binding of I reported in Ref. 7 may be an overestimate resulting from the use of a mixed aqueous solvent system and albumin-only protein solutions. The low temperature centrifugation conditions used (7) also would increase the fraction of drug bound to albumin (11).

The present 85% value for the total binding of I in 4% (w/v) human serum albumin and 1.16% human γ-globulin (Table II) agrees with the 91% value determined in human plasma reported elsewhere (10). There was no significant variation in the percent binding of I or II to the serum proteins over the drug concentration range investigated. However, Fig. 1 shows that the binding of I and II increased proportionally with increasing albumin concentration.

A causal relationship between hydrophobicity (lipophilicity) and drug–protein binding was demonstrated for steroids (15) and other drugs (16, 17). The fact that the distribution coefficient of I is 7–10 times greater than that of II (Table I) correlates with the 32% superiority in protein binding of I over II (Table II). Scatchard plots (7, 18–20) of I and II in 4% (w/v) human serum albumin (Figs. 2 and 3) were prepared from:

$$\gamma/D_f = K(n - \gamma) \qquad \text{(Eq. 4)}$$

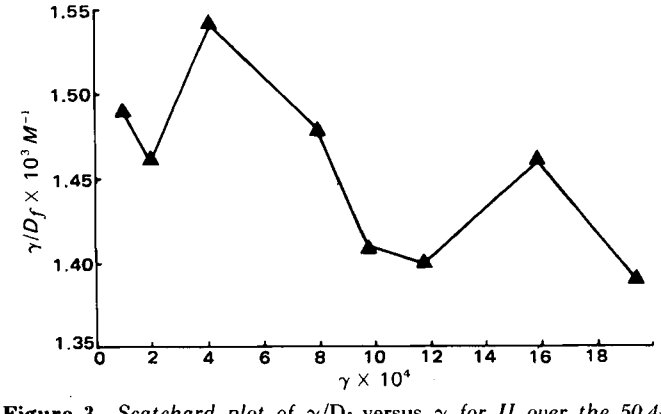

Figure 3—*Scatchard plot of* γ/D_f *versus* γ *for II over the 50.4–1006.0-ng/ml range (1.3–25.1 × 10⁻⁷ M) in 4% (w/v) (5.8 × 10⁻⁴ M) human serum albumin at pH 7.4 and 37°.*

where γ is the ratio of the concentration of albumin-bound spirolactone to that of albumin, D_f is the concentration of unbound spirolactone at dialysis equilibrium, K is the binding constant at equilibrium, and n is the number of a single type of binding sites. The previous investigators (7) apparently obtained linear plots of γ/D_f *versus* γ for several spirolactones. The concentrations of spirolactone used (7) appear to have been ~30–800 times higher than the peak serum concentrations of I and its metabolites detected in human males given a 200-mg oral dose (10).

The observed absence of linearity, together with the complexity of the Scatchard plots for I and II, strongly suggests diverse protein–ligand binding characteristics. Such deviations were reported to be due to cooperative ligand interaction, multiple-contact binding sites, or nonequivalent binding sites (20). Inspection of the Scatchard plots reveals several points of inflection, as well as both concave and convex curvatures at varying concentrations of bound I and II. Such patterns generally are the results of different types of binding sites, each exhibiting cooperative character. Furthermore, individual binding sites for I and II may overlap and also may contribute to the diverse binding pattern observed. Although no absolute conclusion can be drawn regarding which phenomenon dominates the binding pattern of I and II for human serum albumin, a single model of independent, equivalent binding is not applicable for the spirolactones studied.

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Lidocaine Pharmacokinetics in Pregnant and Nonpregnant Sheep

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Abstract □ Lidocaine disposition kinetics were studied in the pregnant ewe following 0.5-, 1.0-, and 2.0-mg/kg iv bolus doses and in the nonpregnant ewe following a 1.0-mg/kg iv bolus dose. Arterial blood was assayed for lidocaine by GLC. The blood lidocaine concentration–time curves were computer fitted to a two-compartment open model. In the pregnant ewe, the total body clearance of lidocaine (38 ml/min/kg) remained constant with increasing dose and was correlated linearly with preinjection cardiac output. The apparent volume of distribution of the central compartment apparently increased with increasing dose. The half-life of the postdistributive phase and the volumes of distribution at steady state and during the postdistributive phase increased as the dose was increased from 0.5 to 1.0 mg/kg. These observations suggest dose-related distribution of lidocaine in the pregnant ewe. The total body clearance of lidocaine in the pregnant ewe was not different from that in the nonpregnant ewe after 1.0-mg/kg doses; however, the volumes of distribution of the central compartment at steady state and during the postdistributive phase and the half-life of the postdistributive phase were greater in the pregnant ewe. The greater total body clearance for lidocaine in sheep as compared to humans is consistent with the greater hepatic blood flow in sheep; calculated hepatic extraction ratios for sheep are similar to hepatic extraction ratios for humans.

Keyphrases □ Lidocaine—pharmacokinetics, pregnant and nonpregnant sheep □ Pharmacokinetics—lidocaine, pregnant and nonpregnant sheep □ Anesthetics—lidocaine, pharmacokinetics, pregnant and nonpregnant sheep

Few literature reports describe drug disposition kinetics in the pregnant individual, but drug pharmacokinetics during pregnant and nonpregnant states may differ markedly. Alterations in drug disposition kinetics could be related to maternal changes, including blood and/or tissue binding of the drug, changes in the rates or distribution of blood flow, and drug metabolism changes. More importantly, drug kinetics during pregnancy may be altered by the addition of the fetal–placental unit with its inherent abilities to distribute, bind, metabolize, and clear drugs.

Lidocaine disposition kinetics at three doses in the pregnant ewe are presented in this report, and a comparison is made with lidocaine disposition kinetics in the nonpregnant ewe.

EXPERIMENTAL

Pregnant and nonpregnant pure or crossbred Suffolk ewes, 64.6 ± 11.3 (mean ± *SD* on the day of surgery) and 66.7 ± 11.9 kg, respectively, were obtained locally. Pregnant ewes were studied from Day 137 to Day 143 of gestation (full term 147–150 days). The ewes were catheterized during sterile surgery under general endotracheal anesthesia, using halothane and oxygen with controlled mechanical ventilation.

Polyethylene catheters were placed into the femoral artery and femoral vein and advanced to the abdominal aorta and inferior vena cava, respectively. The femoral artery catheter was used for continuous blood pressure and heart rate monitoring, and the femoral vein catheter was used for lidocaine administration. A central venous catheter was inserted *via* percutaneous puncture into the external jugular vein and positioned in the right atrium. This catheter was used for the injection of indocya-

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