13 (1959).
(9) F. L. Warren, F. Goulden, and A. M. Robinson, *Biochem. J.*, **42**, 151 (1948).
(10) "The National Formulary," 14th ed., Mack Publishing Co., Easton, Pa., 1975, p. 205.
(11) "The United States Pharmacopeia," 19th rev., Mack Publishing Co., Easton, Pa., 1975, p. 142.
(12) "British Pharmacopoeia 1973," University Printing House, Cambridge, England, 1973, p. 443.
(13) J. E. Sinsheimer and E. F. Salim, *J. Anal. Chem.*, **37**, 566 (1965).
(14) E. F. Salim, P. E. Manni, and J. E. Sinsheimer, *J. Pharm. Sci.*, **53**, 391 (1964).
(15) N. M. Omar and N. A. El-Rabbat, *ibid.*, **67**, 779 (1978).
(16) R. Kuhn and D. Jerchel, *Ber.*, **74**, 949 (1941).
(17) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).
(18) D. Banes, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 669 (1953).
(19) A. M. Mattson and C. O. Jensen, *Anal. Chem.*, **22**, 182 (1950).
(20) H. M. Hashmi, A. S. Adel, A. V. Viegas, and I. A. Ahmad, *Mikrochim. Acta*, **3**, 457 (1970).
(21) P. S. Vassileva-Alexandrova and P. S. Shismanor, *Anal. Chem.*, **47**, 1432 (1975).
(22) R. E. Graham, E. R. Biehl, and C. T. Kenner, *J. Pharm. Sci.*, **66**, 965 (1977).

Tissue Distribution and Metabolism of Drugs V: Effect of Secretin and Pancreozymin on Drug Transport in Rabbit Pancreas

MASATO ARAKAWA *§, KATSUHIKO OKUMURA *, and RYOHEI HORI‡x

Received April 10, 1979, from the **Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan,* and the ‡*Department of Pharmacy, Kyoto University Hospital, Sakyo-ku, Kyoto 606, Japan.* Accepted for publication July 16, 1979. §Present address: General Research Center, Wakunaga Pharmaceutical Co. Ltd., Kotachi, Hiroshima, Japan.

Abstract □ The effect of secretin and pancreozymin on the tissue distribution and penetration of drugs in the rabbit pancreas was studied to clarify hormonally regulated drug distribution. Drugs with high liquid solubility were distributed easily within the pancreas even during secretin or pancreozymin treatment, and these hormones had little effect on drug distribution from the blood to the pancreas. However, secretin increased the concentration ratio of dimethadione in the pancreatic juice (J) to plasma unbound dimethadione (Pf), probably because the pancreatic juice during secretin infusion is alkaline relative to the control. Secretin had no effect on the J/Pf of isonicotinamide and sulfanilamide. Secretin decreased the J/Pf of drugs with low lipophilicity or large molecular weight because the penetration rates of these drugs from cell water to pancreatic juice were not rapid enough to reach equilibrium. Pancreozymin was unable to change the J/Pf of any drug tested. These results suggest that the barrier between the blood and the pancreas or the barrier between the pancreas and the pancreatic juice is unchanged by secretin or pancreozymin.

Keyphrases □ Pancreas—drug transport, various drugs, effect of secretin, pancreozymin, pancreatic juice flow □ Pancreatic juice—effect of flow rate on pancreatic drug transport, various drugs □ Drug transport—pancreas, effect of secretin, pancreozymin, pancreatic juice flow, various drugs

Drug distribution has received increasing attention during recent years because such knowledge concerning the blood, organs, and tissues is needed to provide optimal treatment or protection from adverse reactions. Previous papers reported tissue distribution and penetration of drugs in the pancreas (1), lungs, (2, 3), and testes (4).

It was suggested that the lipid barrier in the pancreas plays a dominant role in drug distribution from the blood to the pancreas and that the lipid barrier and the molecular sieve barrier have independent roles in transport from the pancreas to the pancreatic juice. In these experiments, pancreatic juice secretion was stimulated by secretin infusion to maintain a constant juice flow. However, the pancreatic barriers could be changed by endogenous hormones that regulate pancreatic secretion during food digestion.

The purpose of this study was to determine experimentally whether secretin and pancreozymin, typical peptide hormones that stimulate pancreatic juice flow and bicarbonate or enzyme secretion, affect drug distribution and penetration in the pancreas. Some model drugs that exhibit remarkable lipophilicity, molecular size, and pKa were selected as discussed previously (1).

EXPERIMENTAL

Materials—Secretin[1], 3110 CHR U[2]/mg, was used. Pancreozymin[3], dimethadione, isonicotinamide, isonicotinic acid, sulfanilamide, sulfanilic acid, sulfisoxazole, and procainamide hydrochloride were obtained commercially. All other chemicals were analytical grade.

Animals—Male white rabbits, 2.0–3.0 kg, were housed in constant environment rooms and allowed free access to water and food.

Drug Permeation from Blood to Pancreatic Juice—The experimental procedures were almost identical to those described previously (1). Rabbits were anesthetized with pentobarbital sodium (27 mg/kg iv). Pancreatic juice was collected by cannulation into the pancreatic duct as described in the literature (5). Plasma drug concentrations were established and maintained by a suitable combination of priming injections and continuous intravenous infusion.

As the control, pancreatic juice was collected over 75 min prior to the administration of secretin or pancreozymin. The secretin effect was studied by a priming injection (1 CHR U/kg) and continuous intravenous infusion (2 CHR U/kg/hr), after which the pancreatic juice flow was stimulated from 14 (7–24 μl/min) to 39 (15–66 μl/min) μl/min. Pancreozymin also was studied using a priming injection (1 CHR U/kg) and continuous infusion (4 CHR U/kg/hr)[4]. In this experiment, secretin also was infused simultaneously to minimize the effect of secretin contamination in the pancreozymin preparation.

Drug Distribution—The pancreas was removed and homogenized at the end of each permeation experiment, and drug concentrations in the pancreas and blood were measured to determine the distribution ratio.

Analytical Methods—Drug concentrations in the plasma, plasma ultrafiltrate, pancreatic juice, and pancreas homogenate were determined

[1] Supplied by Eisai Co. Ltd., Tokyo, Japan.
[2] Crick, Harper and Raper Unit.
[3] Boots Pure Drug Co. Ltd., Nottingham, England.
[4] Since pancreozymin is less stable than secretin, a higher dose was used.

0022-3549/80/0100-0027$01.00/0

Table I—Effect of Secretin and Pancreozymin on Pancreatic Drug Distribution

Drug	$(Panc/Pf)_{control}$[a]	$(Panc/Pf)_{secretin}$	$(Panc/Pf)_{secretin\ pancreozymin}$
Procainamide	1.20 ± 0.32 (4)	1.18 ± 0.32 (4)	—
Sulfanilamide	0.99 ± 0.16 (3)	0.95 ± 0.12 (5)	0.89 ± 0.08 (3)
Dimethadione	0.50 ± 0.01 (3)	0.55 ± 0.05 (5)	0.53 ± 0.02 (5)
Sulfisoxazole	0.50 ± 0.03 (4)	0.58 ± 0.04 (7)	0.51 ± 0.02 (4)
Sulfanilic acid	0.21 ± 0.01 (3)	0.25 ± 0.02 (4)	—

[a] Panc/Pf indicates the concentration ratio of drug in the pancreas to unbound drug in the plasma. These data were obtained 2 hr after the constant drug infusion and represent the mean ± *SEM*, with the number of animals in parentheses.

according to the procedure described previously (1). The protein content of the pancreatic juice was measured by the Lowry method (6), and the calcium level in the biological fluids was analyzed by the *o*-cresolphthalein-edetate sodium method with a minor modification (7).

RESULTS AND DISCUSSION

Effect of Secretin and Pancreozymin on Drug Distribution in Pancreas—To ascertain the effect of secretin or pancreozymin on drug distribution in the pancreas, drug uptake was studied after constant infusion for 2 hr. Table I illustrates the concentration ratios of drugs in the pancreas to the plasma of unbound drugs, expressed as the distribution ratio (Panc/Pf) under various conditions. Acidic drugs did not distribute into the pancreas as easily as did the cationic or neutral drugs studied previously (1). However, there was no significant difference in distribution ratio between controls and secretin-infused animals or between secretin-infused animals and secretin–pancreozymin-coinfused animals.

Intracellular pH decreases following secretin infusion and ^{14}C-dimethadione (an acidic compound) in male rats and the decrease in the dimethadione distribution have been explained by the pH-partition theory (8). In the present study, no significant difference was detected in dimethadione distribution when the pancreas was stimulated by secretin. The pH change of the pancreatic juice during secretin infusion has been reported as 0.09, from pH 6.86 to 6.77 (8). When the values of 0.53 ml/g for the intracellular water space and 0.22 ml/g for the extracellular water space were adopted[5], the ratio of the secretin-stimulated pancreatic drug level to the control drug level was ≥0.93. This value is so low that it was difficult to detect by the methods used.

Secretin increased the pancreatic juice flow by 2.8 times, and pancreozymin increased the protein and calcium secretion by 6 times. Furthermore, significant decreases were detected in the physiological calcium

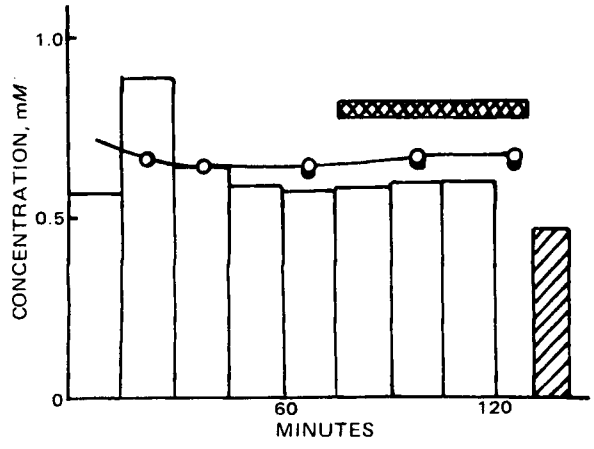

Figure 1—*Isonicotinamide concentration profile. Key: ○, total plasma drug concentration; ●, plasma free drug concentration; and ⊠, secretin infusion period. Open and hatched columns denote drug concentration in the pancreatic juice and in the pancreas, respectively.*

[5] Unpublished data.

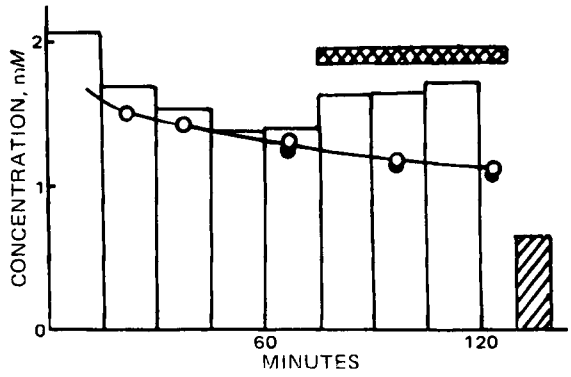

Figure 2—*Dimethadione concentration profile. Key: ○, total plasma drug concentration; ●, plasma free drug concentration; and ⊠, secretin infusion period. Open and hatched columns denote drug concentration in the pancreatic juice and in the pancreas, respectively.*

content of the rat pancreas during pancreozymin infusion (Panc/Pf of calcium content decreased from 1.41 ± 0.05 to 1.14 ± 0.08). This result probably was due to accelerated calcium secretion into the pancreatic juice during pancreozymin administration; the decreased pancreatic calcium could not be compensated fully by calcium from the blood. Thus, calcium apparently does not play a role in the distribution of these drugs in the pancreas. These results suggest that there is no correlation between drug distribution in the pancreas and pancreatic juice secretion or between the enzyme and calcium secretion.

Effect of Secretin and Pancreozymin on Drug Permeation from Blood to Pancreatic Juice—The effect of secretin on drug transport from the blood to the pancreatic juice was examined to clarify the transport barrier located in the luminal side of the pancreas. Drug levels in plasma and in the pancreatic juice following constant intravenous infusion of isonicotinamide, dimethadione, and isonicotinic acid with or without secretin are presented in Figs. 1–3 as a function of time. The ratios of pancreatic juice drug concentration to unbound plasma drug concentration (J/Pf) during the control period and during secretin infusion are listed in Table II, in which the percent change of J/Pf following secretin infusion also is indicated.

The values were calculated from the data obtained during the control period (45–75 min) and during secretin infusion (90–120 min). With isonicotinamide and sulfanilamide, whose molecular sizes are comparatively small, the J/Pf concentration ratios were almost unity and hardly were affected by secretin infusion (Fig. 1 and Table II). Dimethadione, whose molecular size and lipophilicity resemble those of isonicotinamide, exhibited significant concentration ratio increases (J/Pf) with secretin treatment (Fig. 2 and Table II).

Isonicotinic acid, which possesses structural units similar to isonicotinamide and has lower lipophilicity, showed smaller J/Pf ratios during secretin infusion than those of the controls (Fig. 3 and Table II). Almost the same results were obtained with sulfanilic acid, which has lower lipophilicity than sulfanilamide. Sulfisoxazole and procainamide, compounds with large molecular size compared with sulfanilamide and dimethadione, showed smaller J/Pf ratios during secretin infusion.

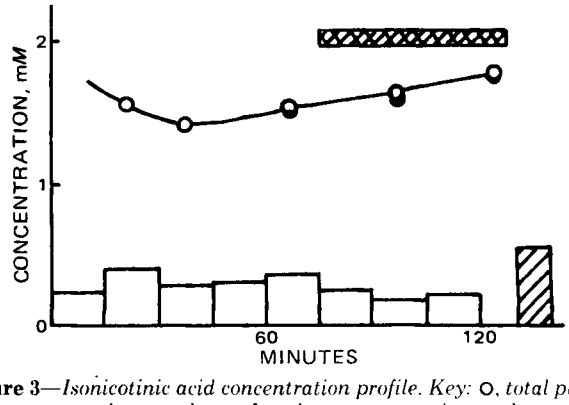

Figure 3—*Isonicotinic acid concentration profile. Key: ○, total plasma drug concentration; ●, plasma free drug concentration; and ⊠, secretin infusion period. Open and hatched columns denote drug concentration in the pancreatic juice and in the pancreas, respectively.*

Table II—Effect of Secretin on Drug Permeation into Pancreatic Juice

Drug	$(J/Pf)_{secretin}$[a]	$(J/Pf)_{control}$	Percent Change[b]	Statistics[c]
Dimethadione	1.83	1.41	+31 ± 6 (3)	$p < 0.05$
Isonicotinamide	0.91	0.92	−2 ± 2 (3)	NS
Sulfanilamide	1.08	1.23	−12 ± 2 (4)	$p < 0.05$
Isonicotinic acid	0.17	0.29	−40 ± 8 (3)	$p < 0.05$
Sulfanilic acid	0.03	0.08	−58 ± 6 (3)	$p < 0.01$
Sulfisoxazole	0.14	0.35	−57 ± 7 (3)	$p < 0.01$
Procainamide	0.09	0.19	−51 ± 6 (3)	$p < 0.01$

[a] J/Pf indicates the concentration ratio of drug in the pancreatic juice to unbound drug in the plasma. [b] Percent change was calculated from the individual data obtained during the control period and during the secretin infusion period. Data are presented with the standard error of the mean (*SEM*), and with the number of animals in parentheses. [c] The statistical test was the *t*-test of percent change.

These results suggest that drugs whose J/Pf ratios are almost unity easily penetrate the blood–pancreas barrier even during secretin treatment, unless there is a change in charge. Eventually, the pancreatic transport of drugs with high permeability would be independent of secretin treatment and of the pancreatic juice flow rate. However, with dimethadione, a weak acid with pKa 6.1, the high J/Pf ratios during secretin infusion could not be explained by its high permeability alone. One possible reason for this finding was the difference between the pancreatic juice pH during secretin treatment and that in the controls. According to the generally accepted view, increased pancreatic juice flow induced by secretin is linked with bicarbonate concentration increments (9).

If complete equilibrium were attained in the concentration ratio between the blood and the pancreatic juice, the transport ratio of acidic drugs would increase with the increase in juice alkalization by secretin infusion. Therefore, the increased transport ratio (J/Pf) of dimethadione caused by secretin infusion could be due to pancreatic juice alkalization and to the rapid partition. With acidic drugs such as isonicotinic acid, sulfanilic acid, and sulfisoxazole, the transport ratios decreased following secretin infusion. Since the transport ratio of acidic drugs theoretically would be greater than unity, as mentioned previously, the difference between the transport ratios of dimethadione and other acidic drugs might arise from differences in lipophilicity and molecular size. Procainamide was the model for basic drugs; the small J/Pf ratio and its decrease by secretin may be due to the alkaline state of pancreatic juice or to the bulky nature of the molecule.

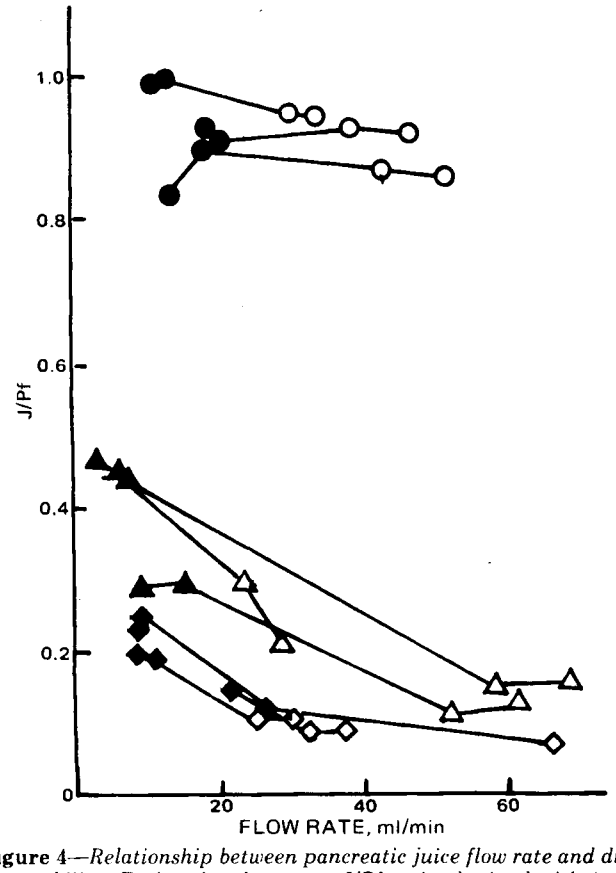

Figure 4—*Relationship between pancreatic juice flow rate and drug permeability. Each point denotes a J/Pf ratio obtained with (open symbol) or without (closed symbol) secretin. Key: ●—○, isonicotinamide; ▲—△, sulfisoxazole; and ♦—◇, procainamide.*

Table III—Effect[a] of Pancreozymin on Drug Permeation into Pancreatic Juice

Drug	$(J/Pf)_{secretin\ pancreozymin}$[b]	$(J/Pf)_{secretin}$	Percent Change[c]
Dimethadione	1.72	1.49	15 ± 4 (3)
Sulfanilamide	1.04	1.04	0 ± 4 (3)
Sulfisoxazole	0.09	0.10	−10 ± 9 (3)

[a] Data were not significant. The statistical test on the *t*-test of percent change. [b] See footnote *a* of Table II. [c] Percent change was calculated from the individual data obtained during secretin infusion period and during the secretin–pancreozymin coinfusion period. Data are presented with the standard error of the mean (*SEM*) and with the number of animals in parentheses.

To demonstrate the role of juice flow on drug movement from the blood to the pancreatic juice, the relationship between the transport ratio (J/Pf) and the pancreatic juice flow rate was examined (Fig. 4). The transport ratios (J/Pf) of procainamide and sulfisoxazole decreased as the juice flow increased, while the isonicotinamide J/Pf was unchanged. It was inferred that the transport ratios of drugs that cannot penetrate easily from the blood to the pancreatic juice due to their low lipophilicity and bulky molecular size decrease as pancreatic juice flow increases, probably because the penetration rates of these drugs are too slow to reach equilibrium before secretion.

A model of salicylic acid transfer from salivary gland cells into saliva by slower diffusion *via* lipid solvation was proposed (10, 11). Salicylic acid diffusion from cell water to saliva was not rapid enough to reach equilibrium, thus decreasing the salivary flow rate, which increased the saliva-to-plasma unbound salicylic acid concentration ratio by about one-third; dimethadione movement was rapid enough to reach equilibrium prior to excretion. In a study of the penetration of six water-soluble chemicals in bile, the permeation rate of small molecules was unchanged during bile flow stimulation while the penetration rate of large molecules decreased as bile flow increased (12). Based on these findings, it is speculated that the molecular sieve barrier and the lipid barrier exist independently even in secretin treatment. The effects of pancreozymin infusion on the permeation of dimethadione, sulfanilamide, and sulfisoxazole from the blood to the pancreatic juice are shown in Table III by the transport ratio (J/Pf) during pancreozymin treatment and during the control experiment.

The percent change of these transport ratios also is presented in Table III. During pancreozymin infusion, the protein and calcium contents of pancreatic juice increased 3.1- and 1.3-fold, respectively, while juice flow increased slightly. No significant difference was observed in the transport rate of these three drugs by pancreozymin infusion. Since no drug binding to enzyme protein in the pancreatic juice was detected by ultracentrifugation, these drugs must exist in the pancreatic juice as unbound molecules. Thus, the data given in Table III indicate that drug transport from the blood to the pancreatic juice is not changed by pancreozymin infusion. The data obtained using a limited number of model drugs support the suggested transport model of drug transfer from the blood to the pancreatic juice through a two-step barrier system: (*a*) a lipoidal barrier from the blood to the pancreas, and (*b*) a molecular sieve barrier and a lipoidal barrier from the cell to the pancreatic juice that coexist independently near the pancreatic duct surface. These barriers probably are rigid even during secretin or pancreozymin treatment.

REFERENCES

(1) R. Hori, M. Arakawa, and K. Okumura, *Chem. Pharm. Bull.*, **26**, 1135 (1978).

(2) K. Okumura, H. Yoshida, and R. Hori, *J. Pharm. Dyn.*, **1**, 230 (1978).

(3) R. Hori, H. Yoshida, and K. Okumura, *Chem. Pharm. Bull.*, **27**, 1321 (1979).

(4) K. Okumura, I. P. Lee, and R. L. Dixon, *J. Pharmacol. Exp. Ther.*, **194**, 89 (1975).

(5) T. Matsunaga, A. Yoshitani, T. Chin, H. Yazawa, M. Toh, M. Saiki, H. Kamogawa, and K. Egami, *Nihon. Univ. Dent. J.*, **45**, 575 (1971).

(6) E. Mizuno, T. Nishina, and M. Kitamura, *Rinsho Byori*, **19**, 427 (1971).
(7) H. V. Connerty and A. R. Broggs, *Am. J. Clin. Pathol.*, **45**, 290 (1966).
(8) C. D. Russell, R. J. Beschi, and R. H. Lindsay, *Int. J. Nucl. Med. Biol.*, **3**, 77 (1976).
(9) G. M. Makhlouf and A. L. Blum, *Gastroenterology*, **59**, 896 (1970).
(10) J. W. Putney, Jr., and J. F. Borzelleca, *J. Pharmacol. Exp. Ther.*, **182**, 515 (1972).
(11) J. F. Borzelleca and J. W. Putney, Jr., *ibid.*, **174**, 527 (1970).
(12) E. L. Forker, *Am. J. Physiol.*, **215**, 56 (1968).

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

ANTHONY S. NG *, RONALD B. KLUZA, and DAVID W. NEWTON ˣ

Received February 22, 1979, from the *Department of Pharmaceutics, Massachusetts College of Pharmacy, Boston, MA 02115.* Accepted for publication July 19, 1979. *Present address: Pharmacy Department, Children's Hospital of San Francisco, San Francisco, CA 94199.

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.

0022-3549/80/0100-0030$01.00/0