effect of salicylic acid and phenylbutazone is due to the change in either the permeability characteristics of intestinal membrane or the rate of mesenteric blood flow, the exsorption rate of sulfanilamide to the rabbit small intestine also will be affected more or less by salicylic acid and phenylbutazone. Nevertheless, as shown in Fig. 4, the exsorption rate of sulfanilamide was not affected at all. The results of these experiments indicate that only the unbound fraction of sulfonamides in plasma is available for the exsorption of the drugs to the rabbit small intestine. Therefore, it is concluded that the absorption of sulfadimethoxine from the rabbit small intestine is reduced as a result of the marked increase of unbound drug in plasma due to the displacement of the drug from its plasma-protein binding sites by salicylic acid and phenylbutazone.

Many investigators[16–18] have pointed out the binding of drugs to plasma proteins is one of important physicochemical factors affecting drug absorption. However, there is no evidence on the basis of the experimental results. In the present study, we confirmed experimentally the importance of plasma-protein binding for the intestinal absorption of sulfonamides in rabbits.

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Chem. Pharm. Bull. 25(12)3405—3408(1977)

UDC 615.31.011.5.076.9 : 547.962.3.04

Protein Binding of Sulfonylureas. I. Interaction of Some Substituted Benzenesulfonyl Propylureas to Bovine Serum Albumin

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(Received April 30, 1977)

Binding affinities of five 4-substituted benzenesulfonyl propylureas to bovine serum albumin (BSA) were investigated by equilibrium dialysis method. Those of chlorpropamide (CPU) to BSA was also investigated by dynamic dialysis method using ^{35}S labelled CPU. Results of both methods agreed very well.

Binding paramethers of these compounds to BSA were obtained assuming that there were two classes of binding sites. CPU and 4-iodobenzenesulfonyl propylurea were bound to BSA stronger than benzenesulfonyl, *p*-toluenesulfonyl and 4-aminobenzenesulfonyl propylureas with regard to the primary binding site.

Keywords——protein binding; chlorpropamide; benzenesulfonyl propylurea; *p*-toluenesulfonyl propylurea; 4-aminobenzenesulfonyl propylurea; 4-iodobenzenesulfonyl propylurea; equilibrium dialysis; dynamic dialysis

Chlorpropamide (4-chlorobenzenesulfonyl propylurea, CPU), one of the hypoglycemic agents, differs significantly from tolbutamide (*p*-toluenesulfonyl butylurea, TBU) in that CPU is not readily metabolized to a physiologically inactive compound as TBU is to carboxytolbutamide.[2] This would partly be due to the difference of the binding affinity of these sulfonylureas to serum or cell proteins. Johnson, *et al.* determined the rate of disappearanec

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of CPU from blood[3] and per centage of its protein binding.[4] Wishinsky, *et al.* compared the fractions of CPU which were bound to bovine serum albumin (BSA) and human serum albumin (HSA) and those of TBU.[5] Judis investigated the binding affinities of CPU and TBU to HSA by equilibrium dialysis method.[6] Crooks and Brown also investigated the binding of CPU and TBU to HSA by dynamic dialysis method.[7] Hsu, *et al.* calculated the binding parameters of some sulfonylureas from Klotz ,*et al.*[8] equation using 1-anilinonaphthalene-8-sulfonic acid as fluorescence probe.[9]

In order to evaluate the further accurate binding parameters of substituted benzenesulfonyl alkylureas, some 4-substituted benzenesulfonyl propylureas were used in this study, *i.e.*, benzenesulfonyl propylurea (BPU), *p*-toluenesulfonyl propylurea (TPU), 4-aminobenzenesulfonyl propylurea (APU), 4-iodobenzenesulfonyl propylurea (IPU) and CPU. *n*-Propyl group was chosen as alkyl group attaching to urea. These compounds have the same fundamental chemical structure of benzenesulfonyl propylurea, so the affinities of protein binding should be reflected by the effect of substituents introduced to the benzene ring.

The binding of 4-substituted benzenesulfonyl propylureas to 4.29×10^{-5} M BSA were determined in 1/15 M phosphate buffer at pH 7.4 by equilibrium dialysis method.[10] The dialysis was carried out at 10° for 3 days with Visking cellulose tubing. The extent of binding was calculated on the basis of following equation.

$$[\text{drug bound}] = [\text{drug total}] - [\text{drug free}]$$

The data in the form of the moles of bound drug per mole of BSA (r), and equilibrium concentration of free drug (c) were subjected to nonlinear least squares fit by means of a Facom 230—28 computer according to the work of Keresztes-Nagy, *et al.*[11] The typical Scatchard plots indicated that there are at least two classes of binding sites as shown in Fig. 1 assuming that there are no interaction between sites, and between free and bound drugs. Therefore, r is defined as

$$r = \frac{n_1 k_1 c}{1 + k_1 c} + \frac{n_2 k_2 c}{1 + k_2 c}$$

where n_1, n_2 and k_1, k_2 represent the numbers of binding sites and the association constants of 4-substituted benzenesulfonyl propylurea-BSA complex of each class, respectively. The binding parameters of the 4-substituted benzenesulfonyl propylureas were summarized in Table I.

To ensure these data, the study of interaction of CPU and BSA (8.66×10^{-5} M) was carried out by dynamic dialysis method[12] using ^{35}S labelled CPU at room temperature. The data of dynamic dialysis method were in good agreement with those of equilibrium dialysis method as shown in Fig. 2.

The primary binding parameters of TPU to BSA were in good agreement with reported data on TBU to HSA where $k = 4.06 \times 10^4$ M^{-1}, $n = 1.36$,[6] and $k = 9.04 \times 10^4$ M^{-1},[9] but differed significantly from those by dynamic dialysis method of $k_1 = 8.504 \times 10^5$ M^{-1} and $n_1 = 2.27$.[7]

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Table I. Binding Parameters of Sulfonylureas to BSA at pH 7.4[a)]

Sulfonylureas	n_1	$k_1 \times 10^{-4}$ (M^{-1})	n_2	$k_2 \times 10^{-2}$ (M^{-1})	S.S.[b)]
Benzenesulfonyl propylurea (BPU)	0.91	7.86	8.04	2.45	3.256
p-Toluenesulfonyl propylurea (TPU)	1.40	5.01	5.53	7.72	0.1327
4-Aminobenzenesulfonyl propylurea (APU)	1.03	7.45	5.77	6.37	0.3007
4-Chlorobenzenesulfonyl propylurea (CPU)	1.06	12.3	9.20	8.96	0.5063
4-Iodobenzenesulfonyl propylurea (IPU)	1.75	14.4	7.69	4.10	0.7878

a) 1/15 M phosphate buffer at 10°. *b*) Sum of squared deviations in *r*.

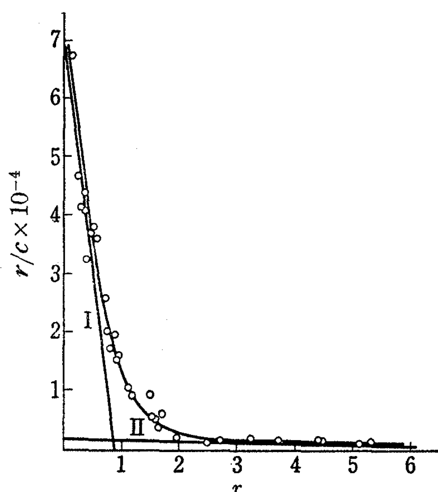

Fig. 1. Scatchard Plot for Binding of Benzenesulfonyl Propylurea to BSA

Line I: plot for primary class; n_1=0.91; k_1=7.86 $\times 10^4$. Line II: plot for second class,; n_2=8.04; k_2=2.45$\times 10^2$. Curve: sum of above lines.
○: experimental.

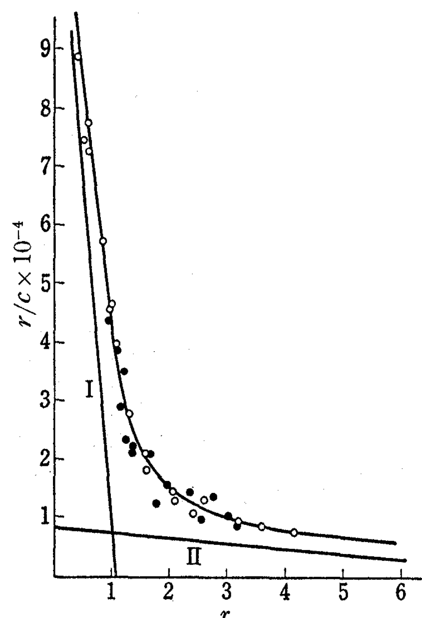

Fig. 2. Scatchard Plot for Binding of 4-Chlorobenzenesulfonyl Propylurea to BSA

Line I: plot for primary class; n_1=1.06; k_1=1.23 $\times 10^5$. Line II: plot for second class; n_2=9.20, k_2= 8.96$\times 10^2$. Curve: sum of above lines.
○: equilibrium dialysis method.
●: dynamic dialysis method.

The primary binding parameters of CPU to BSA were not in agreement with those of CPU to HSA where k=1.0873$\times 10^4$ M^{-1}, n=1.641,[6)] k_1=1.849$\times 10^5$ M^{-1}, n_1=2.20[7)] and k=2.02$\times 10^4$ M^{-1}.[9)] The secondary binding parameters of TPU and CPU to BSA were nearly the same as the reported values of TBU and CPU to HSA where k_2=6.65$\times 10^2$ M^{-1}, n_2=8.22 and k_2=6.52$\times 10^2$ M^{-1}, n_2=8.22, respectively.[7)] Since it has been shown that association constants of greater than 1×10^4 M^{-1} can effect on drug distribution,[13)] it is interesting to compare the primary binding parameters of these 4-substituted benzenesulfonyl propylureas. 4-Halogenated benzenesulfonyl propylureas, IPU and CPU, were more strongly bound to BSA than BPU, TPU and APU in contrast with that TBU were more strongly bound to HSA than CPU.[7)]

Experimental

Materials——BPU, TPU, APU and IPU were synthesized in this laboratory according to the known method.[14)] BPU was synthesized from benzenesulfonamide and *n*-propyl isocyanate. mp 115° (Lit. 118—

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120°[15]). *Anal.* Calcd. for $C_{10}H_{14}N_2O_3S$: C, 49.57; H, 5.82; N, 11.56. Found: C, 49.22; H, 5.83; N, 11.58. TPU was synthesized from *p*-toluenesulfonamide and *n*-propyl isocyanate. mp 151° (Lit. 151—152°,[16] 150—151°[17]). *Anal.* Calcd. for $C_{11}H_{16}N_2O_3S$: C, 51.55; H, 6.29; N, 10.93. Found: C, 51.17; H, 6.37; N, 10.77. APU was obtained by hydrolysis of 4-acetylaminobenzenesulfonyl *n*-propylurea which was synthesized from 4-acetylaminobenzenesulfonamide and *n*-propyl isocyanate. mp 135—136°. *Anal.* Calcd. for $C_{10}H_{15}N_3O_3S$: C, 46.68; H, 5.88; N, 16.33. Found: C, 46.53; H, 5.89; N, 16.19. IPU was synthesized from 4-iodobenzenesulfonamide and *n*-propyl isocyanate. mp 155—156° (Lit. 155—156°[18]). *Anal.* Calcd. for $C_{10}H_{13}IN_2O_3S$: C, 32.60; H, 3.50; N, 7.61. Found: C, 32.69; H, 3.48; N, 7.42. CPU was purchased from Toyama Chem. Ind. Tokyo. BSA was purchased from Sigma Chem. Co., St. Louis. The specific activity of ^{35}S labelled CPU which was obtained from The Radiochemical Centre, Amersham, was 13.7 mCi per mM.

Measurements——The concentration of total and free CPU, IPU, APU were determined with a Shimazu double beam spectrophotometer, model UV-200, at 231, 246 and 254 nm, respectively. Those of BPU and TPU were determined with Hitachi double wavelength spectrophotometer model 356 using the wavelength pairs of 220—280 nm and 226—280 nm, respectively.

The dynamic dialysis was carried out in a glass flow cell of which inner volume was 7 ml. The dialyzer flow rate was controled to 0.42 ml per minute. The radio activities of ^{35}S labelled CPU in dialyzers were determined with Aloka liquid scintillation counter model LSC-602 using counting cocktail of PPO (7 g) and POPOP (0.1 g) in a mixture of ethanol (310 ml) and toluene (690 ml).

The pH measurements were carried out with Hitachi-Horiba pH meter model M-7 calibrated with standard buffer solution.

Acknowledgement The authors wish to thank Dr. J.M. Becktel, Midwest Research Support Center, Veterans Administration Hospital, for his kind information on the computer program.

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[Chem. Pharm. Bull. 25(12)3408—3409(1977)] *UDC 547.918.02 : 581.192*

Pharmacognostical Studies on Gleditsia. III.[1] Flavonoidal Constituents in the Leaves of *Gleditsia japonica* Miquel and *G. sinensis* Lamarck

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(Received May 4, 1977)

Six flavonoids; luteolin-7-glucoside, isoquercitrin, vitexin, isovitexin, orientin, and homo-orientin were isolated from the leaves of *Gleditsia japonica* Miquel and *G. sinensis* Lamarck.

Keywords——*Gleditsia japonica* Miquel; *Gleditsia sinensis* Lamarck; flavonoid glycosides; luteolin-7-glucoside; isoquercitrin; vitexin; isovitexin; orientin; homo-orientin

Gleditsia japonica Miquel (Japanese name: Saikachi) is a deciduous tall tree of the family Leguminosae, distributed in Japan, Korea, and China. In preceding paper,[1] it was reported

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