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# Binding of Diuretics to Human Serum Albumin and to Human Serum from Healthy Adults and Patients with Renal Failure[1a,b]

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It was found by *in vitro* equilibrium dialysis method that there is a small difference in the binding percentage of three diuretics, hydrochlorothiazide, furosemide, and ethacrynic acid, to bovine serum albumin and that to human serum albumin (between two animal species). The binding of three diuretics to human serum was examined in healthy adults and in patients with renal failure. In the healthy adults, binding of three diuretics was dependent on the degree of dilution of human serum. The same type of binding percentage curves was obtained when crystalline human serum albumin and healthy adult serum were diluted with M/15 phosphate buffer. This fact may suggest the possibility that the albumin level in human serum was closely related to the diuretics binding process. In the renal failure, the binding of hydrochlorothiazide and furosemide was correlated with the albumin concentration in human serum. However, the renal failure had reduced the binding of ethacrynic acid due to decreased serum binding capacity. Calculation of binding percentages would be required to assess the clinical importance of decreased binding of diuretics to serum of patients with renal failure.

**Keywords**——diuretic; binding to human serum proteins; serum from patient with renal failure; equilibrium dialysis; human serum albumin; binding capacity; electrophoretic pattern of human serum

It has been discussed in many reviews and original articles that the binding of drugs to serum proteins usually affects their pharmacological activities, distribution in the body, rate of metabolism, and excretion rate. It is generally accepted that only the unbound fraction has the pharmacological activities, and that the degree of drug binding may be influenced by the molecular structure, lipid solubility, dissociation constant, presence of drugs administered concomitantly, endogeneous substances, and desease states.

Our preceding paper[1b] reported the binding of seven commercial diuretics to bovine serum albumin (BSA) and it was shown that there is quite a large difference among the binding abilities of diuretics. The binding constants of hydrochlorothiazide (HCT) and hydroflumethiazide (HFT) to primary binding site of BSA molecule were relatively small compared to those of other diuretics. On the other hand, furosemide (FM) had the maximum binding constant among seven commercial diuretics examined. Ethacrynic acid (EA) was one of exceptional examples because its binding was found to increase considerably with decreasing temperature, and a large value of the evolution of heat (the standard change of enthalpy, $\Delta H^\circ$) was obtained to the others.

The present study on the protein binding of diuretics was undertaken to investigate (1) the extent of binding to human serum albumin (HSA), (2) comparison of experimental results with that with BSA, and also (3) the extent and nature of binding to normal human serum and to serum of patients with renal failure. For this study, the above three representative diuretics (HCT, FM, and EA), except HFT, were used.

1) *a*) This paper forms part V of series entitled "Interaction between Drugs and Blood Components." *b*) Part IV: S. Goto, Y. Odawara, M. Nakano, and Y. Araki, *Chem. Pharm. Bull.* (Tokyo), **26**, 2298 (1978).

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## Experimental

**Materials**——Bovine serum albumin (BSA), fraction V (Armour Pharmaceutical Co., U.S.A.) and human serum albumin (HSA), fraction V (Sigma Chemical Co., U.S.A.) were used, and their molecular weights were assumed to be 69000. Reagent grade $KH_2PO_4$ and $Na_2HPO_4$ were used to prepare the pH 7.4 phosphate buffer solution (M/15). Three diuretics as shown in Chart 1, BSA, and HSA solutions were prepared immediately before use for equilibrium dialysis.

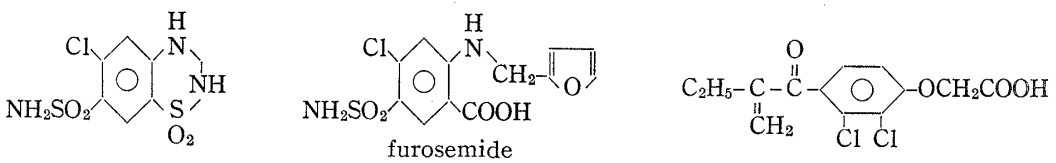


Chart 1. Diuretics used in This Study

Table I. Clinical Data of Healthy Adults and Patients with Renal Failure

| Patient (P) or healthy adult (N) | Sex | Age (yr) | Diagnosis[a] | Disease state[b] | Urine protein (g/day) | Blood red cell count in urine segment (HPF) | Cholesterol (mg/dl) | BUN (mg/dl) | Drugs[c] |
|---|---|---|---|---|---|---|---|---|---|
| N-1 | Male | 35 | N | | — | — | — | — | — |
| N-2 | Male | 35 | N | | — | — | — | — | — |
| N-3 | Male | 30 | N | | — | — | — | — | — |
| N-4 | Male | 26 | N | | — | — | — | — | — |
| P-1 | Male | 60 | CRF | + | 1.0 | — | 130 | 49 | — |
| P-2 | Female | 16 | CGN | + | 1.5 | 10 | 173 | 13 | Inteban SP |
| P-3 | Female | 28 | CGN | + | 0.1 | — | 164 | — | Arlef |
| P-4 | Female | 18 | CGN | ++ | 0.1 | 30 | 194 | — | Arlef |
| P-5 | Female | 42 | CGN | ++ | 3.0 | 4 | 150 | — | Opyrin |
| P-6 | Male | 19 | NS | + | — | — | 250 | — | Predonine, Endoxan |
| P-7 | Female | 25 | NS | + | 1.3 | — | 273 | — | — |
| P-8 | Male | 44 | NS | + | 2.0 | — | 336 | — | — |
| P-9 | Male | 52 | NS | ++ | 4.0 | 2 | 254 | — | — |
| P-10 | Female | 36 | NS | +++ | 2.0 | 3 | 248 | 18 | Predonine, Persantin |
| P-11 | Male | 59 | NS | ++ | 8.0 | 3 | 499 | 16 | — |
| P-12 | Female | 39 | CGN | ++ | 0.3 | 30 | 235 | 17.2 | Arlef, Transamin |
| P-13 | Female | 33 | CGN | + | 0.3 | 6 | 243 | — | Inteban SP |
| P-14 | Female | 25 | CGN | + | 0.8 | 20 | 187 | 31 | Indacin Suppo. |
| P-15 | Male | 33 | CGN | + | 1.2 | 30 | 169 | 24 | Opyrin |
| P-16 | Male | 52 | CGN | + | 2.0 | 10 | 205 | — | Mezolin Suppo. |
| P-17 | Female | 43 | CGN | + | 1.0 | 5 | 240 | 16 | Periactin |
| P-18 | Female | 33 | CRF | + | 2.7 | — | 216 | 24 | |
| P-19 | Male | 22 | CGN | + | 0.3 | 30 | 261 | 21 | Inteban SP, Leftose |
| P-20 | Male | 50 | CGN | + | 2.0 | — | 188 | — | — |
| P-21 | Female | 27 | CGN | + | 0.5 | 30 | 133 | 19 | Urokinase |

*a*) N; normal (healthy adult), CRF; chronic renal failure (clinical syndrome resulting from the failure of excretion of waste products, and the failure of conservation of needed substances consequent to primary disorders of the kidney), CGN; chronic glomerulonephritis (chronic condition usually following acute glomerulonephritis, manifesting proturiuria, hematuria, hypertension and edema and increase in blood urea nitrogen appears in the terminal stage), NS; nephrotic syndrome (characterized by profuse proturiuria, hypoalbuminemia, hyperlipemia and generalized edema).
*b*) + mild, ++ moderate, +++ severe.
*c*) Other drugs administered for treatment.

**Equilibrium Dialysis Method**——The general technique and treatment of data of this study were the same as described previously.[3] The diuretic concentration in the diffusate ranged from 1 to $10\times10^{-4}$ M before dialysis, and the albumin concentration was $2\times10^{-4}$ M, constantly. The binding study using serum (none, 1/2, 1/4, and 1/8 dilutions with M/15 phosphate buffer) was made at diuretic level of $5\times10^{-4}$ M.

**Determination of Diuretics**[1b]——The protein-free compartment in the equilibrium cell was analyzed for free diuretics. A Hitachi Spectrophotometer Model 181 was used for analysis, at 273 nm for hydrochlorothiazide (HCT), at 271 nm for furosemide (FM), and at 277 nm for ethacrynic acid (EA) using M/15 phosphate buffer as a blank. FM was assayed using a Hitachi spectrofluorometer Model MPF-4 in the range of lower concentrations. Two ml of 0.5 N HCl was added to 2 ml sample solution. FM solution has an excitation wavelength of 345 nm and an emission wavelength of 410 nm.

**Patients and Serum**——Serum was obtained from 4 healthy drug-free male adults and from patients with various renal failures. Heparin (0.1 mg in 10 ml serum) was used as the only anticoagulant. All serum samples was kept at approximately −25° for some days before the experiment. The patient number, diagnosis, and clinical and blood test values are given in Table I. All protein and albumin concentrations in serum were determined in 25 healthy adults and patients by Lowry's method[4] and cellulose acetate by the electrophoresis method.

**Electrophoretic Procedure**——All electrophoretic patterns were developed using Tōyō cellulose acetate–electrophoresis apparatus Model SE-2. Cellulose acetate sheets (Separax, Jōkō Sangyo Co., Tokyo) were promptly wetted with a Veronal buffer (pH 8.6 and $\mu=0.07$) before use. The sheets were supported on the stage of a bridge in the electrophoresis apparatus, and a strip of filter paper (Tōyō Roshi No. 2) immersed in the developping solvent (pH 8.6, $\mu=0.07$, Veronal buffer), was placed on it, and this was allowed to stand until equilibrium was reached. A sample (0.001 ml of serum) was applied to the sheet in 1.2 cm width. The current and voltage were regulated by means of a Powerstat (Tōyō elepos Model PS-310) to produce uniform, 0.4—0.8 mA/cm and 110—120 V, during 50 min. An extensive migration was done at 5°, and at pH 8.6 and $\mu=0.07$. Upon completion of electrophoretic pattern, the sheet was soaked in 0.4% Ponceau 3R–3% trichloroacetate mixture for about 30—60 seconds to detect the presence of proteins. The sheet was washed repeatedly with 5% acetate solution and dried naturally in the room. Autoanalysis of electrophoretic patterns was made by moving of the electrophoresis sheet, treated with decaline to become transparent, in a densitometer, Atago Densitomaster Quick, at 500 nm wavelength.

**Other Clinical Tests**——Clinical test values were supplied from the Central Clinical Unit, University Hospital, Okayama University.

## Results and Discussion

### Diuretic–HSA Binding

The binding of three diuretics to crystalline HSA was evaluated by the equilibrium dialysis technique described previously.[3] The Scatchard plots of the data indicated that HCT has only one class of binding site, but the presence of two classes of binding sites must be considered in other two diuretics, FM and EA (Figs 1, 2, and 3). Furthermore, it is obvious from Figs 1, 2, and 3 that the degree of binding to HSA is slightly small compared with that to BSA.

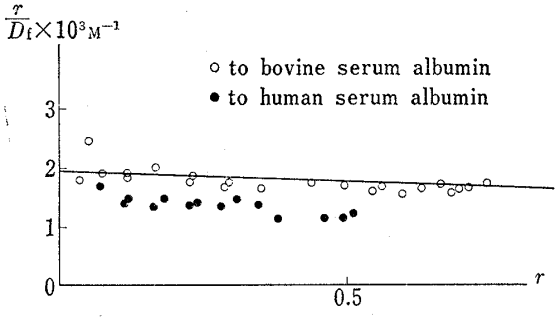


Fig. 1. Scatchard Plots for Binding of Hydrochlorothiazide to Bovine Serum Albumin ($2\times10^{-4}$ M) and Human Serum Albumin ($2\times10^{-4}$ M) at 30° and pH 7.4

All points are experimental while the solid line is computed from the binding parameter in the case of bovine serum albumin.

Kucera and Bullock[5] compared the binding of salicylate to plasma protein from various animal species. They found that the binding percentages of 5.3% of total protein at 50 μg/ml ($3.62\times10^{-4}$ M) of salicylate showed a considerable difference according to animal species (for example, 72% for human and 45% for dog), and such a low binding percentages of dog plasma might be due to the low binding affinity of albumin of the dog.

3) S. Goto, H. Yoshitomi, and M. Kishi, *Yakugaku Zasshi*, **98**, 236 (1978).
4) O.H. Lowry, N.J. Rosebrough, R.J. Farr, and J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
5) J.L. Kucera and F.J. Bullock, *J. Pharm. Pharmacol.*, **21**, 293 (1969).

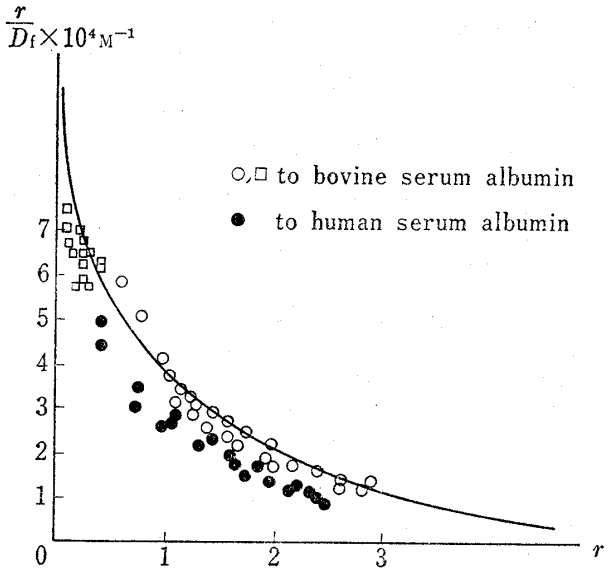


Fig. 2. Scatchard Plots for Binding of Furosemide to Bovine Serum Albumin ($2\times10^{-4}$M) and Human Serum Albumin ($2\times10^{-4}$M) at 30° and pH 7.4

*a*) All points are experimental while the solid line is computed from the binding parameter in the case of bovine serum albumin.
*b*) Spectrophotometric (○, ●) and spectrofluorometric (□) analyses were used for the determination of furosemide after equilibration.

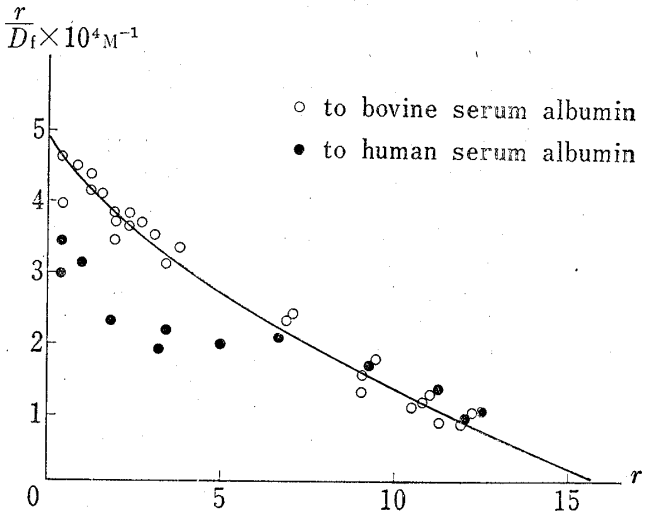


Fig. 3. Scatchard Plots for Binding of Ethacrynic Acid to Bovine Serum Albumin ($2\times10^{-4}$M) and Human Serum Albumin ($2\times10^{-4}$M) at 30° and pH 7.4

All points are experimental while the solid line is computed from the binding parameter in the case of bovine serum albumin.

Table II. Binding Percentages and Parameters of Hydrochlorothiazide, Furosemide, and Ethacrynic Acid ($1\times10^{-4}$M) to BSA ($2\times10^{-4}$M) and HSA ($2\times10^{-4}$M) at 30° and pH 7.4

| | Binding (%) | | Binding parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | BSA | | | | HSA | | | |
| | BSA Mean±S.D. | HSA Mean±S.D. | $K_1$ $\times10^3$ M$^{-1}$ | $K_2$ $\times10^2$ M$^{-1}$ | $n_1$ | $n_2$ | $K_1$ $\times10^3$ M$^{-1}$ | $K_2$ $\times10^2$ M$^{-1}$ | $n_1$ | $n_2$ |
| Hydrochlorothiazide | 35.0±11.3 | 23.2±3.2 | — | 0.3 | — | 7.8 | — | 0.6 | — | 2.5 |
| Furosemide | 95.3± 0.5 | 87.0±3.9 | 375 | 4.2 | 0.7 | 4.9 | 26.8 | 5.9 | 1.3 | 2.2 |
| Ethacrynic acid | 90.4± 1.4 | 86.2±0.8 | 5.3 | 2.1 | 4.8 | 10.6 | 4.3 | 0.7 | 3.9 | 20.3 |

Table III. Analysis of Human Serum Protein and Binding Percentages of Hydrochlorothiazide to Human Serum Protein[a)]

| Patient (P) or healthy adult (N) | Total protein (g/dl) | Albumin (g/dl) | Protein fraction (%) | | | | | Binding (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | | | | |
| | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | |
| N-1 | 8.3 | 5.1 | 62.4 | 11.0 | | 12.2 | 14.5 | 22.4 |
| N-2 | 8.4 | 5.2 | 61.6 | 2.6 | 9.3 | 12.1 | 14.6 | 22.8 |
| N-3 | 8.0 | 5.9 | 73.6 | 2.0 | 7.4 | 7.4 | 9.5 | 22.0 |
| P-1 | 5.5 | 3.3 | 60.5 | 2.3 | 10.8 | 8.8 | 17.7 | 20.8 |
| P-2 | 7.8 | 5.2 | 66.1 | 1.3 | 6.9 | 11.2 | 14.6 | 22.4 |
| P-3 | 9.3 | 5.8 | 62.3 | 2.9 | 5.8 | 8.0 | 21.0 | 24.0 |
| P-4 | 8.2 | 5.4 | 65.7 | 9.1 | | 10.0 | 15.2 | 24.0 |
| P-5 | 7.5 | 4.2 | 56.5 | 1.7 | 8.8 | 10.0 | 22.6 | 23.6 |

*a*) Human serum was diluted with M/15 phosphate buffer (pH 7.4) to 1/2 concentration, keeping the concentration of diuretics constant ($5\times10^{-4}$M).

The calculation of binding was made at diuretic level of $1\times10^{-4}$ M, and the result, expressed in terms of percentages of the drug bound, is presented in Table II. It is clearly recognized that there are very small difference in the percentages of both bindings for all diuretics and also that the binding decreasing in the order is FM>EA>HCT.

**Binding of Diuretics with Healthy Adult Serum**

Protein binding of diuretics with sera from 4 healthy drug-free male adults was studied at an initial concentration of diuretics of $5\times10^{-4}$ M at 30°. There was no marked individual difference in the bound fraction of diuretics as shown in Tables III, IV and V.

TABLE IV. Analysis of Human Serum Protein and Binding Percentages of Furosemide to Human Serum Protein[a)]

| Patient (P) or healthy adult (N) | Total protein (g/dl) | Albumin (g/dl) | Protein fraction (%) | | | | | Binding (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | | | | |
| | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | |
| N-1 | 7.7 | 5.5 | 70.9 | 1.9 | 6.9 | 8.4 | 11.9 | 87.3 |
| N-2 | 8.2 | 5.2 | 63.1 | 2.3 | 9.2 | 13.0 | 12.5 | 88.2 |
| N-4 | 8.4 | 5.8 | 69.2 | 2.0 | 8.9 | 8.6 | 11.4 | 86.6 |
| P-2 | 7.2 | 4.1 | 57.1 | 2.5 | 11.5 | 12.6 | 16.3 | 84.8 |
| P-6 | 7.1 | 4.7 | 66.8 | 2.9 | 8.1 | 11.2 | 11.2 | 85.1 |
| P-7 | 6.6 | 4.3 | 65.5 | 2.3 | 8.8 | 10.3 | 13.0 | 84.1 |
| P-8 | 7.1 | 4.7 | 65.6 | 3.7 | 12.5 | 11.0 | 7.2 | 84.9 |
| P-9 | 6.8 | 3.8 | 56.2 | 3.2 | 15.4 | 11.4 | 13.8 | 89.3 |
| P-10 | 7.2 | 4.0 | 55.0 | 3.0 | 15.4 | 11.3 | 15.4 | 81.4 |
| P-11 | 5.8 | 2.5 | 42.5 | 24.8 | | 21.8 | 10.8 | 60.8 |
| P-12 | 7.4 | 4.2 | 56.3 | 2.8 | 9.6 | 12.2 | 19.1 | 87.5 |
| P-13 | 8.6 | 5.0 | 57.6 | 3.1 | 9.6 | 11.9 | 18.0 | 90.0 |
| P-14 | 8.5 | 5.3 | 61.8 | 1.8 | 8.0 | 9.9 | 18.4 | 88.8 |
| P-15 | 7.4 | 4.7 | 63.0 | 2.7 | 10.0 | 9.5 | 14.8 | 86.6 |
| P-16 | 8.9 | 4.5 | 51.0 | 8.1 | | 7.8 | 33.2 | 83.6 |
| P-17 | 8.4 | 5.3 | 62.5 | 10.0 | | 8.8 | 18.5 | 88.7 |
| P-18 | 7.6 | 5.0 | 65.8 | 8.6 | | 10.6 | 14.9 | 85.7 |

a) Same as in Table III.

TABLE V. Analysis of Human Serum Protein and Binding Percentages of Ethacrynic Acid to Human Serum Protein[a)]

| Patient (P) or healthy adult (N) | Total protein (g/dl) | Albumin (g/dl) | Protein fraction (%) | | | | | Binding (%) |
|---|---|---|---|---|---|---|---|---|
| | | | Albumin | Globulin | | | | |
| | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | |
| N-1 | 7.7 | 5.5 | 70.9 | 1.9 | 6.9 | 8.4 | 11.9 | 90.9 |
| N-2 | 8.2 | 5.2 | 63.1 | 2.3 | 9.2 | 13.0 | 12.5 | 87.7 |
| N-4 | 8.4 | 5.8 | 69.2 | 2.0 | 8.9 | 8.6 | 11.4 | 91.0 |
| P-19 | 5.5 | 3.6 | 65.5 | 3.4 | 7.8 | 7.3 | 16.0 | 71.6 |
| P-20 | 4.5 | 2.5 | 56.5 | 3.4 | 8.4 | 9.2 | 22.7 | 65.2 |
| P-21 | 6.4 | 4.5 | 68.4 | 2.0 | 6.3 | 10.2 | 13.2 | 76.0 |

a) Same as in Table III.

The binding of diuretics in various diluted sera was measured, and the bound fraction of each drug decreased gradually with increasing dilution with M/15 phosphate buffer, pH 7.4 (Figs 4, 5, and 6).

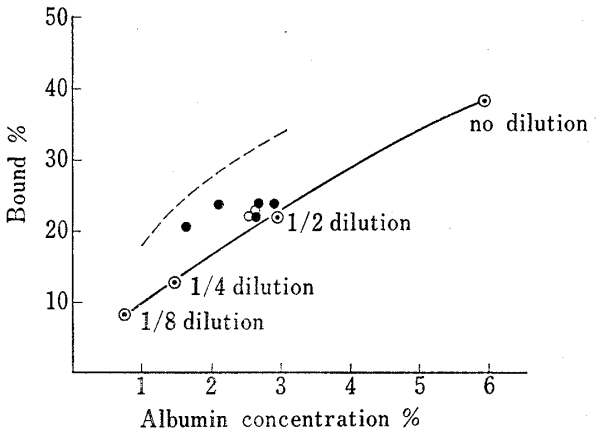


Fig. 4. Hydrochlorothiazide Binding Percentages to Human Serum Protein and Effect of Diluting the Human Serum on the Hydrochlorothiazide Binding

Concentration of hydrochlorothiazide; $5 \times 10^{-4}$M, pH 7.4 and 30°
○ : to 1/2 diluted serum from healthy adult.
● : to 1/2 diluted serum from patient.
—⊙—: to serum (none, 1/2, 1/4, and 1/8 dilutions) of healthy adult.
--------: to HSA.

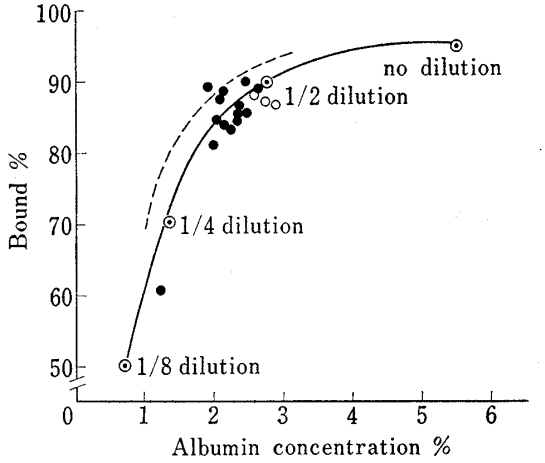


Fig. 5. Furosemide Binding Percentages to Human Serum Protein and Effect of Diluting the Human Serum on the Furosemide Binding

Concentration of furosemide; $5 \times 10^{-4}$M, pH 7.4 and 30°
○ : to 1/2 diluted serum from healthy adult.
● : to 1/2 diluted serum from patient.
—⊙—: to serum (none, 1/2, 1/4, and 1/8 dilutions) of healthy adult.
--------: to HSA.

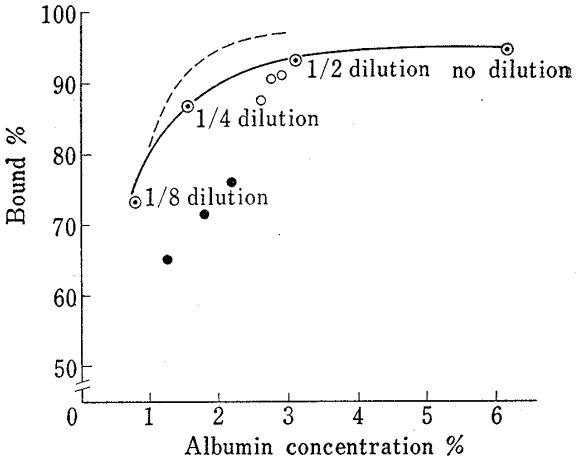


Fig. 6. Ethacrynic Acid Binding Percentages to Human Serum Protein and Effect of Diluting the Human Serum on the Ethacrynic Acid Binding

Concentration of ethacrynic acid; $5 \times 10^{-4}$M, pH 7.4 and 30°
○ : to 1/2 diluted serum from healthy adult.
● : to 1/2 diluted serum from patient.
—⊙—: to serum (none, 1/2, 1/4, and 1/8 dilutions) of healthy adult.
--------: to HSA.

The same type of curve was obtained when crystalline HSA was diluted to the same extent as shown by the broken lines in Figs. 4, 5, and 6. The above experiments with dilution of serum gave results similar to those of Anton[6] obtained with sulfanilamides and of Lunde *et al.*[7] obtained with diphenylhydantoin. The dilution state of serum corresponds to the bleeding of patients with subsequent substitution of the lost fluid with plasma expanders. The free fraction of diuretics may increase markedly in such a condition, and this may be of clinical and toxicological importance. While the protein to which diuretics is bound could not be identified from the present experiments, parallel relationship between the solid lines for dilution of serum from healthy adults and the broken lines for dilution of crystalline HSA in Figs 4, 5, and 6 may indicate the participation of albumin level in human serum for drug bindings. However, about 10% discrepancy of the two lines, as shown in Figs. 4, 5, and 6, cannot be explained exactly. This fact may suggest the possibility of competitive phenomena between diuretic molecules and endogeneous substances on human proteins. In addition to these facts, a comparison was made of the binding of diuretics to serum and to plasma from healthy

6) A.H. Anton, *Clin. Pharmacol. Ther.*, **9**, 561 (1968).
7) P.K.M. Lunde, A. Rane, S.J. Yaffe, L. Lund, and F. Sjöqvist, *Clin. Pharmacol. Ther.*, **11**, 846 (1970).

adult. Because close agreement between them was obtained, the human serum fraction was used in the present experiment.

**Binding of Diuretics in Serum from Patient with Renal Failure**

Protein binding of drugs in serum from patients with renal failure has been stated in many clinical reports.[8-12] It was found that the protein binding of acetylsalicylic acid, salicylic acid, phenylbutazone, diphenylhydantoin, digitoxin, and other drugs was decreased and this reduced binding could only partly be explained by the lower concentration of albumin in the serum from such patients. Moreover, a defect in the drug-binding sites on the proteins has been considered as a possible explanation for the reduced protein binding.[9] In the present study, patients, 9 men and 12 women, were aged between 16 and 60 years. They had suffered from the nephrosis symdrome, chronic glomerulonephritis, and chronic renal failure, but they were not serious cases. Diagnosis of all the patients examined is listed in Table I.

Total protein and albumin concentrations and protein fractions were determined in the healthy adults and patients, and experimental results are shown in Tables III, IV and V. Although a difference between healthy adult and patient can be observed in the percentages of protein fraction from the detailed analysis, the total proteins and albumin concentrations are almost the same except for few patients. Student's t-test was made on each average of total protein and albumin concentrations in healthy adults and patients for three diuretics. As shown in Tables III, IV and V, no statistical difference was observed ($p>0.05$).

The binding percentages of three diuretics (HCT, FM, and EA) to sera from 21 patients with renal failure are shown in the last column in Tables III, IV and V. The concentration of diuretics was $5\times10^{-4}$ M in all experiments while the serum concentration was diluted to 1/2 with M/15 phosphate buffer to maintain pH 7.4. No difference was observed in the serum binding of HCT except in patient P-1. The smallest binding percentages in the cases of FM and EA were patients P-11 and P-20, respectively. Albumin level in serum from these three patients was lower than that from healthy adults and other patients. Figs. 4, 5, and 6 show the diuretics binding at 1/2-dilution serum samples from 21 patients and serum samples from 4 healthy adults at none, 1/2, 1/4, and 1/8-dilutions. The values of binding to patient serum can roughly be put on the curve obtained from the healthy adult serum samples (none, 1/2, 1/4, and 1/8 dilutions), as indicated in Figs. 4 and 5. However, a small discrepancy is noted in the case of EA and its binding with patient serum albumin is less than with the healthy adult serum albumin, there being about 15% difference. According to Andreasen and Jakobsen,[13] furosemide would be exclusively bound to albumin and it was possible that patient's serum albumin had a defective drug-binding mechanism. Andreasen[8] also found that a similar apparent defect existed for other drugs, acetylsalicylic acid, salicylic acid, phenylbutazone, diphenylhydantoin, sulfadiazine, and thiopental, and that the protein-bound fraction of drugs was found to be increased in blood plasma after *in vitro* dialysis of plasma from patients suffering from acute renal failure. The increase of binding could be caused only by the removal of substances (molecular weight higher than *ca.* 700) capable of inhibiting the binding of drugs. Such a phenomenon was also observed after the dialysis of normal-plasma. Shoeman and Azarnoff[12] studied the binding ability of digoxin and diphenylhydantoin to plasma from uremic patients. They found that digoxin was bound 86% to plasma from patients while plasma from healthy volunteers bound 90%,

8) F. Andreasen, *Acta Pharmacol. Toxicol.*, **32**, 417 (1973).
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and the binding of diphenylhydantoin was also depressed in uremia patients. They isolated albumin by curtain electrophoresis from plasma of uremic and normal individuals, and isoelectric focussing revealed a difference in microheterogeneity among albumins isolated from normal volunteers or uremic patients and commercial crystalline HSA. Their conclusion was that this inhibitor might be a protein which is closely associated with or very closely resembles albumin.

There was no significant difference in the binding percentage of diuretics to serum from patients with renal failure and that from healthy adults and the presence of competitively or noncompetitively bound endogeneous inhibitor as stated in other reports could not be expected in this study except in the case of EA.

Conclusion in the present investigation is that the decreased diuretic binding to serum in some patients may partly be due to a lower protein or albumin concentration. Determination of total proteins and albumin concentration from patients with renal failure should always be made for clinical therapy. If these values are relatively small compared with normal, the concentration of unbound drug in patient then must be considered to be higher than that in healthy adults with normal renal function. It is imperative that this fact be considered in devising dosage regimens for patients with renal failure in order to prevent toxic effects.

Other drugs used in medical practice were also studied for the effect on diuretics binding to serum but significant displacing effect exerted was not observed in the present investigation.

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