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# Protein Binding of Sulfonamides and Displacing Effects of Sulfinpyrazone on the Bound Sulfonamides in Nephrotic Patients in the Remissive Stage[1)]

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The binding of sulfamethoxazole and sulfaphenazole to the serum protein of 10 nephrotic patients in the remissive stage, receiving prednisolone medication and alimentotherapy, was studied by equilibrium dialysis, and the results were compared with those for 10 normal subjects. The binding percentages of the two sulfonamides were found to be slightly impaired in the patients' serum as compared with normal serum. The binding characteristics of the patients' serum albumin were examined by calculating the percentage displacement of bound sulfonamides by sulfinpyrazone. The percentage displacement was considerably greater in the patients' serum than in normal serum. However, a proportional relationship between the percentage displacement and serum albumin concentration could not be found. The reason for the increased percentage displacement in the patients' serum remains unclear.

**Keywords**——protein binding; sulfonamides; nephrotic patients; remissive stage; displacing effect; sulfinpyrazone; percentage of displacement; equilibrium dialysis

The nephrotic syndrome is well known to be a disorder characterized by an increased protein loss *via* the kidney with subsequent hypoproteinemia and dysproteinemia.[3,4)] However, in the remissive stage of nephrotic syndrome, the albumin concentration in the serum reaches a normal or near-normal level.

In the present investigation, the protein binding of sulfamethoxazole (SMX) and sulfaphenazole (SPH) in the serum of nephrotic patients in the remissive stage was studied by the equilibrium dialysis method, and the results were compared with those observed in normal serum.

Furthermore, the displacing effects of sulfinpyrazone (SPZ) on the binding of SMX and SPH to serum protein were compared in nephrotic patients and in normal subjects, in order to develop an appropriate method for evaluating the binding activity of the serum of nephrotic patients.

## Experimental

**Materials**——Reagent-grade $KH_2PO_4$, $Na_2HPO_4$ and NaCl were used to prepare 0.1 M isotonic phosphate buffer solution. All other substances were purchased from commercial sources. Sulfamethoxazole, sulfaphenazole and sulfinpyrazone were recrystallized before use. Sulfamethoxazole (SMX) mp 168—171°; sulfaphenazole (SPH) mp 180—182°; sulfinpyrazone (SPZ) mp 131—132°.

**Patients and Normal Subjects**——Ten nephrotic patients and 10 normal subjects participated in this study. All the patients were inpatients at the Pediatric Unit, National Sanatrium of Nishi-Sapporo Hospital in Sapporo.

1) A part of this study was presented at the 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August, 1979.
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3) R. Gugler and D.L. Azarnoff, *Clin. Pharmacokinet.*, **1**, 25 (1976).
4) T. Kawai, "The Plasma Proteins," Igaku Shoin Ltd., Tokyo, 1969, p. 407.

Table I. Clinical Data for Nephrotic Patients[a,b]

| Patients No. | Sex | Age (year) | BUN[d] (mg/dl) | Cholesterol[d] (mg/dl) | Medication[c] Prednisolone (mg/every other day) | Medication[c] Other drugs |
|---|---|---|---|---|---|---|
| No. 1 | Male | 15 | 11.8—15.6 | 178—180 | — | Methylephedrin 0.1, Bisolvon 3T, Asverin 0.1 } 2 days |
| No. 2 | Male | 14 | 9.6— 9.6 | 135—163 | 50 mg | Flucort cream 5 g |
| No. 3 | Male | 10 | 10.2— 6.5 | 236—169 | 35 mg | —— |
| No. 4 | Male | 9 | 8.3— 8.7 | 186—187 | 35 mg | —— |
| No. 5 | Male | 8 | 10.8— 8.7 | 151—159 | 35 mg | —— |
| No. 6 | Male | 9 | 6.7— 6.7 | 171—189 | 35 mg | —— |
| No. 7 | Male | 14 | 9.8—10.4 | 137—178 | 45 mg | Josamycin 6T, Brufen 3T } 8 days |
| No. 8 | Male | 14 | 10.2— 9.6 | 224—195 | 45 mg | Josamycin 6T, Astomin 6T } 8 days |
| No. 9 | Female | 8 | 9.1— 7.3 | 177—173 | 5 mg | Methylephedrin 0.07, Bicillin G 1.0 } 7 days |
| No. 10 | Female | 12 | 9.4—10.3 | 157—161 | — | —— |

*a*) All patients were in the remissive stage, and no urinary protein was detected throughout the experimental period.
*b*) All clinical data shown in this table were obtained during the experimental period.
*c*) All medication was continued during the experimental period.
*d*) Numerical values connected by hyphens represent data at two different times during the experimental period.

Nephrotic patients that participated in this study were from 8 to 15 years of age. Their disease conditions were in the remissive stage, and they were undergoing prednisolone medication and alimentotherapy.

The diagnosis, results of clinical laboratory tests and drugs given to the patients are listed in Table I. All clinical laboratory tests were carried out by the Clinical Pathological Unit, National Sanatorium of Nishi-Sapporo Hospital.

Normal subjects were 10 healthy adults aged from 19 to 20 years, consisting of student nurses at the National Sanatorium of Nishi-Sapporo Hospital.

**Serum**——Sera from the patients and normal subjects were obtained by centrifugation of blood samples and were used for drug protein binding experiments. All serum samples were stored at 4° and were used within one week. Protein and albumin contents were determined by electrophoresis using a Densitron 20 M machine (Joko Co.).

**Binding Experiment**——The extent of binding of drugs to serum was determined by the equilibrium dialysis method as described previously.[5] The medium used was 0.1 M isotonic phosphate buffer at pH 7.4. The osmotic pressure of the buffer was determined with a Knauer semimicro osmometer. The outer compartment contained the isotonic phosphate buffer together with a drug at a concentration of 100 μg/ml in a total volume of 2 ml. The inner compartment (seamless cellulose tubing: Visking Co.) contained 0.5 ml of serum. Incubation was carried out for 24 hr at 37° with constant mild shaking. Upon attainment of equilibrium, the content of the outer compartment was removed. The sulfonamides were analyzed by diazotization.[6]

**Method of Evaluating the Sulfonamide-Displacing Activity of a Drug**——Sulfinpyrazone (SPZ) was used as a displacer, and its ability to interfere with the binding of sulfonamides to serum protein was compared in nephrotic patients and in normal subjects. The parameter used is referred to as "percentage displacement" and is defined as follows.[7]

$$\beta = \frac{B - B_i}{B} \times 100\ (\%)$$

Where $\beta$ is the percentage displacement *in vitro*; $B$ is the percentage of SMX or SPH bound in the absence of competing drug; $B_i$ is the percentage of SMX or SPH bound in the presence of competing drug. All the drugs were used at a level of 100 μg/ml in this work.

5) T. Arita, R. Hori, M. Takada, and A. Misawa, *Chem. Pharm. Bull.*, **19**, 930 (1971).
6) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 413 (1964).
7) Y. Kaneo, A. Nishikawa, Y. Kato, and S. Kiryu, *Yakugaku Zasshi*, **98**, 1452 (1978).

## Results

### Serum Binding of Sulfonamides in Nephrotic Patients and in Normal Subjects

The protein binding of SMX and SPH in the serum of 10 nephrotic patients in the remissive stage and in the serum of 10 normal subjects was investigated in 0.1 M isotonic phosphate buffer at pH 7.4. Calculation of the binding was based on a sulfonamide concentration of 100 μg/ml, and the results were expressed as percentages of the drugs bound. The data are listed in Table II.

Table II. Analytical Data on Serum Protein and Binding Percentages of Sulfamethoxazole and Sulfaphenazole in Nephrotic Patients in the Remissive Stage and in Normal Subjects

| | Sulfamethoxazole | | | Sulfaphenazole | | |
|---|---|---|---|---|---|---|
| | Total protein (g/dl) | Albumin (g/dl) | SMX binding (%) 100 μg/ml | Total protein (g/dl) | Albumin (g/dl) | SPH binding (%) 100 μg/ml |
| Patients | | | | | | |
| No. 1 | 6.60 | 4.19 | 71.6 | 6.20 | 4.07 | 88.3 |
| No. 2 | 6.30 | 4.00 | 66.3 | 6.20 | 3.85 | 88.7 |
| No. 3 | 6.40 | 4.35 | 67.3 | 5.90 | 4.01 | 89.4 |
| No. 4 | 6.40 | 4.08 | 66.7 | 7.00 | 4.62 | 89.7 |
| No. 5 | 6.00 | 3.77 | 64.3 | 6.20 | 3.78 | 87.3 |
| No. 6 | 6.70 | 4.26 | 68.6 | 6.60 | 4.09 | 90.2 |
| No. 7 | 5.90 | 4.25 | 62.4 | 6.40 | 4.23 | 91.6 |
| No. 8 | 6.20 | 4.60 | 69.3 | 6.10 | 4.38 | 88.4 |
| No. 9 | 6.30 | 4.02 | 68.4 | 6.90 | 4.73 | 88.4 |
| No. 10 | 7.10 | 4.92 | 73.3 | 6.90 | 4.77 | 90.9 |
| Mean | 6.39 | 4.24 | 67.8[a] | 6.44 | 4.25 | 89.3[b] |
| ±S.D. | ±0.35 | ±0.33 | ±3.2 | ±0.39 | ±0.36 | ±1.3 |
| Normal subjects | | | | | | |
| No. 1 | 7.80 | 5.82 | 73.4 | 7.80 | 5.82 | 90.9 |
| No. 2 | 7.40 | 5.41 | 69.9 | 7.40 | 5.41 | 90.8 |
| No. 3 | 7.50 | 4.99 | 69.6 | 7.50 | 4.99 | 91.6 |
| No. 4 | 7.80 | 5.65 | 69.3 | 7.80 | 5.65 | 93.1 |
| No. 5 | 9.00 | 5.97 | 72.3 | 9.00 | 5.97 | 90.5 |
| No. 6 | 8.00 | 5.58 | 72.7 | 8.00 | 5.58 | 92.8 |
| No. 7 | 7.90 | 5.16 | 71.8 | 7.90 | 5.16 | 90.0 |
| No. 8 | 7.70 | 5.68 | 68.4 | 7.70 | 5.68 | 93.3 |
| No. 9 | 7.50 | 5.80 | 70.4 | 7.50 | 5.80 | 92.8 |
| No. 10 | 7.80 | 5.35 | 70.9 | 7.80 | 5.35 | 90.2 |
| Mean | 7.84 | 5.54 | 70.9 | 7.84 | 5.54 | 91.6 |
| ±S.D. | ±0.45 | ±0.31 | ±1.6 | ±0.45 | ±0.31 | ±1.3 |

*a*) Significantly different from the normal group, $p<0.005$.
*b*) Significantly different from the normal group, $p<0.005$.

It is clear that the levels of total protein and albumin concentration of nephrotic patients in the remissive stage appear to be within the normal range, but are lower than those of the normal subjects that participated in this experiment. The binding percentages of both sulfonamides to the serum of nephrotic patients were slightly lower than those to normal serum as shown in Table II. Both nephrotic patients and normal subjects exhibited remarkable consistency among individuals in the binding of both sulfonamides to serum albumin, as shown in Table II.

**The Displacing Effects of SPZ on the Binding of Sulfonamides to Sera of Nephrotic Patients and Normal Subjects**

It is well known that serum albumin binds SPZ strongly, and SPZ readily displaces certain acidic drugs.[8] In this experiment, SPZ was used to assess the binding characteristics of serum of nephrotic patients. The results are shown in Table III.

Table III. Displacing Effects of Sulfinpyrazone on the Protein Binding of Sulfamethoxazole and Sulfaphenazole in Nephrotic Patients in the Remissive Stage and in Normal Subjects

| | Sulfamethoxazole | | Sulfaphenazole | |
|---|---|---|---|---|
| | Albumin (g/dl) | Percentage displacement[a] (%) 100 μg/ml | Albumin (g/dl) | Percentage displacement[a] (%) 100 μg/ml |
| Patients | | | | |
| No. 1 | 4.19 | 43.2 | 4.07 | 14.8 |
| No. 2 | 4.00 | 32.7 | 3.85 | 12.9 |
| No. 3 | 4.35 | 28.1 | 4.01 | 12.0 |
| No. 4 | 4.08 | 36.0 | 4.62 | 6.4 |
| No. 5 | 3.77 | 39.0 | 3.78 | 10.0 |
| No. 6 | 4.26 | 34.3 | 4.09 | 12.1 |
| No. 7 | 4.25 | 27.7 | 4.23 | 10.0 |
| No. 8 | 4.60 | 32.6 | 4.38 | 11.1 |
| No. 9 | 4.02 | 26.5 | 4.73 | 4.6 |
| No. 10 | 4.92 | 31.4 | 4.77 | 8.6 |
| Mean ±S.D. | 4.24 ±0.33 | 33.2[b] ±5.2 | 4.25 ±0.36 | 10.3[c] ±3.1 |
| Normal subjects | | | | |
| No. 1 | 5.82 | 19.4 | 5.82 | 6.2 |
| No. 2 | 5.41 | 14.6 | 5.41 | 3.6 |
| No. 3 | 4.99 | 23.0 | 4.99 | 7.1 |
| No. 4 | 5.65 | 27.4 | 5.65 | 7.7 |
| No. 5 | 5.97 | 14.5 | 5.97 | 7.6 |
| No. 6 | 5.58 | 16.2 | 5.58 | 4.8 |
| No. 7 | 5.16 | 27.2 | 5.16 | 6.6 |
| No. 8 | 5.68 | 23.0 | 5.68 | 5·9 |
| No. 9 | 5.80 | 20.5 | 5.80 | 8.5 |
| No. 10 | 5.35 | 20.2 | 5.35 | 7.6 |
| Mean ±S.D. | 5.54 ±0.31 | 20.6 ±4.7 | 5.54 ±0.31 | 6.6 ±1.5 |

*a*) Percentage displacement=$[(B-B_1)/B]\times 100$ (%), wher $B$ is the binding percentage of the sulfonamide alone, and $B_1$ is that in the present of the displacing agent (SPZ).
*b*) Significantly different from the normal group, $p<0.005$.
*c*) Significantly different from the normal group, $p<0.005$.

As shown in Table III, SPZ shows strong displacing activity towards protein-bound SMX, and substantially displaced bound SMX in the patients' serum (33.2±5.2% at 100 μg/ml). In normal serum, the percentage displacement of bound SMX was considerably lower (20.6± 4.7% at 100 μg/ml). The percentage displacement for each patient exceeded the maximum value of normal subjects except in one patient (No. 9). Table III also indicates that SPZ effectively displaces protein-bound SPH, but the percentage displacement of bound SPH by SPZ is much lower than that of bound SMX. The mean percentage displacement in the patients' serum considerably exceeded that in normal serum (10.3±3.1% for the patients and 6.6±1.5%

8) A.H. Anton, *J. Pharmacol. Exp. Ther.*, **134**, 291 (1961).

for the normal subjects) and the individual values for the patients exceeded the maximum value of normal subjects except in two patients (No. 4 and No. 9).

**Effect of Serum Albumin Concentration**

The differences of total protein and serum albumin concentration between the nephrotic patients and normal subjects are marked, as shown in Tables II and III. We thus examined the effect of serum albumin concentration on the binding percentage and percentage displacement for SMX and SPH, using two normal groups with different total protein and serum albumin concentrations. The mean value of serum albumin in Group A was about 4.4 g/dl and that in Group B was about 5.4 g/dl. The results are shown in Table IV.

Table IV. Effects of Serum Albumin Concentration on Binding Percentage and Percentage Displacement for Sulfamethoxazole and Sulfaphenazole

| | Total Protein (g/dl) | Albumin (g/dl) | Sulfamethoxazole | | Sulfaphenazole | |
|---|---|---|---|---|---|---|
| | | | Binding (%) 100 μg/ml | Percentage displacement[a] (%) 100 μg/ml | Binding (%) 100 μg/ml | Percentage displacement[a] (%) 100 μg/ml |
| (Group A) Normal subjects | | | | | | |
| No. 1 | 7.00 | 4.48 | 66.1 | 25.1 | 88.0 | 6.4 |
| No. 2 | 6.75 | 4.11 | 66.8 | 23.2 | 85.6 | 9.2 |
| No. 3 | 6.86 | 4.46 | 68.0 | 17.7 | 85.1 | 5.9 |
| No. 4 | 6.50 | 4.07 | 70.3 | 16.8 | 87.9 | 5.7 |
| No. 5 | 7.90 | 4.77 | 70.8 | 14.1 | 89.6 | 6.0 |
| Mean ±S.D. | 7.00 ±0.53 | 4.38 ±0.29 | 68.4 ±2.1 | 19.4 ±4.6 | 87.2 ±1.9 | 6.6 ±1.5 |
| (Group B) Normal subjects | | | | | | |
| No. 6 | 8.00 | 5.20 | 69.8 | 18.6 | 91.8 | 7.1 |
| No. 7 | 8.20 | 5.20 | 72.5 | 16.0 | 90.8 | 7.1 |
| No. 8 | 7.80 | 5.42 | 73.2 | 18.4 | 91.0 | 6.3 |
| No. 9 | 8.05 | 5.51 | 71.8 | 20.1 | 92.2 | 6.5 |
| No. 10 | 8.90 | 5.87 | 66.4 | 19.3 | 91.8 | 7.0 |
| Mean ±S.D. | 8.19 ±0.42 | 5.44 ±0.28 | 70.7 ±2.7 | 18.5 ±1.5 | 91.5 ±0.6 | 6.8 ±0.4 |

a) Percentage displacement=$[(B-B_i)/B] \times 100$ (%), where $B$ is the binding percentage of the sulfonamide alone, and $B_i$ is that in the presence of the displacing agent (SPZ).

The difference in percentage displacement between the two groups was very slight, and no correlation was apparent between the percentage displacement and the serum albumin concentration in the range studied.

## Discussion

Several reports have been published on reduced drug protein binding in nephrotic syndrome.[3,9–12] The reduced drug protein binding in nephrotic patients is thought to be mainly due to hypoalbuminemia resulting from the urinary loss and leakage into the interstitial fluid of serum albumin.

9) J. Prandota and A.W. Pruitt, *Clin. Pharmacol. Therap.*, **17**, 159 (1975).
10) M.M. Reidenberg and M. Affrime, *Ann. New York Acad. Sci.*, **226**, 115 (1973).
11) L.P. Tillement, F. Lhoste, and J.F. Giwdicelli, *Clin. Pharmacokinet.*, **3**, 144 (1978).
12) M. Nakano, K. Fuji, and S. Goto, *Chem. Pharm. Bull.*, **27**, 101 (1979).

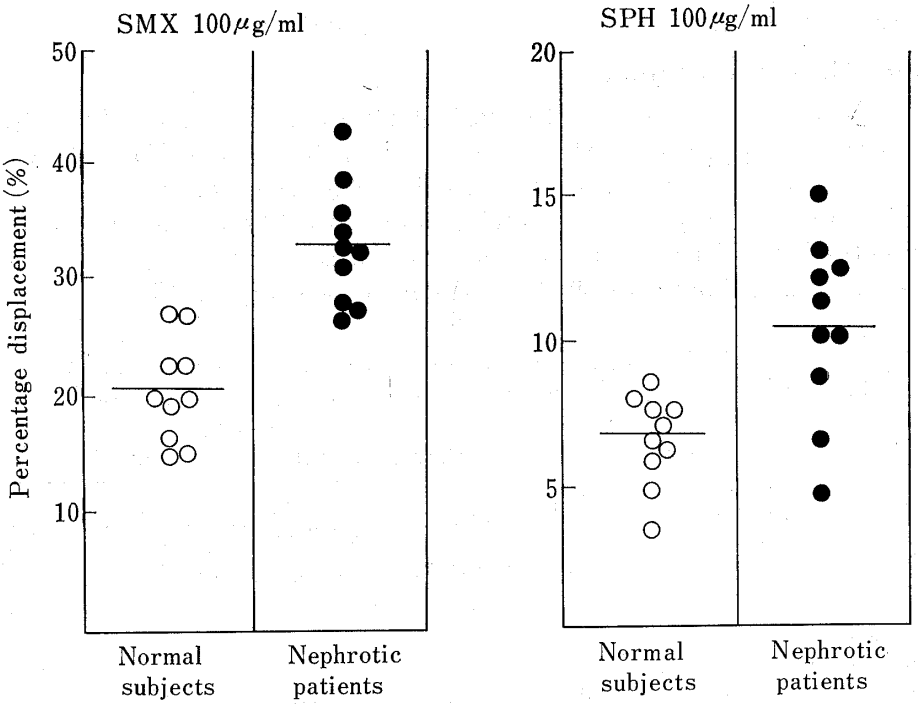


Fig. 1. Comparison of Percentage Displacements of Bound Sulfonamides by Sulfinpyrazone in Nephrotic Patients in the Remissive Stage and in Normal Subjects

Key: ○, normal serum.
●, nephrotic serum.
The solid horizontal lines represent the mean percentage displacement for each group.

In normal subjects, the albumin concentration in serum is usually 3.5 g/dl or higher, but it can be as low as 1 g/dl in nephrotic patients.[3,13] It is also generally accepted that serum albumin molecules in nephrotic patients are normal both electrophoretically and immunologically.[3]

Nephrotic patients participating in this experiment were all in the remissive stage, receiving prednisolone medication and alimentotherapy, and their serum albumin concentrations were all over 4.0 g/dl throughout this experimental period, as shown in Tables II and III. Reduced percentage binding in the nephrotic serum was observed for both sulfonamides. The differences in binding percentage between the patients' serum and normal serum are very slight, but are statistically significant.

SPZ had a greater displacing effect on bound sulfonamides in the patients' serum as compared with those in normal serum. The displacing effect of SPZ on the bound sulfonamides is illustrated in Fig. 1.

From the results in Tables II and III, it appears that there may be a direct relationship between the magnitude of displacement and serum albumin concentration. The data in Table IV, however, do not support a proportional relationship between the percentage displacement and serum albumin concentration.

The mechanisms leading to such an increased percentage displacement in the serum of nephrotic patients in the remissive stage are unclear.

13) T. Kawai, "The Plasma Proteins," Igaku Shoin Ltd., Tokyo, 1969, p. 131.