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On the Mechanism of Increased Renal Tubular Reabsorption of Drugs in Alloxan Diabetic Rabbits[1,2]

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A study was made on the mechanisms of increased renal tubular reabsorption of chlorpropamide, sulfadimethoxine and N^4-acethylsulfadimethoxine in diabetic rabbits.

Urinary acidification with NH_4Cl increased the renal tubular reabsorption of the drugs. Reabsorption coefficients of undissociated and dissociated molecules of the drugs were calculated using data of renal clearance of the drugs in alloxan diabetic rabbits and in NH_4Cl-treated rabbits.

Excellent accordance of the reabsorption coefficients as well as apparent reabsorption ratios in diabetic rabbits with those in NH_4Cl-treated rabbits was observed. It was concluded that renal tubular reabsorption of drugs depended exclusively on the urinary pH in the renal tubules.

Parameters of renal tubular reabsorption *in vivo* could be well expressed by the parameters of distribution between $CHCl_3$–CCl_4 (1: 1 by volume) containing soy bean lecithin and buffer solution.

Keywords——alloxan diabetic rabbits; renal tubular reabsorption and urinary pH; NH_4Cl-treated rabbits; chlorpropamide; sulfadimethoxine

Previously,[4] it was reported that the renal clearance of chlorpropamide (CPA), sulfadimethoxine (SDM) and N^4-acethylsulfadimethoxine (N^4-AcSDM) decreased in alloxan diabetic rabbits and that the decreased proximal tubular secretion and the increased tubular reabsorption were responsible for the reduced renal clearance of these drugs. The increased reabsorption was subjected to the acidification of urine due to acidosis in alloxan diabetes.

Presently, to make clear the mechanism, a comparison was made on tubular reabsorption under alloxan diabetic condition and condition which urinary acidification was artificially performed by the administration of ammonium chloride.

Experimental

Materials——CPA was given from Taito Pfizer Pharmaceutical Co., Ltd. SDM on market was used after recrystalization from aqueous ethanol. N^4-AcSDM was synthesized after Uno *et al.*[5] Probenecid was given from Kaken Pharmaceutical Co., Ltd. Commercially available Soy bean lecithin was used (Wako Pure Chemicals Co.). Other reagents were of reagent grade.

Methods——Male rabbits weighing 2.0—3.0 kg were used. Preparation of alloxan diabetes and administration of insulin were made as reported previously.[6] Renal clearance of inulin and drugs, plasma protein binding of drugs, and distribution of drugs into red blood cells were performed as reported previously.[4] Analysis of a drugs in plasma and urine samples were also made with a high pressure liquid chromatography reported in the previous paper.[4]

Acidification of Urine in Normal Rabbits——Acidification of urine was performed by an oral administration of ammonium chloride. Four percent aqueous solution of NH_4Cl was orally administered to normal rabbits once a day at a dose of 7.5 ml/kg for three or seven days before the clearance experiment. The last dose was administered just prior to the clearance experiment. Urinary pH was measured with a pH-meter immedeately after the collection of urine with a urethal catheter.

1) This report forms Part IV of "Pharmacokinetic Behavior od Drugs in Diseased State."
2) This work presented at the Annual Meeting of Pharmaceutical Society of Japan held Tokyo, 1977.
3) Location: *133-1, Yamada-kami, Suita, Osaka 565, Japan.*
4) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 3353 (1978).
5) T. Uno and M. Ueda, *Yakugaku Zasshi*, **80**, 1785 (1960).
6) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2238 (1978).

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Distribution of Drugs between Organic Solvent and Buffer——A mixture of chloroform and carbon tetrachloride (1: 1 by volume) containing soy bean lecithin at a concentration of 100 mg/ml was used as an organic solvent. Sörensen phosphate buffers with the pH range of 5 to 8 were used. Ionic strength of each buffer solution was adjusted to 0.1 by the addition of NaCl. Each drug was dissolved in a buffer solution at three concentrations of 100, 200, and 300 μM. The organic solvent and buffer solution were saturated each other before use.

Five ml of the organic solvent and equivolume of the buffer solution containing a drug were taken into a L-shaped test tube. The tube was shaken on a locking shaker for 120 min at 5 cycles/min in a water bath controlled at 37°±0.1°. One half ml of the aqueous phase was analysed for the drug and apparent distribution ratio was routinely calculated.

Determination of Acidic Dissociation Constants of CPA, SDM and N⁴-AcSDM——Acidic dissociation constants of CPA, SDM and N⁴-AcSDM were spectrophotometrically determined after Robinson,[7] employing Shimadzu Multipurpose Recording Spectrophotometer Model—50 L. Each drug was dissolved in a buffer solution at the concentration of 100 μM. The buffer systems used were follows: CH_3COOH–CH_3COONa for pH 4.0—6.0, Na_2HPO_4–NaH_2PO_4 for pH 6.0—7.5. Ionic strength of the buffer solution was adjusted to 0.1 with NaCl.

Results and Discussion

Renal Tubular Reabsorption in Diabetic and NH_4Cl-treated Rabbits

In the previous paper,[4] it was reported that renal clearance of CPA, SDM and N⁴–AcSDM was markedly decreased under diabetic condition while inulin clearance was not influenced by the diabetic condition. These finding suggest that a blockade of proximal tubular secretion and/or increased tubular reabsorption due to urinary acidification accompanied to diabetic acidosis may be responsible for the results. Urinary pH of alloxan diabetic rabbits

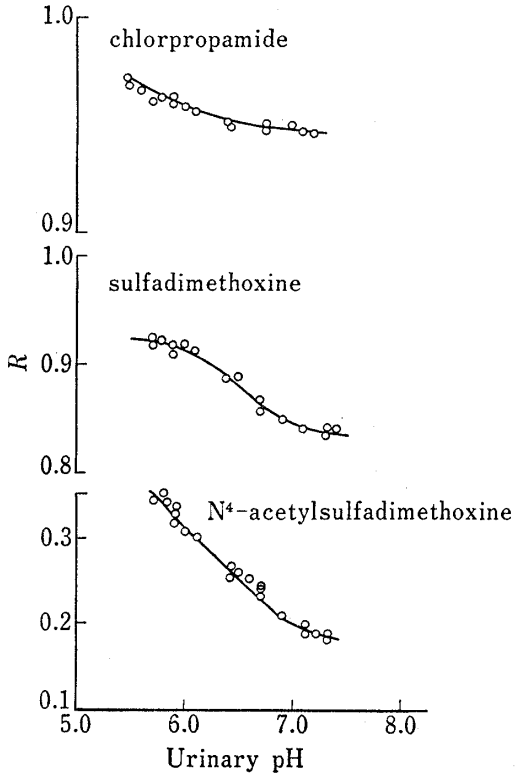

Fig. 1. Relations between Apparent Renal Tubular Reabsorption Ratio (R) and Urinary pH

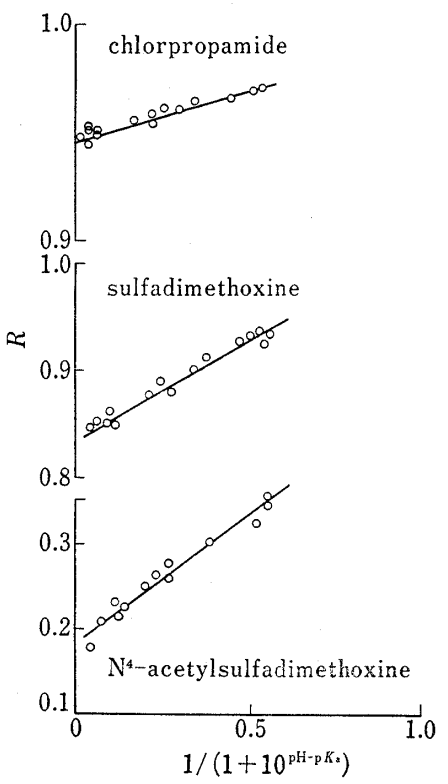

Fig. 2. Plotting Apparent Renal Tubular Reabsorption Ratio (R) against $1/(1+10^{pH-pK_a})$ following Eq. 7

7) E.J. Robinson and B. W. Madsen, *J. Am. Chem. Soc.*, **67**, 1186 (1945).

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varied depending on the degree of the disease and especially on the concentration of acetone bodies in blood. Acidification of urine in diabetic rabbits was enhanced by an increase in the concentration of acetone bodies in blood. The renal clearance of drugs under diabetic condition decreased with an increase in the levels of acetone bodies in blood.

In normal and diabetic rabbits with various degree of the disease, relations between the ratios of renal tubular reabsorption and urinary pH were examined.

Plotting the ratios of renal tubular reabsorption against pH of urine, a sigmoid curve was obtained (Fig. 1).

To make clear the concentration of hydrogen ion concentration in the urine to the renal reabsorption, an analysis was made.

Assuming that both undissociated and dissociated forms of a drug are reabsorbed through renal tubules, apparent reabsorption ratio (R) will be expressed by eq.1.

$$R = R_M + R_I \qquad \text{eq. 1}$$

where R_M and R_I represents apparent reabsorption ratios of undissociated and dissociated forms, respectively.

Using F, which is a fraction of undissociated drug molecules in the renal tubules, R_M will be rewritten by eq. 2

$$R_M = P_M F \qquad \text{eq. 2}$$

where P_M represents the reabsorption coefficient of undissociated molecules.

Similarly, eq, 3 will be derived in terms of dissociated molecules.

$$R_I = P_I(1-F) \qquad \text{eq. 3}$$

where P_I represents the reabsorption coefficient of dissociated molecules.

Substituting eq. 2 and eq. 3 into eq. 1, one can obtain eq. 4.

$$F = \frac{R}{P_M - P_I} - \frac{P_I}{P_M - P_I} \qquad \text{eq. 4}$$

In case of an acidic drug, eq. 5 will be derived from the Henderson-Hasselbalch's equation.

$$\frac{[A^-]}{[HA]} = 10^{pH-pK_a} \qquad \text{eq. 5}$$

F also can be described by eq. 6.

$$F = \frac{[HA]}{[HA]+[A^-]}$$

$$1/F = 1 + [A^-]/[HA] \qquad \text{eq. 6}$$

Substituting eq. 5 into eq. 6 and then replacing F by that in eq. 4, one obtains eq. 7.

$$R = \frac{P_M - P_I}{1+10^{pH-pK_a}} + P_I \qquad \text{eq. 7}$$

Eq. 7 represents that the apparent reabsorption ratio of a drug from renal tubules depends on the urinary pH in the tubules and the dissociation constant of a drug.

Plotting the apparent reabsorption ratio against $1/(1+10^{pH-pK_a})$ employing data in Fig. 1, a straight line was obtained with correlation coefficient of 0.95 (Fig. 2).

From these findings, it will be concluded that the renal tubular reabsorption of a drug depends mainly on the urinary pH. From Fig. 2, P_M and P_I can be calculated from the values of slope and intercept of the line (Table I).

Dissociated species of CPA and SDM proved to be well reabsorbed through renal tubules. Reabsorption of dissociated N^4-AcSDM was less than that of CPA or SDM.

In NH_4Cl-treated rabbits, similar results were obtained (Table I). Values of P_M and P_I of each drug obtained in NH_4Cl-treated rabbits were almost same as those in diabetic rabbits.

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Table I. Values of Renal Tubular Reabsorption Coefficient of Undissociated (P_M) and Dissociated (P_I) Molecules in Alloxan Diabetic Rabbits and NH_4Cl-treated Rabbits

	P_M	P_I
Alloxan diabetic rabbits		
Chlorpropamide	0.991±0.005	0.949±0.003
Sulfadimethoxine	0.915±0.033	0.840±0.031
N^4-Acetylsulfadimethoxine	0.429±0.024	0.197±0.011
NH_4Cl-treated rabbits		
Chlorpropamide	0.996±0.009	0.943±0.007
Sulfadimethoxine	0.899±0.025	0.824±0.018
N^4-Acetylsulfadimethoxine	0.458±0.023	0.196±0.020

Table II. Parameters of Renal Clearance of Inulin and Drugs in NH_4Cl-treated Rabbits

	Normal	$NH_4Cl^{a)}$	$NH_4Cl^{b)}$	Normal$^{c)}$
Chlorpropamide				
Inulin clearance (ml/min)	2.55± 0.06	2.51± 0.05	2.51± 0.07	2.59± 0.05
GFR·P_f	100			
S	258.4 ±35.2	276.1 ±38.3	270.5 ±45.2	297.0 ±40.8
A	333.6 ±33.6	353.8 ±35.9	355.0 ±43.8	371.4 ±40.4
UV	22.8 ± 2.9	22.4 ± 2.3	15.4 ± 1.9	25.6 ± 1.0
Reabsorption ratio (%)	93.1	94.1	96.0	93.6
Sulfadimethoxine				
Inulin clearance (ml/min)	2.67± 0.06	2.72± 0.06	2.70± 0.07	2.69± 0.04
GFR·P_f	100			
S	—	—	—	—
A	82.8 ± 1.0	87.8 ± 0.4	87.9 ± 0.4	82.6 ± 0.4
UV	17.2 ± 1.0	12.2 ± 0.4	12.1 ± 0.4	17.4 ± 0.4
Reabsorption ratio (%)	82.8	87.8	87.9	82.6
N^4-Acetylsulfadimethoxine				
Inulin clearance (ml/min)	2.67± 0.06	2.72± 0.06	2.70± 0.07	2.69± 0.04
GFR·P_f	100			
S	244.4 ±18.6	277.4 ±21.5	255.3 ±41.2	245.7 ±21.2
A	83.1 ± 4.4	153.0 ±14.5	135.1 ±15.8	79.2 ± 2.8
UV	261.3 ±14.2	221.5 ± 6.7	216.5 ±19.2	256.9 ±12.9
Reabsorption ratio (%)	24.1	40.5	38.0	22.9

Relative amounts of proximal tubular secretion (S), tubular reabsorption (A) and urinary excretion (UV) of drugs were calculated according previous paper[4] on the basis of GFR·P_f value which was not influenced by NH_4Cl-treated condition.
a) NH_4Cl-treated rabbits for 3 days.
b) NH_4Cl-treated rabbits for a week.
c) 2 weeks after withdrawal of NH_4Cl-treatment.

Thus, the increased reabsorption in diabetic rabbits should be predominantly subjected to the acidification of urine.

Parameters of renal clearance of inulin and drugs in NH_4Cl-treated rabbits are presented in Table II.

Inulin clearance and proximal tubular secretion are not influenced by the administration of NH_4Cl.

Thus, it may be concluded that the urinary acidification itself is not responsible for the reduced proximal tubular secretion in diabetic rabbits unlike for the increased tubular reabsorption.

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Pharmacokinetic Parameters of Drugs in NH_4Cl-treated Rabbits

Plasma concentration-time courses of CPA and SDM following intravenous administration of the drugs to NH_4Cl-treated rabbits are represented in Fig. 3. Higher concentrations of CPA and SDM in comparison with concentrations in untreated normal rabbits were observed at the β phase. These findings are similar to the results obtained in diabetic rabbits as reported previously. Pharmacokinetic parameters calculated with two compartment model are summarized in Table III.

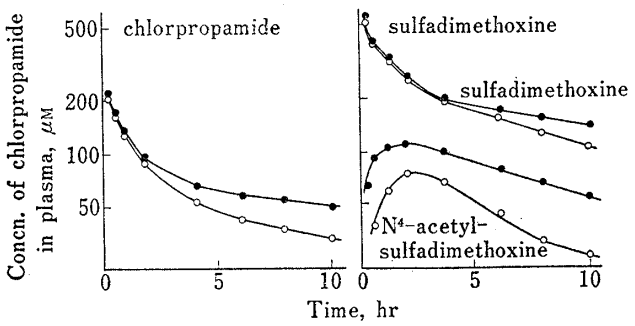

Fig. 3. Time Coarse of the Plasma Concentration of Drugs after Intravenous Administration in NH_4Cl-treated Rabbits

normal: —○—, NH_4Cl-treatment for a week: —●—.

Decreases in elimination rate constant of CPA and SDM are observed. The decrease in elimination of SDM from plasma will be subjected to a decrease in excretion rate because the rate constant of metabolism is not so much influenced by the administration of NH_4Cl. Volume of distribution of the tissue compartment was slightly increased in both drugs, but the magnitude was not so much as the increase in diabetic rabbits reported in the previous paper. Volume of distribution of the central compartment of both drugs was not influenced by the administration of NH_4Cl like that in diabetic rabbits.

Table III. Pharmacokinetic Parameters of Chlorpropamide and Sulfadimethoxine following Intravenous Administration in NH_4Cl treated Rabbits

	Weight (kg)	k_{el} ($\times 10^3$ min^{-1})	k_{12}	k_{21}	V_c (l)	V_t	k_{ex} ($\times 10^3$ min^{-1})	k_m	$k_{ex(M)}$	f	Urine-pH
Chlorpropamide (rabbit No. 251)											
Normal	2.6	3.77	5.86	4.60	0.58	0.74					8.68
NH_4Cl[a]	2.6	2.92	8.03	5.27	0.58	0.89					7.14
NH_4Cl[b]	2.5	1.64	9.03	5.42	0.55	0.84					6.67
Normal[c]	2.6	3.66	6.43	4.81	0.56	0.75					8.28
Sulfadimethoxine (rabbit No. 261)											
Normal	2.8	3.54	18.53	27.73	0.81	0.54	2.20	1.34	6.13	0.21	8.53
NH_4Cl[a]	2.8	3.13	12.63	14.68	0.88	0.75	1.73	1.40	4.28	0.23	6.89
NH_4Cl[b]	2.8	2.37	12.04	12.53	0.91	0.87	1.12	1.25	2.95	0.19	6.42
Normal[c]	2.8	3.59	18.73	27.40	0.80	0.55	2.33	1.26	5.98	0.23	8.40

k_{el} ($=k_{ex}+k_m$): elimination rate constant, k_{ex} and $k_{ex(M)}$: renal excretion rate constant of unchanged drug and metabolite respectively, k_m: metabolic rate constant, f: fraction of metabolite in the tissue compartment.
a) NH_4Cl-treated rabbits for 3 days.
b) NH_4Cl-treated rabbits for a week,.
c) 2 weeks after withdrawal of NH_4Cl-treatment.

Plasma protein binding and distribution into red blood cells were not influenced by the NH_4Cl-treatment.

From these findings, it is clarified that the retardation of plasma clearance of CPA, SDM and N^4–AcSDM may be partly subjected to the increased tubular reabsorption and that the degree of the reabsorption may exclusively depends on urinary pH in the proximal renal tubules.

Distribution of Drugs between Buffer Solution and Organic Solvent

It is generally recognized that the dissociated species of a drug molecule does not distribute to the organic solvent *in vitro* but considerable amounts of the dissociated species are

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absorbed through small intestine *in vivo*. To make up the discrepancy between the results *in vitro* and *vivo*, distribution of a drug into the organic phase involving lecithins have been studied.[8]

In the present study, $CHCl_3$–CCl_4 mixture (1:1 by volume) containing soy bean lecithin (100 mg/ml) was used as an organic phase. Distribution equilibrium of CPA, SDM and N^4–AcSDM between buffer solution and the organic mixture was established after about 60 min. Therefore, a distribution ratio was measured after 100 min.

Distribution of the drugs into organic phase increased with an increase in the concentration of lecithin. However, an emulsion was formed by shaking the organic mixture with buffer solution when the concentration of soy bean lecithin exceeded 150 mg/ml. So, the concentration of 100 mg/ml was adopted.

Distribution ratio of the drugs between the buffer solution and the organic phase with or without soy bean lecithin was plotted against pH of the buffer solution and a comparison was made on the pH-profile of renal reabsorption (Fig. 4). Excellent accordance of pH-profile of tubular reabsorption with that of the distribution ratio between the buffer solution and the organic phase containing lecithin was observed.

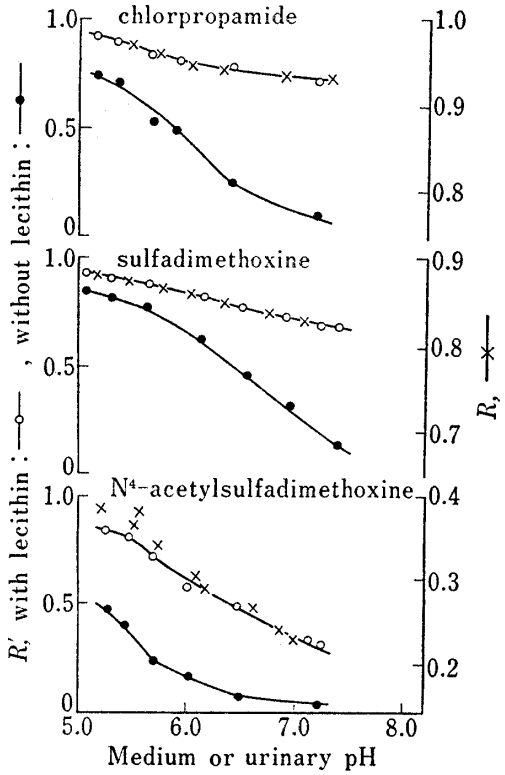

Fig. 4. Comparison of pH Profile of Apparent Renal Tubular Reabsorption and that of Distribution Ratio between the Buffer Solution and Organic Solvent with or without Soy Bean Lecithin

Organic solvent: $CHCl_3$–CCl_4 (1:1 by volume) containing soy bean lecithin at the concentration of 100 mg/ml.
Buffer solution: Sörensen phosphate buffer with the pH range of 5 to 8 (ionic strength of each buffer solution was adjusted to 0.1 by the addition of NaCl).

Supposing that both undissociated and dissociated molecules are distributed into the organic phase containing lecithin, the apparent distribution ratio R' will be described with the same eq. 7 using penetration coefficients of undissociated and dissociated molecules, P'_M and P'_I respectively. From the data in Fig. 4, P'_M and P'_I were calculated and summarized in Table IV.

Similar values of P'_M and P'_I in Table V to those of P_M and P_I in Table I and II were obtained.

These findings suggest that the physicochemical characteristics of renal tubular wall may be represented by those of the organic solvent system containing soy bean lecithin used in the present study.

Table IV. Penetration Coefficient of Drugs into Organic Phase containing Soy Bean Lecithin

	P'_M	P'_I
Chlorpropamide	1.000±0.008	0.768±0.006
Sulfadimethoxine	0.968±0.008	0.698±0.002
N^4-Acetyl-sulfadimethoxine	0.968±0.004	0.308±0.002

Organic solvent: $CHCl_3$–CCl_4 (1:1 by volume) containing soy bean lecithin at the concentration of 100 mg/ml.
Buffer solution: Sörensen phosphate buffer with the pH range of 5 to 8 (ionic strength of each buffer solution was adjusted to 0.1 by the addition of NaCl).

8) S. Furusawa, K. Okumura, and H. Sezaki, *J. Pharm. Pharmac.*, **24**, 272 (1972).

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