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Pharmacokinetic Behavior of Chlorpropamide and Sulfadimethoxine in Alloxan Diabetic Rabbits[1,2]

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Pharmacokinetic behavior of chlorpropamide (CPA) and sulfadimethoxine (SDM) following intravenous administration were studied in rabbits under normal and alloxan diabetic condition.

Plasma concentration of both drugs in the β phase in diabetic rabbits sustained a higher level than those under normal condition. The abnormal pattern of the concentration-time curve of SDM recovered to the normal pattern by the 5-day insulin treatment, but CPA was not the case. Renal clearances of CPA, SDM and N^4-acetylsulfadimethoxine (N^4-AcSDM) were found to be decreased under diabetic condition. The decreased renal clearances of CPA and N^4-AcSDM were subjected to the blockade of proximal tubular secretion of both drugs and to the increased renal tubular reabsorption due to urinary acidification under diabetic condition. The decreased renal clearance of SDM was subjected to the increased renal tubular reabsorption alone. Plasma protein binding and distribution into red blood cells of the drugs were also studied under normal and diabetic conditions.

Keywords——pharmacokinetic behavior under diseased state; chlorpropamide; sulfadimethoxine; blockade of proximal tubular secretion under diabetic condition; increased tubular reabsorption under diabetic condition; alloxan diabetes

In the previous paper,[4] pharmacokinetic behavior of sulfamethoxazole was studied in rabbits under normal and diabetic conditions.

Presently, similar study was made on chlorpropamide and sulfadimethoxine. Chlorpropamide (CPA), an oral antidiabetic drug, is reported as a drug which requires a careful supervision for its administration. An elucidation of pharmacokinetic behavior of CPA under diabetic condition will be instructive for its safety use. Sulfadimethoxine, one of long acting sulfonamides, is considered to be suited as a convenient model drug for the study of renal clearance following single intravenous administration.

Experimental

Materials——Sulfadimethoxine (SDM) on market was used after recrystallization from aqueous ethanol. N^4-Acetylsulfadimethoxine (N^4-AcSDM) was synthesized after Uno.[5]

CPA and probenecid were obtained through the courtesy of Taito Pfizer Co. and Kaken Pharm. Co., respectively.

Insulin zinc suspension J.P. IX on the market was used for insulin treatment.

Animals——Two male white rabbits weighing 2.0—3.0 kg were used for each drug.

Methods——Alloxan diabetic condition was developed as reported previously.[4] In order to confirm the diabetic condition after the administration of alloxan, concentrations of glucose and acetone bodies in blood, and concentrations of protein and albumin in serum were occasionally determined with the method described in the previous paper.[4]

1) This work was presented at the 8th symposium on drug metabolism and action sponsored by the Pharmaceutical Society of Japan, Hiroshima, November 1976.
2) This report forms Part III of "Pharmacokinetic Behavior of Drugs under Diseased States."
3) Location: *133–1, Yamada-kami, Suita, Osaka, 565, Japan.*
4) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2058 (1978).
5) T. Uno and M. Ueda, *Yakugaku Zasshi*, **80**, 1785 (1960).

Five tenths molar aqueous solution of SDM was intravenously administered into a ear vein of rabbits under normal or diabetic condition at a dose of 200 μmol/kg. One tenth molar aqueous solution of CPA was similarly administered at a dose of 50 μmol/kg.

After the administration of a drug, about one ml samples of blood were chronologically taken with a heparinized syringe from the opposite ear vein. Blood samples were immediately centrifuged for 10 min at 3000 rpm and the supernatant plasma layers were kept frozen until analyzed. After collecting the final blood sample, rabbits were separately kept in metabolic cages and urine was collected for 72 hr or 96 hr into reservoirs cooled with ice. Urine samples were also kept frozen until analyzed.

General schedules of experiments under normal and diabetic conditions were similar to those described in the previous paper.[4]

Analytical Procedures of SDM, N^4-AcSDM and CPA——SDM and N^4-AcSDM: Plasma (0.2 ml) or urine (0.5 ml) sample was mixed with one ml of 0.1 M acetate buffer (pH 4.5) in a glass-stoppered centrifuge tube (10 ml), followed by an extraction with 5.0 ml of ethyl acetate for 5 min at 90 strokes/min using a KM-shaker (Iwaki Co. Ltd.). After centrifugation for 5 min at 3000 rpm, four ml of ethyl acetate layer were pipetted into a 15 ml conical-shaped centrifuge tube. The organic layer was evaporated to dryness with a Vapor-Mix (Tokyo Rikakikai Co. Ltd.) under reduced pressure at room temperature. The residue was dissolved in 0.2 ml (for plasma) or 0.5 ml (for urine) of dimethylsulfoxide (DMSO) containing 3-chloro-4-hydroxybenzoic acid (60 μg/ml) as an internal standard. Ten μl of the solution were injected onto the column of a high pressure liquid chromatograph (HPLC). Analytical conditions of the HPLC are summarized in Table I. The analytical conditions were also well applicable for the separation of probenecid which was used in the inhibitory experiments of renal clearance of SDM and N^4-AcSDM.

CPA: Extraction of CPA from plasma or urine samples with ethyl acetate was similarly performed with change in pH of the buffer. Acetate buffer (0.1 M) of pH 3 was used for extraction of CPA. Four ml of organic layer were similarly evaporated to dryness. The residue was dissolved in 0.2 ml (for plasma) or 0.5 ml (for urine) of ethanol containing tolbutamide (60 μg/ml) as an internal standerd. Ten μl of the solution were injected onto the column of HPLC. Analytical conditions of HPLC were summarized in Table II. For the separation of probenecid, 0.1 M acetate buffer of pH 3 was used as a mobile phase with a flow rate of 1.0 ml/min.

Table I. Analytical Condition of High Pressure Liquid Chromatography for the Determination of Chlorpropamide (CPA)

	Condition I		Condition II	
Instrument	Shimadzu LC-841			
Column	500 mm × 3.15 mm			
Column packing	Permaphase ODS			
Mobile phase	0.1 M-Acetate buffer (pH 4.5)		0.1 M-Acetate buffer (pH 3.0)	
Flow rate	0.75 ml/min		1.0 ml/min	
Detector	UV 240 nm			
Retention time	CPA	5.4 min	CPA	2.5 min
	Tolbutamide	9.4 min	Probenecide	7.5 min
			Tolbutamide	11.5 min

Table II. Analytical Condition of High Pressure Liquid Chromatography for the determination of Sulfadimethoxine (SDM) and N^4-Acetylsulfadimethoxine (N^4-AcSDM)

Instrument	Shimadzu LC-841	
Column	500 mm × 3.15 mm	
Column packing	Zipax SAX	
Mobile phase	0.05 M-Acetate buffer (pH 4.6)	
Flow rate	2.2 ml/min	
Detector	UV 267 nm	
Retention time	SDM	3.6 min
	N^4-AcSDM	7.0 min
	3-Chloro-4-hydroxybenzoic acid	16.4 min
	Probenecid	11.2 min

Renal Clearance of Inulin——Renal clearance of inulin was determined as follows following Yamamoto[6] with some modifications: Twenty per cent aqueous solution of inulin was subcutanously administered at the retrorenal area at a dose of 1 ml/kg. The serum concentration of inulin reached a constant level 3 hr after the administration and sustained for some hr.

Clearance experiment started at the time when serum concentration of inulin reached a constant level. Urine samples were collected every 10 min for half an hour since the beginning of the experiment. One ml of blood was taken at the midpoint of every collection of urine sample. After half an hour, 0.1 M solution of probenecid was administered at a dose of 20 μmol/kg. Twenty min after the administration of probenecid, first samples of blood and urine were collected. Further collection of blood and urine samples were made for another 30 min. Clearance of inulin was calculated from the plasma level of inulin and the amount of inulin in the urine excreted in 1 min. Inulin in plasma and urine samples was determined after Lunt[7] with slight modifications. A half ml of plasma was mixed with one ml of 0.1% resorcinol in 95% ethanol and 2.5 ml of 30% HCl in a glass-stoppered test tube. The test tube was kept in a water bath controlled at $80° \pm 0.5°$ for 25 min. After cooling with running water for 3 min, color of the solution was spectrophotometrically determined at 490 nm. Plasma or urine sample collected before the administration of inulin was similarly treated and used as the respective reference.

Renal Clearance of CPA, SDM and N^4-AcSDM——CPA, SDM and N^4-AcSDM was intravenously administered into a ear vein of unanethetized rabbit at a dose of 100 μmol/kg, 400 μmol/kg and 200 μmol/kg, respectively. Concentrations of CPA, SDM and N^4-AcSDM in plasma or urine were determined with the HPLC method described already. Clearance experiments start after establishment of a distribution equilibrium. At the post distribution phase (β phase, 4 or 6 hr after the administration of a drug), the concentration of these drugs in plasma remained relatively constant for a while enough to study the clearance experiments. At the β phase, urine and plasma samples were collected at pre- and post-administration of probenecid which was used as an inhibitor of renal tubular secretion of drugs. Collections of plasma and urine samples were made similarly in case of the inulin clearance. Since probenecid is an acidic drug, use of this drug will frequently induce aciduria, resulting in changes of excretion pattern of drugs. Although urinary pH will be undoubtedly decreased by excreted CPA, SDM or N^4-AcSDM, further acidification by probenecid should be avoided. The intravenous doses of probenecid without further acidification of urine by itself and sufficient to suppress the renal tubular secretion were found to be 15 μmol/kg for CPA and 20 μmol/kg for SDM and N^4-AcSDM.

Plasma Protein Binding of Drugs——Degree of plasma protein binding of CPA, SDM and N^4-AcSDM was determined with a sephadex gel equilibrium method described previously.[4]

Distribution of a Drug into Red Blood Cells——CPA or SDM was intravenously administered at a dose of 50 μmol/kg or 200 μmol/kg, respectively. Distributions of CPA and SDM into red blood cells were determined at the range of plasma concentration of 100—250 μM and 100—500 μM, respectively. Distribution of N^4-AcSDM into red blood cells was determined at the plasma concentration of 50—150 μM following intravenous administration of SDM. One ml samples of blood were chronologically collected from the marginal ear vein after intravenous administration of a drug. The concentrations of a drug in plasma (P) and whole blood (B) were determined as described in the analytical procedures. Hematocrit (H) was routinely determined. An apparent distribution ratio of a drug was calculated as follows:

$$\text{Apparent distribution ratio } (\%) = \frac{[B-P(1-H)]}{B} \times 100$$

Administration of Insulin to Rabbits under Diabetic Condition——An insulin zinc suspension J.P. was used for insulin treatments. Administration of insulin to alloxan diabetic rabbits was performed by two dosage schedules: One method was to administer the insulin zinc suspension to femoralis muscle once a day for 5 days at a dose of 1 unit/body and the other was to administer the same dose of insulin on alternate days for four weeks.

Results and Discussion

Plasma Concentration and Urinary Excretion of CPA

A typical plasma concentration profile of CPA following an intravenous administration to a rabbit is presented in Fig. 1. CPA levels in plasma declined biexponentially. In diabetic condition, plasma concentrations of CPA at the β phase held on higher values than those in normal condition. Those high plasma levels under diabetic condition were found to be enhanced by the 5 day insulin treatment, although physiological parameters such as

6) K. Yamamoto, H. Yamazoe, and J. Ueda, *Osaka City Med. J.*, **8**, 171 (1962).
7) E. Lunt and D. Sutcliff, *Biochem. J.*, **55**, 122 (1953).

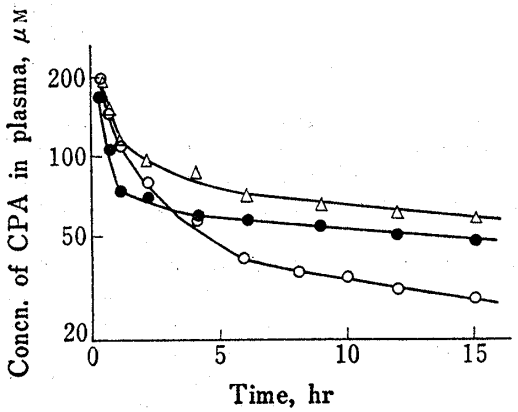

Fig. 1. Plasma Concentration Profiles of Chlorpropamide (CPA) after Intravenous Administration of Chlorpropamide to Rabbit No. 117 under Normal, Alloxan Diabetic Conditions and Insulin Treatment

—○— normal condition, —●— alloxan diabetic condition, —△— insulin treatment.

blood level of glucose and acetone bodies *etc.* showed a recovering trend to the normal values by the treatment.

The reasons of the enhanced higher levels by the 5-day insulin treatment are left unexplained. CPA was excreted in the urine more than 95% of the dose during 96 hr and any metabolite was not found. Pharmacokinetic parameters of CPA were determined with a two compartment model (Table III and IV).

Under the diabetic condition, rate constant, k_{el}, of elimination from plasma was found to be smaller than that under the normal condition. As CPA are almost completely excreted as unchanged form, the elimination rate constant can be regarded as the renal excretion rate constant, k_{ex}. The decrease in k_{ex} is mainly subjected to retardation of urinary excretion due to a possible impariment

Table III. Pharmacokinetic Parameters of Chlorpropamide in Rabbit No. 117 under Normal and Alloxan Diabetic Conditions

Experiment weight (weeks, kg)		k_{el}	k_{12} ($\times 10^3$ min^{-1})	k_{21}	V_c (L)	V_t	Glucose in blood (mg/100 ml)
0	2.2	2.53	10.04	5.89	0.61	1.03	76.85
1	2.2	2.57	9.20	5.95	0.68	1.06	76.42
3	2.3	0.96	14.07	5.83	0.76	1.85	312.61
4	2.2	0.89	15.62	6.19	0.69	1.55	342.19
5	2.2	1.03	15.22	6.79	0.69	1.55	326.31
6-Id	2.2	0.83	5.84	13.48	0.63	0.27	111.22
7	2.2	0.83	13.36	11.79	0.76	0.87	279.78
8-Id	2.2	0.88	4.03	8.32	0.69	0.34	92.68
10	2.1	0.65	10.65	7.52	0.66	0.94	330.58
14-Iw	2.2	1.74	8.42	10.14	0.66	0.52	90.23
17	2.0	0.61	9.86	6.94	0.64	0.91	329.28

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

Table IV. Pharmacokinetic Parameters of Chlorpropamide in Rabbit No. 118 under Normal and Alloxan Diabetic Conditions

Experiment weight (weeks, kg)		k_{el}	k_{12} ($\times 10^3$ min^{-1})	k_{21}	V_c (L)	V_t	Glucose in blood (mg/100 ml)
0	2.5	2.23	8.07	4.55	0.86	1.52	83.41
1	2.5	2.13	6.96	3.98	0.83	1.46	80.94
3	2.5	0.93	12.66	6.37	0.83	1.66	398.75
4	2.4	0.86	27.57	5.44	0.66	3.35	376.58
5-Id	2.4	0.89	8.35	6.53	0.72	0.98	142.18
6	2.2	0.93	38.81	16.27	0.67	1.61	426.13
7-Id	2.3	0.73	6.03	5.33	0.79	0.90	96.28
8	2.3	0.79	25.32	5.34	0.83	3.96	419.36
12-Iw	2.3	1.55	5.23	7.21	0.72	0.52	77.49
15	2.1	0.69	22.73	5.48	0.63	2.62	460.07

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

of renal function. Under the diabetic condition, the distribution volume of the tissue compartment was noted to increase significantly but that of the central compartment did not change. This finding suggests an increase in the distribution of CPA to tissues. Elimination rate constant of CPA under the diabetic condition was not influenced by the 5-day insulin treatment. But the 4-week insulin treatment resulted in a remarkable recovery to the levels at normal condition.

Plasma Concentration and Urinary Excretion of SDM and N^4-AcSDM

Plasma concentration profiles of SDM and N^4-AcSDM following intravenous administration of SDM to the rabbit No. 127 are presented in Fig. 2 as an example. Plasma levels of SDM decreased biexponentilly. Under the diabetic condition, plasma levels at the β phase remained higher levels than those under the normal conditions. The levels of N^4-AcSDM in plasma also showed higher levels than those under the normal condition. The higher levels of SDM and N^4-AcSDM under the diabetic condition showed a recovering trend to the normal levels by the 5-day insulin treatment. SDM was excreted in the urine as unchanged SDM and its metabolite, N^4-AcSDM. Total amount of SDM excreted in the urine within 72 hr after administration was more than 95% of the dose under normal and diabetic conditions. Like the case of CPA, pharmacokinetic parameters of SDM and N^4-AcSDM were determined with the two compartment model described in the previous paper[4] (Table V and VI).

Under the diabetic condition, elimination rate constant of SDM, k_{el}, was markedly decreased. The decrease in k_{el} will be subjected to the decrease in the rate constants of urinary excretion, k_{ex}, and/or of metabolism, k_m.

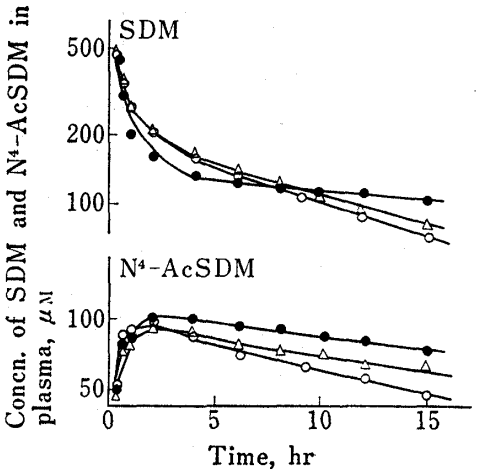

Fig. 2. Plasma Concentration Profiles of Sulfadimethoxine (SDM) and N^4-Acetylsulfadimethoxine (N^4-AcSDM) after Intravenous Administration of Sulfadimethoxine to Rabbit No. 125 under Normal, Alloxan Diabetic Conditions and Insulin Treatment

—○— normal condition, —●— alloxan diabetic condition, —△— insulin treatment.

TABLE V. Pharmacokinetic Parameters of Sulfadimethoxine in Rabbit No. 125 under Normal and Alloxan Diabetic Conditions

Experiment	weight (weeks, kg)	k_{el}	k_{12}	k_{21} ($\times 10^3$ min^{-1})	V_c	V_t (L)	k_m	k_{ex}	$k_{ex(M)}$ ($\times 10^3$ min^{-1})	f	Glucose in blood (mg/100 ml)
0	2.5	2.36	8.18	10.07	1.06	0.86	1.84	0.50	4.92	0.30	74.28
1	2.5	2.38	8.02	9.41	1.05	0.89	1.84	0.53	5.03	0.30	78.95
3	2.3	2.27	33.96	23.01	0.83	1.20	1.91	0.36	3.19	0.21	274.23
4	2.3	1.69	17.86	6.37	0.99	2.77	1.44	0.26	1.78	0.21	310.15
5	2.3	0.77	13.93	5.94	1.04	2.44	0.63	0.15	1.53	0.24	290.42
6-Id	2.3	2.16	14.88	9.34	0.92	1.45	1.61	0.56	2.09	0.24	92.68
7	2.3	1.24	17.68	8.06	0.92	2.03	0.96	0.28	2.21	0.22	318.51
8-Id	2.3	1.60	17.21	11.29	0.91	1.39	1.22	0.38	2.38	0.23	118.67
9	2.2	0.83	20.91	8.15	0.87	2.22	0.65	0.17	2.42	0.24	290.34
13-Iw	2.4	2.18	9.20	11.24	1.01	0.83	1.66	0.52	4.03	0.28	85.74
16	2.1	1.19	14.68	8.16	0.83	1.49	0.88	0.31	2.04	0.26	369.17

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

TABLE VI. Pharmacokinetic Parameters of Sulfadimethoxine in Rabbit No. 127 under Normal and Alloxan Diabetic Conditions

Experiment weight (weeks, kg)		k_{el}	k_{12}	k_{21} ($\times 10^3$ min^{-1})	V_c	V_t (L)	k_m	k_{ex}	$k_{ex(M)}$ ($\times 10^3$ min^{-1})	f	Glucose in blood (mg/100 ml)
0	2.4	2.58	7.53	9.18	1.06	0.86	2.08	0.51	4.42	0.26	85.46
1	2.4	2.42	6.89	8.34	1.08	0.89	1.97	0.46	4.53	0.27	91.27
3	2.4	3.17	27.90	18.25	0.93	1.43	2.80	0.37	3.73	0.27	198.53
4	2.5	1.24	16.63	7.04	1.04	2.46	1.05	0.19	1.98	0.24	220.42
5	2.5	0.90	13.63	6.36	1.19	2.55	0.76	0.15	1.47	0.27	256.65
6-Id	2.5	1.88	8.27	8.74	1.08	1.03	1.39	0.49	2.26	0.25	114.28
7	2.5	1.11	13.72	7.99	1.01	2.36	0.85	0.26	1.68	0.24	278.37
8-Id	2.6	2.11	10.51	8.89	1.05	1.24	1.63	0.48	1.87	0.23	102.65
9	2.5	1.12	13.02	7.45	1.05	1.84	0.86	0.26	1.73	0.23	274.35
13-Iw	2.6	2.45	13.79	19.65	1.08	0.76	1.96	0.49	3.74	0.26	80.75
16	2.4	1.34	13.60	12.43	0.93	1.01	1.13	0.21	2.03	0.26	308.68

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

Urinary excretion rate constant of N^4-AcSDM, $k_{ex(M)}$, also decreased under the diabetic condition. Recovering trend of k_m and k_{ex} to the normal values was observed following the insulin treatment for 5 days. However, the decreased $k_{ex(M)}$ under the diabetic condition was not influenced by the 5-day insulin treatment, while a remarkable recovery to the normal value was observed by the 4-week insulin treatment.

The difference of the effect of insulin treatments on k_{ex} and $k_{ex(M)}$ suggest the difference of the mechanisms in the renal excretion of SDM and N^4-AcSDM.

Volume of distribution of the tissue compartment increased under the diabetic condition. The increase was normalized by insulin treatments. Fraction of N^4-AcSDM in the tissue compartment, f, was not influenced by diabetes like N^4-AcSMX in the previous paper.[4]

Plasma Protein Binding of CPA, SDM and N^4-AcSDM

Profiles of plasma protein binding of CPA in rabbits No. 117 and 118 are shown in Fig. 3(a). Plasma protein binding of CPA increased under the diabetic condition. The enhanced binding may be explained by the fact that the plasma concentrations of total protein and albumin increased under the diabetic condition as reported previously.[8]

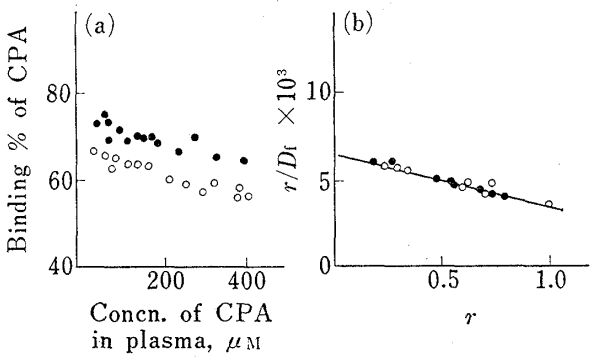

Fig. 3. Plasma Protein Binding of CPA in Rabbits under Normal and Diabetic Conditions

(a) binding % *vs.* plasma concentration.
(b) Scatchard's plot.
○ Normal condition, ● diabetic condition.

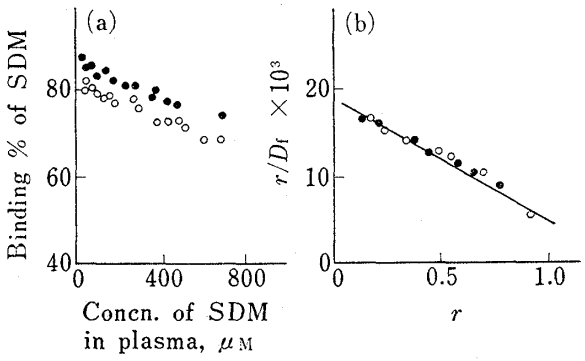

Fig. 4. Plasma Protein Binding of SDM in Rabbits under Normal and Diabetic Conditions

(a) binding % *vs.* plasma concentration.
(b) Scatchard's plot.
○ normal condition, ● diabetic condition.

8) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2238 (1978).

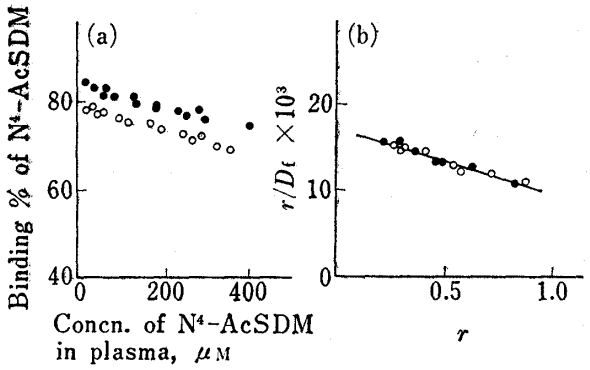

Fig. 5. Plasma Protein Binding of N^4-AcSDM in Rabbits under Normal and Diabetic Conditions

(a) binding % *vs.* plasma concentration.
(b) Scatchard's plot.
○ normal condition, ● diabetic condition.

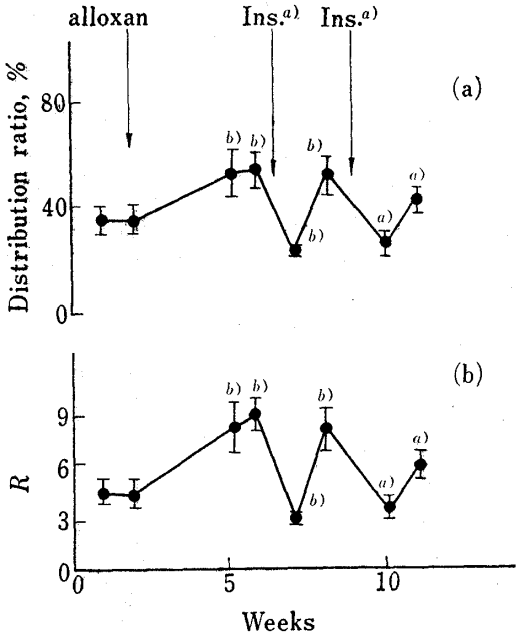

Fig. 6. Distribution of CPA into Red Blood Cells

(a) changes of apparent distribution ratio of CPA.
(b) changes of R in eq. 1 (see text).
Ins.a) means the administration of insulin zinc suspension for 4 days.
a) and *b*); Statistically significant in *t*-test at the level 0.005 ($p<0.005$) and 0.001 ($p<0.001$), respectively.
Six rabbits were used in this experiment. Each plot represents mean±S.D. at various concentrations in the β phase of drug after intravenous administration ($n=45$).

The enhanced binding was remarkably reduced to the normal value by insulin treatment for 4 weeks. Scatchard's plots of these results under three conditions resulted in a single line (Fig. 3(b)) which were obtained on a basis of the experimental fact that CPA was mainly bound to albumin molecules.

Similar results were obtained for SDM and N^4-AcSDM (Fig. 4(a), (b), and 5 (a), (b))

From these findings it may be concluded that the enhanced binding under diabetic conditions may be subjected to an increase in the plasma concentration of albumin, and that structural change in albumin molecules under the diabetic condition may not be a factor of the enhanced binding.

The present results of increased plasma protein binding are considered to be one of factors responsible for the decrease in the elimination of CPA and SDM from plasma.

Distribution of CPA, SDM and N^4-AcSDM into Red Blood Cells

Distribution of CPA into red blood cells is shown in Fig. 6 (a). Under the normal condition, 37—38% of CPA was distributed into red blood cells. Under the diabetic condition, the distribution was markedly increased. The increase in the distribution was reduced to the values less than those under normal condition by the insulin treatment.

Assuming that only unbound CPA in plasma is effective for the distribution into red blood cells, ratio of apparent concentration of CPA in red blood cells (B_{app}) to the plasma concentration of unbound CPA (P_f) can be described by eq. 1.

$$R = \frac{B_{app}}{P_f} = \frac{[B-P(1-H)]}{H}\ \frac{1}{P_f} \qquad \text{eq. 1}$$

where B, and H are the same defined already. Changes in R under three conditions are shown in Fig. 6(b).

Under the normal condition, the ratio was around three, but under the diabetic condition, it was observed to increase to about 2 folds of the normal value. The ratio was markedly decreased to the value less than that of normal condition by the insulin treatment. This finding suggests that the increase in the tissue distribution volume is supposed to be predominantly subjected to an increase in the distribution into red blood cells which possess the major part of the tissue compartment in the present pharmacokinetic analysis. A remarkable

decrease in the distribution of CPA into red blood cells and tissues by insulin treatment also suggest that when CPA is administered to diabetic patients, CPA distributed into red blood cell and other tissue will be easily liberated into plasma and cause a marked increase in its plasma concentration by the administration of other drugs, resulting in severe hypoglycemia. The marked differences of distribution of CPA into red blood cells and other tissues between under normal and diabetic conditions may be one of the reasons of the difficulties in the therapeutic use of CPA for diabetic patients.

Distributions of free SDM and N^4–AcSDM in plasma into red blood cells are shown in Fig. 7 and 8.

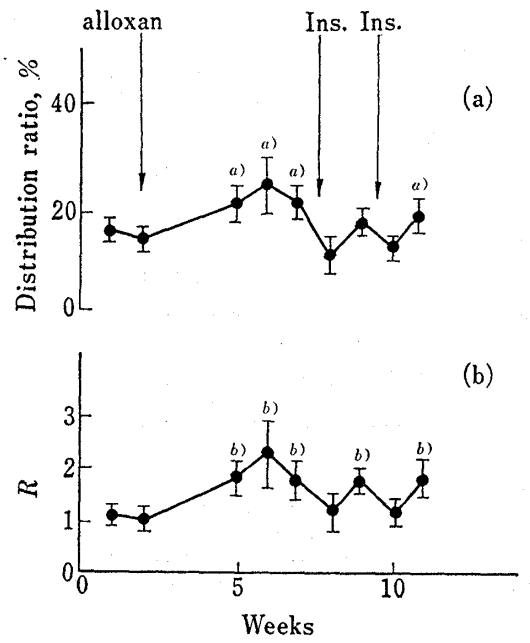

Fig. 7. Distribution of SDM into Red Blood Cells

(a) changes of apparent distribution ratio of SDM.
(b) changes of R in eq. 1 (see text).
Ins.$^{a)}$ means the administration of insulin zinc suspension for 4 days.
a) and *b*); Statistically significant in t-test at the level 0.005 ($p<0.005$) and 0.001 ($p<0.001$), respectively.
Six rabbits were used in this experiment. Each plot represents mean±S.D. at various concentrations in the β phase of drug after intravenous administration ($n=45$).

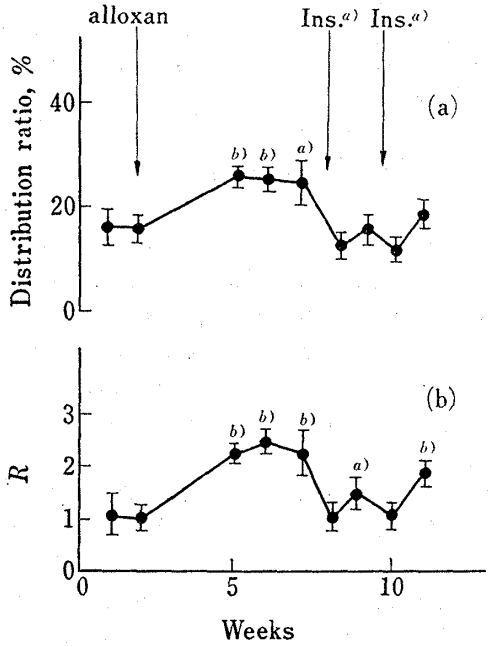

Fig. 8. Distribution of N^4-AcSDM into Red Blood Cells

(a) changes of apparent distribution ratio of N^4-AcSDM.
(b) changes of R in eq. 1 (see text).
Ins.$^{a)}$ means the administration of insulin zinc suspension for 4 days.
a) and *b*); Statistically significant in t-test at the level 0.005 ($p<0.005$) and 0.001 ($p<0.001$), respectively.
Six rabbits were used in this experiment. Each plot represents mean±S.D. at various concentrations in the β phase of drug after intravenous administration ($n=45$).

Unlike that of CPA, distribution ratios of both drugs under the diabetic condition recovered to the normal value by insulin treatment.

Plasma Clearance of CPA and SDM

As described already, the elimination of CPA, SDM and N^4–AcSDM from plasma was retarded under the diabetic condition. As one of reasons of the retardation, enhanced plasma binding of these drugs should be considered. Levy *et al.*[9] discussed the plasma clearance of drugs on the basis of unbound form in plasma using eq. 2 and 3.

$$UV = k_{ex} V_c P_f \qquad \text{eq. 2}$$

$$C_t = UV/P_t \qquad \text{eq. 3}$$

9) G. Levy and A. Jacobi, *J. Pharm. Sci.*, **63**, 805 (1974).

where, UV means the amount of a drug excreted in urine. k_{ex} and V_c represent the urinary excretion rate constant and volume of central compartment in terms of unbound drug in plasma, respectively. P_t and P_f mean the concentrations of total and unbound drug in plasma, respectively. C_t means the plasma clearance of a drug.

Substituting eq. 2 into eq. 3, eq. 4 will be derived.

$$C_t = k_{ex} V_c (P_f/P_t) \qquad \text{eq. 4}$$

Representing C_t on the basis of plasma clearance per unit body weight, C_t', C_t' will be written by eq. 5.

$$C_t' = C_t/W = k_{ex}(V_c/W)(P_f/P_t) \qquad \text{eq. 5}$$

where, W means the body weight. Substituting $P_f/P_t = F$ and $k_{ex}(V_c/W) = k'$ into eq. 5, eq. 6 will be derived.

$$C_t' = k'F \qquad \text{eq. 6}$$

Eq. 6 means that plasma clearance per body weight depends on only the free fraction on a drug in plasma. C_t' will be calculated with k_{ex}, V_c, P_f and P_t using eq. 2 and 3. Thus, values of C_t' under normal and diabetic conditions and insulin treatment were calculated.

If the plasma clearance depends on only the free fraction of a drug in plasma, plots of C_t' against free fraction of the drug will result in a single line. As shown in Fig. 9, the value of C_t' of CPA under the diabetic condition deviates remarkably from the line connected C_t' value under normal condition with the origin. Insulin treatment for 5 days also did not influence the value, but the treatment for 4 weeks changed the value to that near the line.

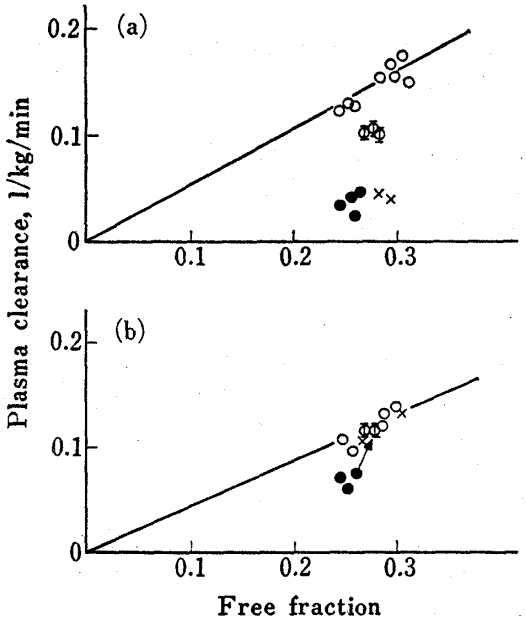

Fig. 9. Relationship between total Plasma Clearance of a drug and Its Free Fraction in Plasma

(a) CPA, (b) SDM.
○ normal, ● Alloxan diabetic condition.
× insulin treatment for 5 days.
Φ insulin treatment for 4 weeks.

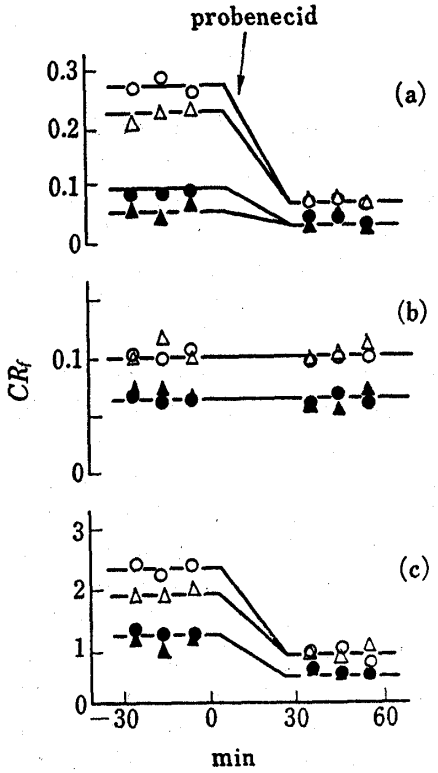

Fig. 10. Changes of Clearance Ratio Corrected for Protein Binding in a Rabbit under Normal and Diabetic Conditions

(a) CPA, (b) SDM, (c) N^4-AcSDM
○ normal, ● diabetes, △ 4-week-insulin treatment to diabetic rabbit, ▲ three weeks after withdrawal of insulin treatment.

The value of C_t' for SDM obtained under the diabetic condition also deviated from the line obtained under the normal condition, but by the insulin treatment, the value shifted to that on the line, which was presented by an arrow in Fig. 9(b). Those results coincide with the change of elimination rate constants under three conditions.

From these findings, it will be concluded that other factor (s) except plasma protein binding should be considered for the retardation of elimination of CPA and SDM from plasma. As one of other factors, functional changes of renal excretion will be suggested.

Renal Clearance of CPA, SDM and N^4-AcSDM

Characteristics of renal excretion of a drug can be represented by clearance ratio corrected for protein binding, CR_f, *i.e.*, the ratio of renal clearance corrected for protein binding to inulin clearance, GFR.

Clearance ratios of CPA, SDM and N^4-AcSDM before and after blockade of proximal tubular secretion are shown in Fig. 10. CR_f of CPA and N^4-AcSDM under the normal condition decreased markedly by the administration of probenecid. The findings suggest the existence of renal tubular secretion of CPA and N^4-AcSDM.

CR_f values of CPA and N^4-AcSDM under diabetic condition were smaller than those under the normal condition. Further decrease in the CR_f values were observed by the administration of probenecid. CR_f values under the diabetic condition showed a marked recovery by the insulin treatment for 4 weeks.

CR_f value of SDM under the normal condition was not influenced by the administration of probenecid, suggesting no existence of renal tubular secretion of SDM in rabbits. Under the diabetic condition, CR_f value of SDM was markedly lower than that under the normal condition. This low CR_f value was not influenced by the administration of probenecid. The decreased CR_f value under the diabetic condition showed a complete recovery by the insulin treatment for 5 days.

Relative amounts of proximal tubular secretion (S), tubular reabsorption (A) and urinary excretion (UV) of drugs were calculated according to Arita *et al.*[10] on the basis of GFR$\cdot P_f$ value which was not influenced by disease condition.

The results are shown in Table VII—IX. Tubular reabsorption ratio seems to be increased by diabetic condition. The increased ratio was reduced to the normal value by insulin treatment. The increase in tubular reabsorption will be subjected to the acidification of urine due to the diabetic acidosis. Further study on the mechanisms of an increase in the reabsorption will be discussed elsewhere.

Proximal tubular secretions of CPA and N^4-AcSDM showed a marked decrease under the diabetic condition. The decreased secretion did not recover by the 5-day insulin treatment but recovered by the 4-week insulin treatment. Thus, it will be concluded that the retardation of urinary excretion of CPA, SDM and N^4-AcSDM will be mainly subjected to a decrease in the proximal tubular secretion and/or an increase in the reabsorption. The decreased urinary excretion rate constant of SDM showed a complete recovery by the 5-day insulin treatment, while the 4-week insulin treatment were required for CPA and N^4-AcSDM. These findings will concide with the results of renal clearance experiments.

Due to the absence of proximal tubular secretion of SDM, retardation of urinary excretion of SDM under the diabetic condition will be subjected to an increase in the tubular reabsorption alone, because glomerular filtration will not be influenced by the diabetic condition. The altered reabsorption was normalized by the 5-day insulin treatment. This findings will be reflected in the recovery of k_{ex} by the 5-day insulin treatment. On the other hand, proximal tubular secretion of CPA and N^4-AcSDM was not recovered by the 5-day insulin treatment but recovered by the 4-week insulin treatment. Altered tubular reabsorption of

10) T. Arita, R. Hori, E. Owada, and K. Takahashi, *Chem. Pharm. Bull.* (Tokyo), **17**, 2526 (1969).

CPA and N^4–AcSDM was recovered by the 5-day insulin treatment. These findings will be reflected in the requirement of the 4-week insulin treatment for the recovery of excretion rate constant of CPA and N^4–AcSDM.

Table VII. Parameters of Renal Clearance of CPA under Normal and Diabetic Conditions

	Normal	AD	AD-Id	AD-Iw
Inulin clearance (ml/min/kg)	2.68	2.68	2.73	2.78
GFR·P_f	100.0	100.0	100.0	100.0
S	449.1	126.7	90.6	328.8
A	523.3	218.6	180.7	404.0
UV	25.3	7.9	9.9	24.8
R (%)	95.4	96.5	94.8	94.2

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

Table VIII. Parameters of Renal Clearance of SDM under Normal and Diabetic Condition

	Normal	AD	AD-Id	AD-Iw
Inulin clearance (ml/min/kg)	2.50	2.53	2.71	2.71
GFR·P_f	100.0	100.0	100.0	100.0
S	0.0	0.0	0.0	0.0
A	91.3	94.4	90.3	90.9
UV	8.7	5.6	9.7	9.1
R (%)	91.3	94.4	90.3	90.9

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

Table IX. Parameters of Renal Clearance of N^4-AcSDM under Normal and Diabetic Conditions

	Normal	AD	AD-Id	AD-Iw
Inulin clearance (ml/min/kg)	2.50	2.53	2.71	2.58
GFR·P_f	100.0	100.0	100.0	100.0
S	226.6	48.2	53.7	258.1
A	69.5	56.1	37.1	101.2
UV	257.1	92.1	116.5	258.7
R (%)	26.5	35.3	24.2	28.3

Id: insulin treatment to diabetic animal for 5 days.
Iw: insulin treatment to diabetic animal for 4 weeks.

It is reported that the administration of a large dose of alloxan will cause irreversible impairement of renal function. Since the partial blockade of proximal tubular secretion in CPA and N^4–AcSDM was recovered by the 4 week insulin treatment, the direct irreversible impairement due to alloxan will not be the reason of the blockade of proximal tubular secretion found in the present experiments.

It will be reasonable to consider that the impairement of proximal tubular secretion is caused by diabetes itself.