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Pharmacokinetic Behavior of Sulfamethoxazole in Alloxan Diabetic Rabbits[1,2]

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Pharmacokinetic behaviors of sulfamethoxazole (SMX) and its main metabolite, N^4-acetyl-sulfamethoxazole (N^4-AcSMX) in rabbits were studied under normal and diabetic condition following intravenous administration of SMX. The pharmacokinetic parameters were calculated employing a two compartment model.

Under diabetic condition, the elimination rate constant of SMX from plasma showed a marked decrease. Renal excretion rate constants of SMX and N^4-AcSMX also decreased under diabetic condition. Distribution of SMX to the tissue compartment increased in diabetic condition but that in the central compartment remained at the same value of normal condition. Changes in parameters under diabetic condition showed a recovering trend by insulin treatment. Plasma Protein binding of SMX and N^4-AcSMX were enhanced under diabetic condition. The increase in protein binding was subjected to an increase in the concentration of albumin under diabetic condition. Insulin treatment caused a slight recover of the binding to the values of nornal condition. Thus, it was concluded that the decrease in renal excretion rate constant of SMX and the increase in plasma protein binding of SMX and N^4-AcSMX are attributable to a decrease in elimination rate of SMX from plasma.

It was also suggested that the decrease in renal excretion of SMX and N^4-AcSMX might be caused by changes of the physiological factors such as blood glucose and acetone bodies.

Keywords——pharmacokinetic behavior; sulfamethoxazole; alloxan diabetic rabbit; plasma protein binding; HPLC analysis; decrease in renal excretion; increase in tissue distribution

It has been well recognized that dosage schedules of a drug for individual patients should be based on pharmacokinetic behavior of the drug in each patient.[4] Hence, it is required to study the pharmacokinetic behavior of drugs in patients under diseased state.

Alloxan diabetes is one of the experimentally produced diseases.[5] Alloxan diabetic animals have been used to study the pathology of diabetes and to evaluate antidiabetic drugs.[5] However, few reports have been presented in terms of pharmacokinetic behavior of drugs in alloxan diabetic animals.

In the previous paper,[6] it was reported that alloxan diabetic rabbits at the stages of hyperglycemia and hyperalbuminemia were considered to be suitable for the study of pharmacokinetic behavior of drugs under diabetic condition because the picture of diabetes remained almost constant.

Presently, the pharmacokinetic behavior of sulfamethoxazole was studied in alloxan treated rabbits being afflicted with diabetes at the stages of hyperglycemia and hyperalbuminemia.

1) This work was presented at the 96th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo. April 1976.
2) This report forms Part II of "Pharmacokinetic Behavior of Drugs uncer the Diseased States."
3) Location: *133-1, Yamada-Kami, Suita, Osaka.*
4) L. Dettle, Translation of Pharmacokinetics to Clinical Medicine p. 75 in Pharmacology and Pharmacokinetics (eds by T. Teorell, R.L. Dedrick and P.G. Condiliffe, Plenum Press N.Y., 1972).
5) C.C. Rerup, *Pharmacol. Rev.*, **22**, 485 (1970).
6) T. Nishihata, N. Yata, and A. Kamada, *Chem. Pharm. Bull.* (Tokyo), **26**, 2238 (1978).

Experimental

Materials——Sulfamethoxazole (SMX) on the market was used after recrystallization from aqueous ethanol. N^4-Acetylsulfamethoxazole (N^4-AcSMX) was prepared according to the method of Uno, *et al.*[7] Insulin zinc suspension J.P. IX was used in case of insulin treatment. Other chemicals were reagent grade.

Methods——Alloxan diabetic rabbits were prepared as reported in the previous paper.[6] Levels of blood glucose, serum protein, serum albumin, blood acetone bodies, and urinary pH value were determined as reported previously.[6] Changes in pharmacokinetic behavior of SMX in three rabbits (weighing 2.0—3.0 kg) were studied under the conditions of health, alloxan diabetes and insulin treatment of diabetes. To simplify the consideration, the results of individual rabbits were analyzed for each rabbit in terms of the condition of disease.

SMX was intravenously administered into a ear vein at a dose of 200 μmol/kg. One half ml samples of blood were chronologically collected from a ear vein for the analysis of SMX and N^4-AcSMX. Rabbits were individually kept in metabolic cages after taking final blood sample and urine was collected during the experimental period of 48 hours after the administration of SMX.

Pharmacokinetic parameters of SMX for rabbits under normal condition were determined once a week for two weeks. Then, on the next day after the second determination of the parameters, an intravenous administration of alloxan at a dose of 100 mg/kg was made. This treatment was sufficient to secure the diabetic condition at hyperglycemia and hyperalbuminemia.[6] At the fourth week (tentatively symbolized as AD_4-1) and since then, pharmacokinetic parameters of SMX were repeatedly determined once a week for another 19 or 23 weeks (AD_{23}-19 or AD_{27}-23). At eighth week and tenth week, insulin treatments were given to the rabbit No. 105 of diabetic condition. They were symbolized as AD_8-In-1 and AD_{10}-In-2, respectively. Similary, insulin was administered to rabbit No. 107 and No. 108 under diabetic condition at the 9th week (AD_9-In-1) and 11th week (AD_{11}-In-2). Insulin was administered at a dose of 1 IU/body once a day for 5 days.[8] Pharmacokinetic parameters of SMX after insulin administration were determined 24 hr after the final administration of insulin.

High Pressure Liquid Chromatography Analysis of SMX and N^4-AcSMX——One ml of 0.1 M acetate buffer (pH 4.5) was added to a half ml of plasma or urine sample. The mixture was extracted with 5 ml of ethyl acetate being shaken for 5 min with a KM Shaker (Iwaki Co., Ltd.) at room temperature. After centrifugation of the mixture for 5 min at 300 rpm, four ml of the organic layer were taken into a conical centrifuge tube and evaporated to dryness with a Vapor-Mix (Tokyo Rikakikai Co., Ltd.) at room temperature under reduced pressure. The residue was dissolved with 0.5 ml of dimethyl sulfoxide solution of an internal standard just prior to the analysis. Five μl of the solution were injected onto the column. A Shimadzu LC 841 high pressure liquid chromatographed equipped with a ultraviolet (UV) absorption detector (Shimadzu Model 202 UV Spectrophotometer) was used for the analysis. Zipax SAX packing material was used for stationary phase. The column was a 3.15-mm stainless tube with a 50-cm length. The mobile phase was 0.05 M acetate buffer (pH 4.5). Other condition are summarized in Table I. The recoveries of SAX and N^4-AcSMX from Plsama or urine samples were 80% employing the present extraction and analytical procedures. The sensitivities for the analyses of SMX and N^4-AcSMX in plasma or urine samples were 5 μmol/l and 10 μmol/l, respectively.

Table I. Analytical Conditions of High Pressure Liquid Chromatography for the Determination of Sulfamethoxazole and N^4-Acetylsulfamethoxazole

Instrument	Shimadzu LC-841	
Column	500 mm × 3.15 mm	
Column packing	Zipax SAX	
Mobile phase	0.05 M Acetate buffer (pH 4.5)	
Flow rate	1.0 ml/min	
Detector	UV 255 nm	
Retention time	SMX	7.0 min
	N^4-AcSMX	14.5 min
(Internal standard	N^4-Acetylsulfadimethoxine (60 μg/ml)	22.0 min)

Plasma Protein Binding of SMX and N^4-AcSMX *in Vivo*——Plasma protein binding of SMX and N^4-AcSMX was determined following a Sephadex-gel equilibrium method[9] with slight modifications. One ml of blood samples was taken from an ear vein at appropriate intervals following intravenous administra-

7) T. Uno and M. Ueda, *Yakugaku Zasshi*, **80**, 1785 (1960).
8) S. Miki, T. Nakazima, M. Toshioka, and G. Chiba, *Yakugaku Zasshi*, **88**, **166** (1968).
9) M. Hirose and Y. Kano, *Biochim. Biophys. Acta*, **251**, 376 (1971).

tion of SMX. The sample was taken into a small centrifuge tube and centrifuged for 5 min at 3000 rpm. Three tenth ml of the plasma were taken into a glass-stoppered test tube which contained 100 mg of Sephadex® G 25 (coarse, Pharmacia Fine Chemicals) and 0.3 ml of phosphate buffer (1/15 M, pH 7.4). The tube was kept for 5 hours at 37°±0.5° in water bath to establish an equilibrium between inner and outer phase of the gel. Then, 200 μl of the outer phase were taken with a microsyringe. The solution was analyzed for SMX and N⁴-AcSMX with the method described previously. The degree of binding of SMX and N⁴-AcSMX to plasma protein was obtained following Hirose's method.[9]

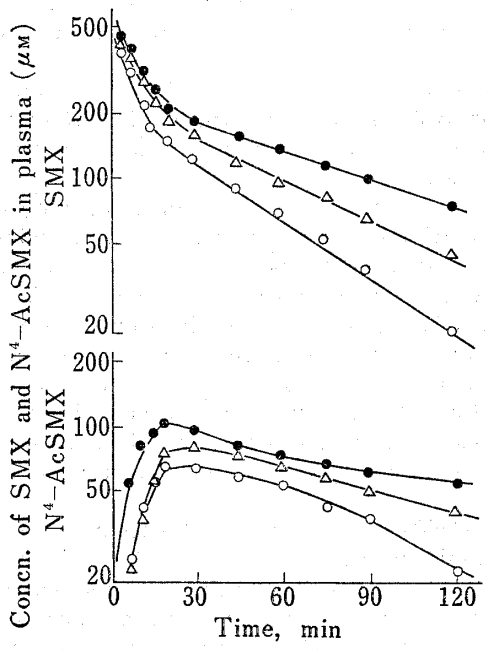

Fig. 1. Plasma Concentration Profiles of Sulfamethoxyazole (SMX) and N⁴-Acetylsulfamethoxazole (N⁴-AcSMX) after Intravenous Administration of Sulfamethoxazole to Rabbit under Normal, Alloxan Diabetic Conditions and Insulin Treatment

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

Results and Discussion

Plasma Concentration of SMX and N⁴-AcSMX in Diabetic Rabbits after Intravenous Administration of SMX

The plasma concentration profiles of SMX and N⁴-AcSMX in the rabbit No. 107 at three conditions following intravenous administration of SMX at each condition are presented in Fig. 1. In alloxan diabetic condition, plasma concentrations of SMX held on higher values than those in normal condition. These high plasma levels under diabetic condition seemed to recover to the normal levels by insulin treatment of the diabetic animal. Other two rabbits No. 105 and 108 also showed a similar results.

Pharmacokinetic parameters of unchanged SMX were determined with a residual method[10] employing a two compartment model (Chart 1). Generally, the elimination of a drug from plasma is sum of elimination through various routes such as hepatic, renal and others.

In the present study of SMX, more than 95% of the dose were found in the urine within 48 hrs as unchanged SMX and its metabolite, mainly N⁴-AcSMX, under normal and diabetic conditions. Thus, the elimination rate constant, k_{el}, of SMX from plasma is considered to be the sum of the rate constant for excretion, k_{ex}, and metabolism, k_m. The rate constant for metabolism can be obtained by eq. 1.

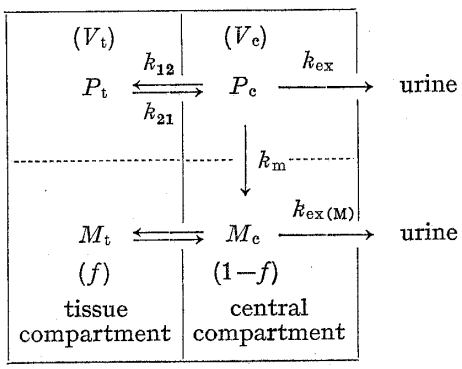

Chart 1. Pharmacokinetic Model

P_c and M_c: amount of unchanged drug and metabolite in the central compartment at time t in the β phase, respectively
P_t and M_t: amount of unchanged drug and metabolite in the tissue compartment at time t in the β phase, respectively
V_c and V_t: volume of distribution in the central compartment and the tissue compartment, respectively
f: fraction of metabolite in the tissue compartment
k_{ex} and $k_{ex(M)}$: renal excretion rate constant of unchanged drug and metabolite, respectively
k_m: metabolic rate constant
k_{el} $(=k_{ex}+k_m)$: elimination rate constant

10) J. Swarbrick ed., "Current Concepts in the Pharmaceutical Sciences. Dosage Form Design and Bioavailability," Lea & Febiger, Philadelphia, 1973, p. 13.

$$k_m = k_{el} \times (\text{fraction of metabolite(s) in the urine}) \quad \text{eq. 1}$$

Renal excretion rate constant and fraction in the tissue compartment of N^4-AcSMX ($k_{ex(M)}$ and f) are obtained as follows:

Using fraction of metabolite in tissue compartment, f, amount of the metabolite in tissue compartment is described by eq. 2.

$$M_t = M_c \times \frac{f}{1-f} \quad \text{eq. 2}$$

where, M_t and M_c are the same as those defined in Chart 1. After small interval of Δt, *i.e.*, at the time of $t+\Delta t$, if the amounts of the unchanged SMX and its metabolite in the central compartment are defined as P_c' and M_c' respectively, M_c' can be described by eq. 3.

$$M_c' = \left(M_c + k_m P_c \Delta t - k_{ex(M)} M_c \Delta t + M_c \frac{f}{1-f}\right)(1-f) \quad \text{eq. 3}$$

Thus, eq, 4 is derived.

$$\frac{M_c' - M_c}{1-f} + k_{ex(M)} M_c \Delta t = k_m P_c \Delta t \quad \text{eq. 4}$$

As shown in Table II, the volume of distribution of metabolite in the central compartment, which was calculated with the two compartment model following an intravenous administration of authentic N^4-AcSMX, was almost same as that of unchenged SMX under normal and diabetic conditions.

Therefore, dividing eq. 4 by the volume of distribution in the central compartment, V_c, one obtains eq.5.

$$\frac{(M_c' - M_c)/V_c}{1-f} + k_{ex(M)} \Delta t M_c / V_c = k_m \Delta t P_c / V_c \quad \text{eq. 5}$$

Equation 5 can be rewritten as follow:

$$\frac{(C_M' - C_M)}{1-f} + k_{ex(M)} \Delta t C_M = k_m \Delta t C \quad \text{eq. 6}$$

where, C_M' is the plasma concentration of the metabolite at time $t+\Delta t$. C and C_M are the plasma concentration of unchanged SMX and its metabolite at time t.

The values of $C_{M'}$, C_M, C, and k_m in eq. 6 can be experimentally determined. Thus, with eq. 6, $k_{ex(M)}$ and f can be calculated with the least squares method.

Pharmacokinetic parameters of each rabbits are summarized in Tables III—V.

Physiological factors such as concentrations of glucose, acetone bodies, total protein, and albumin in blood or serum and pH value of urine were measured at the time when pharmacokinetic parameters were determined (Tables VI—VIII).

Table II. Comparison of the Distribution Volume of SMX and N^4-SMX in the Central and Tissue Compartments under Normal or Diabetic Condition

Weeks,	Symbol	SMX		N^4-AcSMX		
		V_c (L)	V_t (L)	V_c (L)	V_t (L)	f
1	Normal	0.94	0.62	—	—	—
1.5	Normal	—	—	0.95	0.27	0.22
4	AD_4-1	1.00	0.84	—	—	—
4.5	$AD_{4.5}$-1.5	—	—	0.96	0.24	0.20
5	AD_5-2	0.82	0.80	—	—	—
5.5	$AD_{5.5}$-2.5	—	—	0.98	0.92	0.22
6	AD_6-3	0.93	0.96	—	—	—

a) Data was obtained using a rabbit No. 108.
Volume of distribution of N^4-AcSMX was obtained by intravenous administration of authentic N^4-AcSMX (dose: 200 μmol/kg).

Table III. Pharmacokinetic Parameters of Sulfamethoxazole in Rabbit No. 105 under Normal and Alloxan Diabetic Conditions

Weeks, Symbol		k_{el}	k_{12} ($\times 10^2$ min^{-1})	k_{21}	V_c (L)	V_t	k_m	k_{ex} ($\times 10^2$ min^{-1})	$k_{ex(M)}$	f
1	Normal	3.69	5.48	10.98	0.71	0.35	2.68	1.01	3.84	0.28
2	Normal	3.72	5.00	10.79	0.74	0.34	2.72	0.99	3.71	0.29
3										
4	AD_4-1	4.75	13.95	13.73	0.60	0.67	4.13	0.62	3.24	0.24
5	AD_5-2	1.94	8.68	9.21	0.71	0.67	1.57	0.37	3.05	0.25
6	AD_6-3	2.88	7.44	8.03	0.71	0.66	2.39	0.50	2.86	0.21
7	AD_7-4	1.97	8.31	8.01	0.61	0.62	1.58	0.40	2.54	0.25
8	AD_8-5	2.58	10.10	8.60	0.66	0.77	2.08	0.51	2.63	0.26
9	AD_9-In-1	2.29	8.52	12.37	0.69	0.46	1.57	0.71	3.05	0.28
10	AD_{10}-7	1.92	7.89	7.64	0.65	0.67	1.50	0.42	2.63	0.25
11	AD_{11}-In-2	2.44	8.58	13.76	0.70	0.44	1.81	0.63	2.95	0.27
12	AD_{12}-9	1.93	7.21	6.93	0.63	0.65	1.46	0.47	2.29	0.25
25	AD_{25}-22	2.77	9.88	9.60	0.88	0.91	2.18	0.60	2.73	0.24
27	AD_{27}-24	2.18	9.43	9.26	0.82	0.84	1.61	0.75	2.39	0.26

Table IV. Pharmacokinetic Parameters of Sulfamethoxazole in Rabbit No. 107 under Normal and Alloxan Diabetic Conditions

Weeks, Symbol		k_{el}	k_{12} ($\times 10^2$ min^{-1})	k_{21}	V_c (L)	V_t	k_m	k_{ex} ($\times 10^2$ min^{-1})	$k_{ex(M)}$	f
1	Normal	1.48	7.82	13.29	0.82	0.54	0.55	0.94	3.26	0.29
2	Normal	1.54	6.86	10.25	0.93	0.62	0.69	0.95	3.29	0.27
3										
4	AD_4-1	1.09	10.11	11.18	0.71	0.64	0.61	0.48	2.03	0.27
5	AD_5-2	1.28	0.04	8.69	0.74	0.76	0.74	0.54	1.97	0.24
6	AD_6-3	1.32	8.73	8.01	0.79	0.86	0.71	0.61	1.96	0.23
7	AD_7-4	0.99	11.58	11.71	0.71	0.70	0.47	0.52	2.15	0.26
8	AD_8-5	0.95	10.00	8.94	0.70	0.78	0.44	0.51	2.15	0.23
9	AD_9-In-1	1.23	4.59	9.78	0.83	0.38	0.55	0.68	2.87	0.28
10	AD_{10}-7	1.11	8.27	8.07	0.74	0.75	0.52	0.59	2.16	0.22
11	AD_{11}-In-2	1.10	5.13	9.47	0.78	0.42	0.53	0.66	2.37	0.25
12	AD_{12}-9	0.88	9.24	9.03	0.76	0.78	0.42	0.46	2.10	0.25
21	AD_{25}-22	0.91	8.76	7.18	0.80	0.97	0.40	0.51	1.75	0.21
23	AD_{27}-24	0.86	8.95	7.34	0.77	0.94	0.38	0.48	1.82	0.23

Table V. Pharmacokinetic Parameters of Sulfamethoxasole in Rabbit No. 108 under Normal and Alloxan Diabetic Conditions

Weeks, Symbol		k_{el}	k_{12} ($\times 10^2$ min^{-1})	k_{21}	V_c (L)	V_t	k_m	k_{ex} ($\times 10^2$ min^{-1})	$k_{ex(M)}$	f
1	Normal	3.46	8.05	12.19	0.94	0.62	2.30	1.15	4.20	0.26
2	Normal	3.51	8.17	13.33	0.98	0.60	2.30	1.20	4.27	0.24
3										
4	AD_4-1	3.99	5.05	6.04	1.00	0.84	3.25	0.74	3.34	0.25
5	AD_5-2	3.69	9.37	9.59	0.82	0.80	2.91	0.78	2.93	0.22
6	AD_6-3	2.93	8.27	8.08	0.93	0.96	2.30	0.62	2.68	0.18
7	AD_7-4	1.79	7.90	6.29	0.73	0.92	1.26	0.57	2.80	0.21
8	AD_8-In-1	2.85	5.45	10.43	1.09	0.57	1.80	1.05	3.25	0.20
9	AD_9-6	2.82	7.41	7.52	0.97	0.96	2.16	0.66	3.04	0.22
10	AD_{10}-In-2	3.18	4.88	8.16	1.19	0.63	2.14	1.05	3.46	0.20
11	AD_{11}-8	2.33	6.35	6.74	0.93	0.88	1.91	0.41	3.13	0.20

Table VI. Physiological Changes under Normal and Alloxan Diabetic Conditions in Rabbit No. 105

Weeks	Symbol	Weight (kg)	Glucose in blood (mg/100 ml)	Acetone bodies im blood (mg/100 ml)	Total protein in serum (g/100 ml)	Albumin in serum (g/100 ml)	Urine-pH
1	Normal	2.6	72.43	0.76	5.61	2.93	8.28
2	Normal	2.6	74.26	0.94	5.58	3.00	8.46
3							
4	AD_4-1	2.8	164.23	1.14	5.80	3.06	7.42
5	AD_5-2	2.8	156.67	1.68	5.80	3.06	7.42
6	AD_6-3	2.8	186.94	1.70	5.93	3.47	7.52
7	AD_7-4	2.8	240.26	1.74	6.26	3.55	7.68
8	AD_8-In-1	2.9	106.42	0.85	5.88	3.45	7.85
9	AD_9-6	2.8	172.42	1.42	6.03	3.52	7.41
10	AD_{10}-In-2	2.8	106.83	0.98	5.80	3.27	7.68
11	AD_{11}-8	2.7	240.58	1.60	6.20	3.54	7.51
25	AD_{25}-22	2.8	212.47	2.03	6.32	3.54	7.16
27	AD_{27}-24	2.8	196.58	1.84	6.16	3.52	7.08

Table VII. Physiological Changes under Normal and Alloxan Diabetic Conditions in Rabbit No. 107

Weeks	Symbol	Weight (kg)	Glucose in blood (mg/100 ml)	Acetone bodies in blood (mg/100 ml)	Total protein in serum (g/100 ml)	Albumin in serum (g/100 ml)	Urine-pH
1	Normal	1.7	79.78	0.85	4.96	2.86	8.43
2	Normal	1.8	77.56	0.88	5.02	3.00	8.58
3							
4	AD_4-1	1.8	254.87	1.42	5.48	3.28	7.58
5	AD_5-2	1.8	338.97	1.55	5.62	3.64	7.42
6	AD_6-3	1.9	298.96	1.60	5.69	3.67	7.42
7	AD_7-4	1.9	282.47	1.50	5.53	3.67	7.30
8	AD_8-5	1.8	318.56	1.80	5.58	3.52	7.06
9	AD_9-In-1	2.0	102.48	0.95	5.26	3.21	8.05
10	AD_{10}-7	1.9	306.74	1.70	6.22	4.02	7.68
11	AD_{11}-In-2	2.1	128.76	0.85	5.40	3.41	8.24
12	AD_{12}-9	1.9	324.56	1.53	5.78	3.60	7.80
25	AD_{25}-22	1.7	375.29	2.04	5.54	3.60	6.90
27	AD_{27}-24	1.7	395.28	2.01	5.56	3.61	7.13

Table VIII. Physiological Changes under Normal and Alloxan Diabetic Conditions in Rabbit No. 108

Weeks	Symbol	Weight (kg)	Glucose in blood (mg/100 ml)	Acetone bodies in blood (mg/100 ml)	Total protein in serum (g/100 ml)	Albumin in serum (g/100 ml)	Urine-pH
1	Normal	2.8	93.72	1.03	5.63	3.91	8.20
2	Normal	2.8	98.75	0.98	5.60	3.94	8.65
3							
4	AD_4-1	2.6	426.58	1.92	6.00	4.22	7.42
5	AD_5-2	2.6	382.54	2.98	6.45	4.68	7.03
6	AD_6-3	2.6	428.46	2.20	6.32	4.34	6.85
7	AD_7-4	2.5	498.65	2.20	6.35	4.51	7.18
8	AD_8-5	2.4	442.75	2.46	6.35	4.28	6.70
9	AD_9-In-1	2.6	112.38	1.42	6.08	4.07	7.83
10	AD_{10}-7	2.5	426.53	2.13	6.35	4.28	6.70
11	AD_{11}-In-2	2.7	130.15	1.06	5.93	4.06	7.67
12	AD_{12}-9	2.4	441.54	2.52	6.28	4.13	6.74
21	AD_{21}-18	1.9	463.95	2.64	5.42	3.18	6.60
23	AD_{23}-20	1.9	492.78	2.18	5.38	3.23	6.72

Symbols in the column in Tables III—V correspond to those in Tables VI—VIII, respectively.

From the levels of blood glucose and serum albumin (Tables VI—VIII), rabbits No. 107 and No. 108 were considered to be in the diabetic condition two weeks after alloxan treatment. The rabbit No. 105 was considered in the diabetic condition at three weeks after alloxan treatment. So, the pharmacokinetic parameters obtained at second or third week (AD_5-2 for No. 105, and AD_4-1 for No. 107 and 108) and therafter following alloxan treatment were considered to represent the parameters under diabetic condition.

In all rabbits, marked decrease in the values of k_{el}, and $k_{ex(M)}$ were observed in diabetic condition regardless of some fluctuations in the absolute values of each parameter being subjected to individual physiologic differences as well as the differences in the picture of disease. Since SMX were excreted in the urine more than 95% of the dose within 48 hr in normal rabbits as well as in diabetic rabbits, a decrease in the value of k_{el} under diabetic condition was considered to be attributable to the decrease in the values of k_m and/or k_{ex}. It was interesting to note that the values of k_m was decreased by alloxan treatment with some fluctuation at the early period of diabetic condition but they were remained at the smaller values than those in normal condition at the later period. The reason of those fluctuations of k_m value at the early stage of diabetes is left unclarified. The decrease in values of k_{ex} and $k_{ex(M)}$ may suggest a possible impairment of the renal functions in diabetic conditions. The values of k_{ex} and $k_{ex(M)}$ in diabetic condition were slightly recovered by insulin treatment.

These findings strongly support that the renal function may be impaired under diabetic condition but the function is recovered by insulin treatment with the recovery of physiologic factors of blood such as levels of glucose and acetone bodies in the blood (Tables VI—VIII).

Further study is required to clarify the mechanisms of the decrease in values of k_{ex} and $k_{ex(M)}$ under diabetic condition in terms of the physiological function of kidney.

Distribution of SMX to the tissue compartment showed a marked increase under the diabetic condition but that to the central compartment was not influenced by treatments of alloxan and insulin. The increase in V_t under diabetic condition was considered to be subjected to an increase in the distribution of SMX into tissue involving red blood cells. And it was recovered to the normal value by insulin treatment.

Unlike the plasma concentration, the fraction of N^4–AcSMX in the tissue compartment following the administration of SMX did not seem to be influenced by diabetic condition.

This finding will be supported by the results obtained with the authentic N^4–AcSMX. The distribution volumes of N^4–AcSMX in the tissue compartment and in the central compartment were calculated with the two compartment model following intravenous administration of the authentic N^4–AcSMX at a dose of 200 μmol/kg (Table II). The distribution volume of N^4–AcSMX in the tissue compartment under diabetic condition was almost same as that under normal condition.

Therefore, the higher plasma concentration of N^4–AcSMX under diabetic condition following administration of SMX will be subjected to the decrease in the excretion rate constant of N^4–AcSMX. An enhanced protein binding of N^4–AcSMX which will be discussed below will have also a possible influence on the higher plasma level.

Plasma Protein Binding of SMX and N^4-AcSMX

Plasma concentration of total protein and albumin increased under diabetic condition (Tables VI—VIII). It suggests an enhanced protein binding of SMX and N^4–AcSMX in plasma.

Profiles of plasma protein binding of SMX and N^4–AcSMX are presented in Fig. 2 following intravenous administration of SMX to rabbits under normal and diabetic condition and insulin treatment. Plasma protein binding of SMX and N^4–AcSMX was significantly increased

in diabetic condition. And the enhanced binding under diabetic condition was slightly recovered by insulin treatment. Scachard's plots of these results of three conditions resulted in single line (Fig. 3). From these findings it may be concluded that the increase in the plasma protein binding of SMX and its metabolite in diabetic condition is primarily attributed to an increase in albumin concentration and structural change in albumin molecule is not considered to be the factor of the changes in the protein binding.

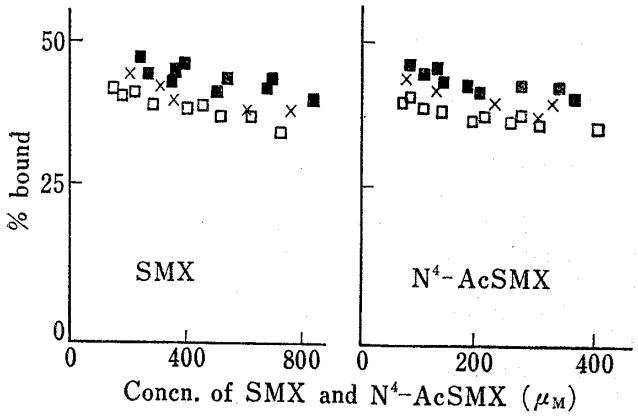

Fig. 2. Plasma Protein Binding of Sulfamethoxazole(SMX) and N⁴-Acetylsulfamethoxasole (N⁴-AcSMX) in Rabbits

key: □; normal condition, ■; alloxan diabetic condition, × ; insulin treatment to diabetic animals.

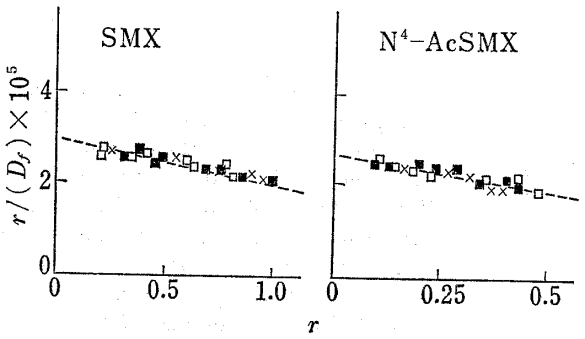

Fig. 3. Scatchard's Plots of Plasma Protein Binding of Sulfamethoxazole (SMX) and N⁴-Acetylsulfamethoxazole (N⁴-AcSMX)

key: □; normal condition, ■; alloxan diabetic condition, × ; insulin treatment to diabetic animals.

This increase in plasma protein binding is considered to be one of other factors responsible for a decrease in the elimination of SMX from plasma.