Chem. Pharm. Bull.
22(4) 894—906 (1974)

UDC 547.771.09 : 615.212.015.4

Urine Data Analysis for Pharmacokinetics of Aminopyrine and Its Metabolites in Man[1]

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(Received September 3, 1973)

Aminopyrine and its metabolites, 4-aminoantipyrine and N-acetyl-4-aminoantipyrine, were orally administered to human subjects and cumulative amounts of 4-aminoantipyrine and N-acetyl-4-aminoantipyrine excreted in urine were determined.

Pharmacokinetic parameters of aminopyrine were estimated by means of least square fit of multi-exponential equations to the urine data.

For a study on pharmacokinetic behavior of a drug in the body (drug absorption, metabolism and excretion), blood concentration time course is the most important source of informations. Repeated venipunctures, however, charge quite a heavy burden to subjects. Besides, determination of drug concentrations in blood are sometimes impeded by an insufficient sensitivity of the analytical method. On the other hand, collections of urine samples and estimations of the amount of drug excreted in urine are performed relatively with ease. As a matter of fact, quite a few pharmacokinetic studies in man deal with urine data alone.[3]

For a pharmacokinetic study of a drug of which metabolic pathways are previously known and linearity is assumable for all of the pharmacokinetic steps, the following procedure is feasible with only the urine data.

At a start point, one of the ultimate metabolites of the drug is administered to subjects, urine samples are collected periodically, amount of the metabolite is assayed and a multiexponential equation is fitted to the data according to the least square method by means of a digital computer, for which a computer program has been reported in the previous paper.[4] After a sufficient period of time for washout, a precursor of the metabolite is administered to the same subjects and a multiexponential equation is fitted to the urine data to obtain an extra exponential parameter, which is a rate constant of the step from the precursor to the metabolite.

Metabolism of aminopyrine have been studied extensively so far,[5] and it is clear that the main metabolic route is de-methylation of dimethylamino group, or formation of 4-aminoantipyrine, and subsequent acetylation of the amino group. Rubazoic acid, methyl rubazoic acid and 4-hydroxyantipyrine are also reported in the urine of subjects who ingested aminopyrine.

The purpose of the present study is to evaluate the rate constants corresponding to the respective steps of the pharmacokinetic model shown in Chart 1, from urine data alone.

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[1] Presented in part to the Meeting of Hokuriku Branch, Pharmaceutical Society of Japan, Kanazawa, December, 1972.

[2] Location: *3190, Gofuku, Toyama, 930, Japan.*

[3] *a*) E. Nelson and I. O'Reilly, *J. Pharmacol. Exptl. Therap.*, 129, 368 (1960); *b*) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964); *c*) G. Levy, *J. Pharm. Sci.*, **54**, 959 (1965); *d*) G. Levy and T. Matsuzawa, *J. Pharmacol. Exptl. Therap.*, **156**, 285 (1967); *e*) A.J. Cummings, M.L. King and B.K. Martin, *Brit. J. Pharmacol.*, **29**, 150 (1967); *f*) M.C.B. Van Oudtshoorn and F.J. Potgieter *J. Pharm. Pharmacol.*, **24**, 357 (1971); *g*) G. Levy and T. Tsuchiya, *Clin. Pharmacol. Therap.*, 13, 317 (1972).

[4] T. Koizumi, M. Ueda, and M. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **21**, 2549 (1973).

[5] *a*) B.B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **99**, 171 (1950); *b*) F.R. Preuss and K.M. Voigt, *Arzneimittel Forsch.*, **15**, 741 (1965); *c*) H. Yoshimura, H. Shimeno, and H. Tsukamoto, *Yakugaku Zasshi*, **90**, 1406 (1970).

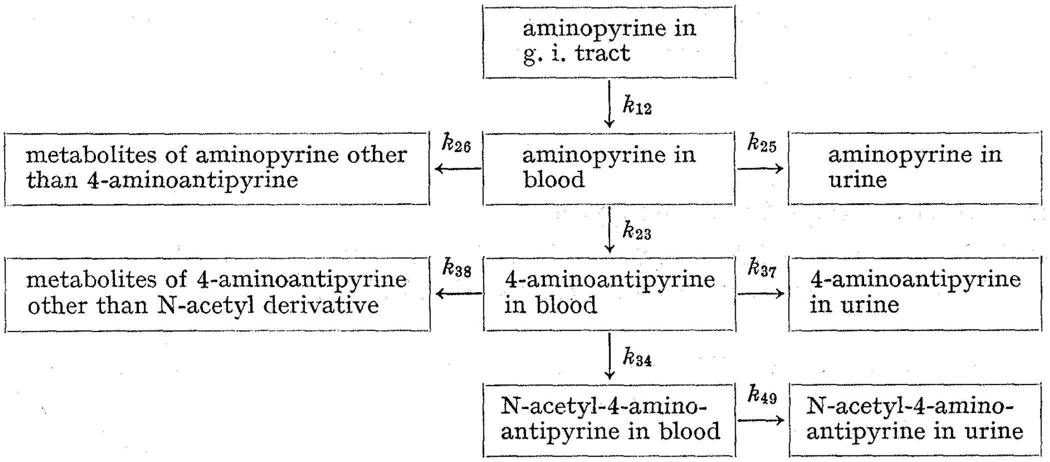

Chart 1

Experimental

Material——Aminopyrine was J.P. grade. 4-Aminoantipyrine was reagent grade and obtained from Nakarai Chemical Co., Ltd. N-Acetyl-4-aminoantipyrine was prepared according to the method reported by Knorr, *et al.*[6] 4-Aminoantipyrine was heated under reflux in glacial acetic acid and recrystallized from chloroform–ligroin, to obtain white crystalline powder, mp 197—199°. UV $\lambda_{\max}^{\text{ethanol}}$ mμ: 245, 275.

Procedure——Two healthy adult human subjects (25, 38 yrs. 52 , 58kg) were used in this study. Dose of aminopyrine, approximately 50 mg, was dissolved in water and administered orally two hours prior to breakfast after control urine samples had been taken. (With such a low dose level as adopted in this experiment, the authors intended to avoid non-linearity in pharmacokinetic processes.) Following breakfast, the subjects were permitted to ingest foods and fluids as desired. Collections of total urinary output were made every one hour for the first two hours, then every two hours for the next ten hours and after that every 2 to 9 hours until 28 to 34 hours after dosage.

At intervals of one week, the same subjects ingested with the similar manner, 4-aminoantipyrine, N-acetyl-4-aminoantipyrine and pyrabital, respectively.

Analytical Method——4-Aminoantipyrine in Urine: Urinary concentration of 4-aminoantipyrine was determined by a modification of the method described by Brodie and Axelrod.[5a)]

Fifteen ml of urine and 1 ml of 1 N NaOH were added to a 25 ml of chloroform in a 50 ml glass-stoppered centrifuge tube. The tube was shaken for 30 minutes and centrifuged for 10 minutes at 3000 rpm. The supernatant aqueous layer was removed by aspiration. Twenty ml of the chloroform phase was transferred to a test tube and chloroform was distilled off in a water bath (75—85°). Three ml of 0.5 N HCl was added to the residue in the test tube and was cooled in ice water. One half ml of 0.2% $NaNO_2$ was added to the test tube, which was shaken vigorously and was left stand for 10 minutes. Then 0.5 ml of 1.0% ammonium sulfamate solution was added and mixed thoroughly. After 3 minutes 1 ml of 1.0% solution of β-naphthol in 2 N NaOH was added, shaken and left at room temperature for 10 minutes. Then 10 ml of chloroform was added and the mixture was shaken vigorously for 30 seconds. The supernatant aqueous layer was removed and 0.5 g of anhydrous sodium sulfate was added to the chloroform phase. The absorbance of the dehydrated chloroform solution was determined at 450 mμ using a spectrophotometer.

Standards were prepared by handling known amounts of 4-aminoantipyrine in the same manner as the unknowns.

An absorbance of approximately 0.365 was obtained in the Hitachi 139 spectrophotometer when 0.1 mg were run through the procedure described above.

N-Acetyl-4-aminoantipyrine: Urinary concentration of N-acetyl-4-aminoantipyrine was determined by a modification of the method described by Brodie and Axelrod.[5a)]

Ten ml of urine and 1 ml of 1 N HCl saturated with NaCl were added to 2.5 g of NaCl and 25 ml of chloroform in a 50 ml glass-stoppered centrifuge tube. The tube was shaken for 30 minutes and centrifuged for 10 minutes at 3000 rpm. The supernatant aqueous layer was removed by aspiration. Twenty ml of the chloroform phase was transferred to a test tube and chloroform was distilled off on a water bath (75—85°). Three ml of 0.5 N HCl was added to the residue and heated in an autoclave (121°, 1.0 kg/cm^2) for 1 hour. Cooled in ice water and estimated the resulting 4-aminoantipyrine as described above.

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6) L. Knorr and F. Stolz, *Ann. Chem.*, 293, 58 (1896).

Standards were prepared by handling known amount of N-acetyl-4-aminoantipyrine in the same manner as the unknowns. An absorbance of approximately 0.315 was obtained in the Hitachi 139 spectrophotometer when 0.1 mg of N-acetyl-4-aminoantipyrine were run through the procedure described above.

Result

Administration of N-Acetyl-4-aminoantipyrine

Cumulative amounts of N-acetyl-4-aminoantipyrine (NA) excreted in urine after oral administration of NA to subjects A and B are listed in Table I.

Table I. Cumulative Amount of NA excreted in Urine after Ingestion of N-Acetyl-4-aminoantipyrine

Subject A 52 kg Dose 69.7 mg		Subject B 59 kg Dose 51.7 mg	
Time hr	NA mg	Time hr	NA mg
2.0	9.17	1.0	2.14
4.0	17.27	2.0	6.70
6.0	25.64	4.0	13.58
8.0	34.13	6.0	19.14
10.0	40.49	8.0	23.02
11.75	44.19	10.0	26.38
15.33	49.94	12.0	29.71
18.42	54.85	14.0	32.74
22.0	59.10	15.38	34.52
25.0	62.19	18.80	37.59
28.0	64.37	23.0	40.24
31.0	65.83	27.0	41.92
33.92	66.88		
rest	3.05	rest	3.33
total	69.93	total	45.25

Taking the fact into consideration that NA is one of the ultimate metabolites of aminopyrine (AM), the simplest model describing the absorption and excretion of the drug would be shown as Chart 2.

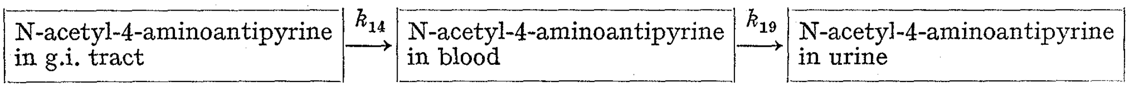

Chart 2

Assuming first order rate processes for absorption and excretion, the cumulative amount of NA excreted in urine is expressed by 2-exponential equation (type 3, grade 1).[7]

The average excretion rates calculated with the data of Table I were plotted at the midpoint of each time interval to give log "Rate" plots.[3e] The rate constant calculated from the slope of the terminal linear portion of the log "Rate" plot was used to obtain "Rest" value (the amount of the drug to be excreted after the collection of urine sample has discontinued).[8] Amount of the drug ultimately excreted in urine was calculated by adding the "Rest" value to the cumulative amount observed and "Sigma minus" plot[3e] was made on a semi-logarithmic scale.

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[7] For the definition of "Type" and "Grade" of multi-exponential equation, see the previous report (4).

[8] "Rest" value is calculated by dividing the excretion rate of the drug at the time when the last urine sample is collected with the excretion rate constant.

Table II. Rate Constants and Coefficients calculated from Least Square Fit of Multi-exponential Equations to Urine Data

	Drug	Subject	Rate const.	Coeff.	SS
(a)	NA	A	2.0661± 0.6132	− 3.0361	5.7340
			0.0847± 0.0032	74.0311	
		B	3.0817± 0.8914	− 1.4034	1.6080
			0.0913± 0.0032	47.3533	
(b)	AA	A	2.5415± 2.5087	1.4094	2.8650
			1.2242± 1.5146	− 6.3395	
			0.0951± 0.0086	43.9345	
		B	3.5000± 2.3770	1.0537	0.7450
			1.6000± 1.0039	− 5.2146	
			0.0950± 0.0027	49.0066	
(c)	AM	A	3.0984± 44.256	− 0.0101	0.1400
			0.7391± 0.6441	1.0623	
			0.2214± 0.0599	− 12.5490	
			0.0652± 0.0132	31.0614	
		B	3.3289± 46.178	− 0.0093	0.1093
			1.1519± 2.3505	0.2467	
			0.0933± 0.0372	− 63.1860	
			0.0579± 0.0266	97.5474	
(d)	PB	A	3.4826± 64.690	− 0.0297	0.5907
			0.8817± 0.5251	4.0455	
			0.5238± 0.4723	− 10.6016	
			0.0597± 0.0123	34.9893	
		B	6.3643±633.55	− 0.0043	0.0736
			3.1497± 64.145	0.0360	
			0.0883± 0.1195	−123.1229	
			0.0719± 0.0998	150.0833	

Two exponential parameters were calculated from the "Sigma minus" plot by means of the method of residual[9] and used as the first estimates necessary for the least square fit of data to 2-exponential equation by the program (LSME-20) reported in the previous paper[4] on a digital computer. Computer results are shown in Table II (a).

Fit of the data to 2-exponential equation is excellent as is evident from the sum of squared differences between observed and calculated values (SS) and from Fig. 1(a).

In most cases the rate constant of drug absorption from gastro-intestinal tract is greater than that of elimination from the blood. Consequently the larger value of the exponential parameters shown in Table IIa (2.07/hr for subject A and 3.08/hr for subject B) may well be assigned to absorption rate constant and the other one (0.085/hr for subject A and 0.091/hr for subject B) to excretion rate constant. This, however, is not definitely concluded yet.

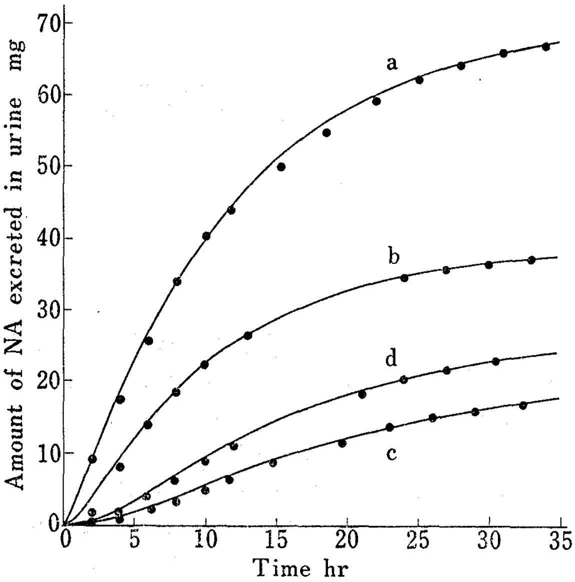

Fig. 1. Cumulative Amount of N-Acetyl-4-aminoantipyrine excreted in Urine after Oral Administration of a) N-Acetyl-4-aminoantipyrine, b) 4-Aminoantipyrine, c) Aminopyrine and d) Pyrabital to Subject A

solid line; least square fit value
circle; observed data

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[9] J.G. Wagner, *Drug Intel.*, **2**, 206 (1968).

Administration of 4-Aminoantipyrine

Cumulative amounts of NA and 4-aminoantipyrine (AA) excreted in urine after oral administration of AA to subjects A and B are listed in Table III.

Table III. Cumulative Amount of AA and NA excreted in Urine after Ingestion of 4-Aminoantipyrine

Subject A 52kg Dose 58.4 mg			Subject B 59 kg Dose 52.9 mg		
Time hr	AA mg	NA mg	Time hr	AA mg	NA mg
2.0	1.47	1.88	1.0	0.40	0.75
4.0	3.54	8.06	2.0	1.80	4.27
6.0	4.18	14.09	4.17	2.86	11.69
8.0	4.44	18.51	6.0	3.13	17.44
10.0	4.60	22.32	8.0	3.25	22.06
13.0	4.73	26.44	10.08	3.34	25.98
24.0	—	34.57	12.0	—	29.55
27.0	—	35.70	14.0	—	31.72
30.0	—	36.52	17.17	—	35.33
33.0	—	37.01	21.08	—	38.16
			24.0	—	39.63
			27.0	—	41.05
			30.0	—	42.03
rest	0.31	1.70	rest	0.25	2.95
total	5.04	38.71	total	3.59	44.98

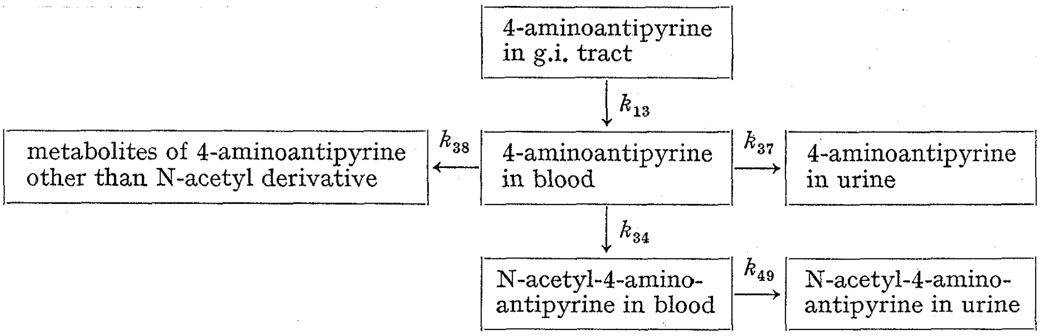

Chart 3

The simplest model describing the absorption and elimination of the drug would be as shown in Chart 3. In this particular case, the cumulative amount of NA excreted in urine is expressed by 3-exponential equation (type 3, grade 2) and that of AA by 2-exponential equation (type 3, grade 1).

Since the excreted amount of NA is ten times greater than that of AA (Table III), least square fit of multi-exponential equation was carried out for NA data first. First estimates for parameters were prepared by applying the method of residual[9] to "Sigma minus" plot. Results are shown in Table II(b) and on Fig. 1(b).

As is elucidated in Appendix (A) of this report, the urinary excretion rate constant of NA is obtained from the cumulative amounts of NA and AA excreted in urine after ingestion of AA by solving convolution for weight function.

Numerical method of Umemura[10] was applied to the data of Table III and results are listed in Table IV. "Sigma minus" plot of $\bar{G}(t)$ values (Fig. 2) gave a rate constant of ap-

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[10] K. Umemura, Abstracts of Papers, 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July 1970, p. IV-46.

proximately 0.1/hr. Therefore, it is reasonable to assign exponential parameter of 0.095/hr (subjects A and B) in Table II(b) to the urinary excretion rate constant of NA. The presumption of the previous section that the larger value of the exponential parameters (2.07/hr and 3.08/hr) correspond to absorption rate constant and the other to excretion rate constant, is now confirmed.

Table IV. Estimation of NA Excretion Rate Constant by Numerical De-convolution Method of Umemura

Time	$X(t)$	$Y(t)$	$\bar{G}(t)$	$\frac{k_{34}}{k_{37}}-\bar{G}(t)$
Subject A				
0.0	0.00	0.00	0.00	7.69
2.0	1.47	1.88	2.65	5.04
4.0	3.54	8.06	3.58	4.11
6.0	4.18	14.09	4.91	2.78
8.0	4.44	18.51	4.49	3.20
10.0	4.60	22.32	—	—
		$\frac{k_{34}}{k_{37}}=\frac{38.71}{5.04}=7.69$		
Subject B				
0.0	0.00	0.00	0.00	12.52
2.0	1.80	4.27	4.00	8.52
4.0	2.86	11.69	5.75	6.77
5.0	3.13	17.44	6.86	5.66
8.0	3.25	22.06	7.92	4.60
10.0	3.34	25.98	—	—
		$\frac{k_{34}}{k_{37}}=\frac{44.98}{3.59}=12.52$		

According to Yamada[11] and Nogami, *et al.*,[12] substituent constants of NH_2- and CH_3CONH- group are −0.15 and −0.16 respectively. This fact suggests that absorption rate constants of AA and NA are almost the same. Therefore, 2.54/hr (subject A) and 3.50/hr (subject B) are assigned to the absorption rate constant of AA. Consequently, the other parameters (1.22/hr for subject A and 1.60/hr for subject B) to the elimination rate constant of AA, which includes acetylation, excretion and formation of metabolites other than NA.

Kinetic model shown in Chart 3 points out that the cumulative amount of excreted AA is expressed by 2-exponential equation and parameters are common to those of NA shown in Table II(b) except the one that corresponds to NA excretion rate constant. As SS values in Table V shows, least square fit of the above mentioned 2-exponential equation to AA data was unexpectedly poor. However, the fit was improved by adding one more exponential term as shown in the right hand side of Table V.

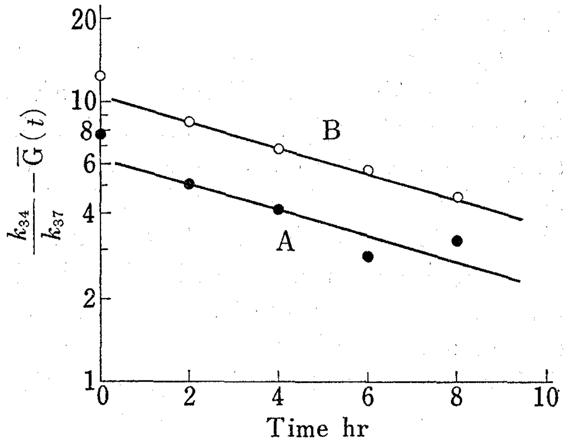

Fig. 2. Estimation of the Excretion Rate Constant of N-Acetyl-4-aminoantipyrine (See Appendix A)

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[11] H. Yamada, *Farumashia*, 3, 739 (1967).

[12] H. Nogami, M. Hanano, and H. Yamada, *Chem. Pharm. Bull.* (Tokyo), 16, 389, 580, 586 (1968).

Table V. Rate Constants, Coefficients and SS Values for Urinary AA Data

		With parameters obtained by least square fit of NA data			With parameters obtained by least square fit of NA data plus one more exponential term		
		Rate const.	Coeff.	SS	Rate const.	Coeff.	SS
(a) AA	A	2.5415	−4.1022	7.2526	0.4054	8.3619	0.1827
		1.2242	8.5163		2.5415	0.6229	
					1.2242	−4.0621	
	B	3.5000	−2.3131	3.2643	0.8923	11.6886	0.0396
		1.6000	5.0599		3.5000	1.6207	
					1.6000	−10.0638	
		3.0984	0.0375		0.1006	3.8123	
(b) AM	A	0.7391	−0.8728	0.4179	3.0984	0.0156	0.0146
		0.2214	2.3894		0.7391	− 0.2415	
					0.2214	− 1.1432	
		3.3289	0.0451		0.2286	− 3.0270	
	B	1.1519	−0.3895	2.5490	3.3289	− 0.0112	0.0282
		0.0579	5.1570		1.1519	0.1908	
					0.0576	− 8.8000	
		3.4826	0.1374		0.1882	5.8502	
(c) PB	A	0.8817	−4.4855	5.2231	3.4826	− 0.0095	0.0140
		0.5238	6.6370		0.8817	1.4810	
					0.5238	− 4.5312	
		6.3643	0.0204		0.0781	−28.5371	
	B	3.1497	−0.0845	0.5561	6.3643	0.0053	0.0197
		0.0719	1.8905		3.1497	− 0.0205	
					0.0719	31.4330	

Administration of Aminopyrine

The pharmacokinetic model for aminopyrine (AM) is much more complicated as shown in Chart 1.

Cumulative amounts of NA and AA excreted in urine after oral administration of AM to subjects A and B are listed in Table VI. Because of low dose level of aminopyrine adopted in the present study and of uncertainty involved in the analytical method especially for urine sample[5a] the authors abandoned the determination of unchanged AM excreted in urine. According to Brodie and Axelrod[5a] urinary excretion of unchanged AM after oral administration of AM to man is 2 to 4% of dose.

In this case, amount of NA in urine is expressed by 4-exponential equation (type 3, grade 3). Computer results of the fit to the data of Table VI is shown in Table II(c). Although sum of squared differences between observed and calculated values are small and apparent fit is excellent as shown in Fig. 1(c), extraordinarily large standard error values proclaim that the fit of 4-exponential equation to the data of Table VI is inappropriate.

The substituent constant for CH_3- group is reported as +0.12.[11,12] This value implies that the absorption rate constant of AM is 1.738 (=antilog of 0.12×2) times greater than that of AA (AM minus two CH_3- group). However, taking into consideration the effect of aminopyrine on gastric empting time reported by Goto, *et al.*,[13] it is reasonable to assign 3.10/hr (subject A) and 3.33/hr (subject B) of Table II(c) to absorption rate constant of AM.

As is explained in Appendix (B), rate constant of AM elimination (de-methylation and other metabolic processes) is estimated by cumulative amount of AA excreted in urine after administration of AA and AM, numerically from convolution. $\bar{G}(t)$ values calculated by the

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[13] *a*) S. Goto, O. Tsuzuki, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **19**, 944 (1971); *b*) S. Goto, O. Tsuzuki and S. Iguchi, *J. Pharm. Sci.*, **61**, 945 (1972).

Table VI. Cumulative Amount of AA and NA excreted in Urine after Ingestion of Aminopyrine

Subject A 52 kg Dose 48.8 mg			Subject B 59 kg Dose 63.3 mg		
Time hr	AA mg	NA mg	Time hr	AA mg	NA mg
2.0	0.10	0.25	1.0	0.00	0.02
3.97	0.45	0.84	2.0	0.00	0.11
6.25	0.64	1.98	4.0	0.20	0.79
8.08	0.91	3.28	6.0	0.55	1.75
10.03	1.15	4.76	8.0	0.84	3.03
11.75	1.39	6.13	10.22	1.28	4.79
14.83	1.67	8.23	12.0	1.81	6.58
19.67	1.91	10.85	14.75	2.35	9.20
23.0	—	12.66	19.50	3.19	13.20
26.0	—	14.08	22.25	3.54	15.52
29.0	—	14.93	25.08	3.83	17.83
32.33	—	15.79	28.0	—	19.98
			31.0	—	21.88
rest	0.18	16.65	rest	1.03	13.05
total	2.09	32.44	total	4.86	34.93

Table VII. Estimation of AM Elimination Rate Constant by Numerical De-convolution Method of Umemura

Time	$X(t)$	$Y(t)$	$\bar{G}(t)$	$\frac{Dy}{Dx}-\bar{G}(t)$
Subject A				
0.0	0.00	0.00	0.000	0.415
2.0	1.47	0.10	0.145	0.270
4.0	3.54	0.45	0.171	0.244
6.0	4.18	0.64	0.194	0.220
8.0	4.44	0.91	0.297	0.118
10.0	4.60	1.15	—	—
		$\frac{Dy}{Dx}=\frac{2.09}{5.04}=0.415$		
Subject B				
0.0	0.00	0.00	0.000	1.354
2.0	1.80	0.00	0.028	1.326
4.0	2.86	0.20	0.169	1.185
6.0	3.13	0.55	0.295	1.059
8.0	3.25	0.84	0.366	0.988
10.0	3.34	1.28	—	—
		$\frac{Dy}{Dx}=\frac{4.86}{3.59}=1.354$		

method of Umemura[10)] are listed in Table VII and "Sigma minus" plot of $\bar{G}(t)$ is shown on Fig. 3. From the slope of the curve, it is clear that the rate constant of aminopyrine elimination is approximately 0.17/hr for subject A and 0.052/hr for subject B. Therefore, 0.221/hr for subject A and 0.058/hr for subject B of Table II(c) were assigned to AM elimination rate constant. Assignment of the other parameters are 0.739/hr (subject A) and 1.15/hr (subject B) to AA elimination, and 0.065/hr (subject A) and 0.093/hr (subject B) to NA excretion.

Evident lack of coincidence between observed urinary data and theoretical values from the model of Chart 1 of excreted AA are observed again, as the case of AA administration, and one more exponential term was necessary to get appropriate fit (Table V(b)).

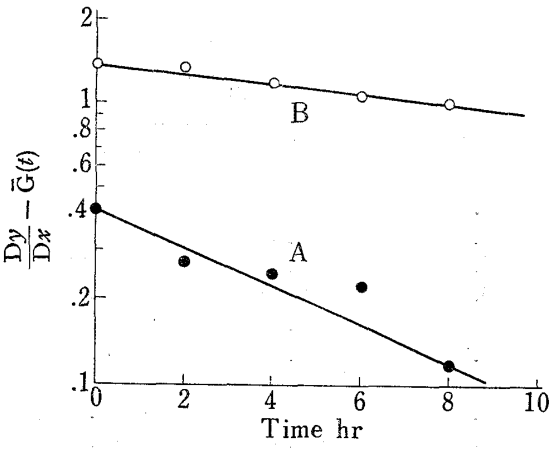

Fig. 3. Estimation of the Elimination Rate Constant of Aminopyrine (See Appendix B)

Administration of Pyrabital

Cumulative amounts of NA and AA excreted in urine after oral administration of pyrabital (PB) to subjects A and B are listed in Table VIII.

The kinetic model is the same as that of AM.

It is reported by Goto, *et al.*[13] that barbital reduces the gastric empting time of aminoyrine. Besides, barbiturates are known to behave as an inducer of the drug-metabolizing enzyme.[14] Therefore, the rate constants of AM absorption and metabolism are expected to increase, or at least be affected.

Results of least square fit of 4-exponential equation to urinary data of NA (Table VIII) are shown in Table II(d). Although SS values are relatively small indicating an excellent fit (also Fig. 1(d)). extraordinarily large standard error values suggest that fit of 4-exponential equation to the data might be unreasonable. Farther assignment of parameters were withheld accordingly. Fit of AA data with 3- and 4-exponential equation is shown in Table V(c).

TABLE VIII. Cumulative Amount of AA and NA excreted in Urine after Ingestion of Pyrabital

Subject A 52 kg Dose 69.7 mg			Subject B 59 kg Dose 74.3 mg		
Time hr	AA mg	NA mg	Time hr	AA mg	NA mg
2.0	0.14	0.48	1.0	0.02	0.06
4.0	0.49	1.79	2.0	0.04	0.22
5.88	1.04	3.98	4.0	0.13	0.75
7.83	1.61	6.19	6.0	0.32	1.94
10.0	1.89	9.08	8.25	0.56	3.52
12.0	2.16	11.29	10.0	0.69	4.72
20.92	2.69	18.06	12.0	0.83	6.20
23.93	—	20.21	14.0	0.96	7.90
27.33	—	21.81	20.5	1.36	12.63
30.33	—	22.83	23.0	—	14.52
			26.0	—	16.31
			29.0	—	17.78
rest	0.21	6.13	rest	0.17	5.74
total	2.90	28.96	total	1.53	23.52

Discussion

All the rate constants assigned in the present study are listed in Table IX. Assuming that gastro-intestinal absorption of AA is complete, the acetylation rate constant was calculated from the elimination rate constant of AA and also shown in Table IX.

Although blood concentrations were not determined in the present study, plasma concentration of AM after intravenous and oral administration are shown on Fig. 4 and 5, respec-

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[14] M. Akagi, "Yakubutsu Taisha no Seikagaku," Nanzando, Tokyo, 1970; H. Kitagawa, T. Noguchi, and R. Ito, "Kusuri no Taisha," Nankodo, Tokyo, 1971.

Table IX. Rate Constants of Aminopyrine Pharmacokinetics calculated from Urine Data

Absorption rate constants (/hr)				
Aminopyrine	A	3.098		
(k_{12})	B	3.329		
4-Aminoantipyrine	A	2.542		
(k_{13})	B	3.500		
N-Acetyl-4-amino antipyrine	A	2.066		
$k(_{14})$	B	3.082		
Elimination rate constants (/hr)				
Aminpoyrine	A	0.221		
($k_{23}+k_{25}+k_{26}$)	B	0.058		
4-Aminoantipyrine	A	0.739	1.224	
($k_{34}+k_{37}+k_{38}$)	B	1.152	1.600	
Acetylation rate constant (/hr)				
4-Aminoantipyrine	A	0.812		
(k_{34})	B	1.360		
Excretion rate constant (/hr)				
N-Acetyl-4-amino antipyrine	A	0.065	0.095	0.085
(k_{49})	B	0.093	0.095	0.091

tively, reproduced from the paper of Brodie and Axelrod.[5a)] The values were read from the graph and re-plotted on a semi-logarithmic scale. The elimination rate constant of AM calculated from the slope of the semi-logarithmic plot was 0.079/hr for intravenous data and 0.05 to 0.21/hr for *per os* data, which coincide to the values shown in Table IX.

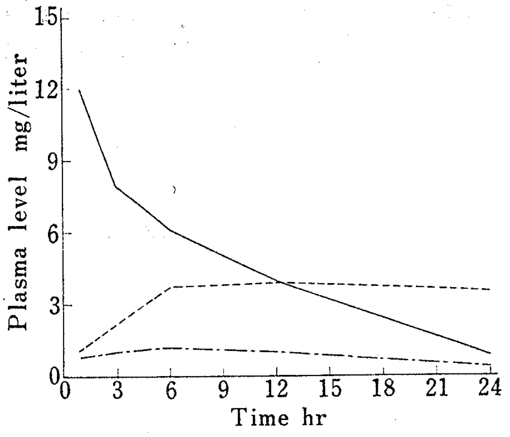

Fig. 4. Plasma Levels of Aminopyrine (solid line) and Its Metabolites, 4-Aminoantipyrine (dashed line) and N-Acetyl-4-aminoantipyrine (dash-dot line) after the IntravenousAdministration of 500 mg of Aminpoyrine to Man, reproduced from the Paper of Brodie and Axelrod

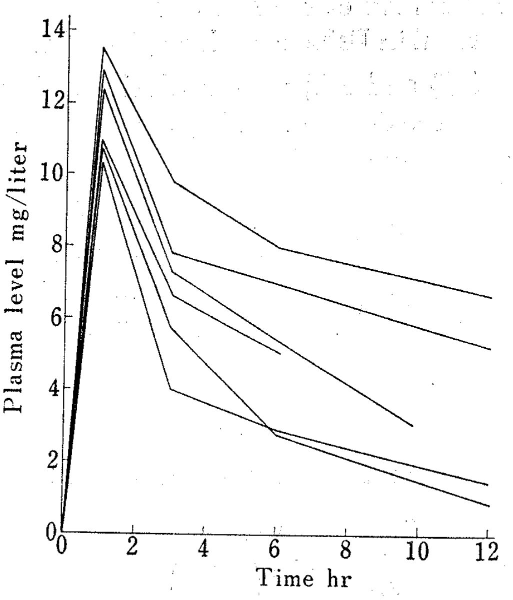

Fig. 5. Plasma Levels of Aminopyrine after Its Oral administration to Various Subjects, reproduced from the Paper of Brodie and Axelrod

Fig. 5 shows that the peak plasma concentration is attained one hour after the dosage. First order rate constant for AM absorption is calculated from Eq. 1.

$$T_{\max}=\frac{\ln k_1-\ln k_2}{k_1-k_2} \qquad \text{Eq. 1}$$

where $T_{\max}$ is the time after dosage when the plasma concentration is maximum, and k's are rate constants for absorption and elimination.

For $T_{\max}=1.0$ hr and $k_2=0.05$—0.21/hr, k_1 was calculated as 3.3—4.3/hr, which is consistent with the results of the present study (Table IX).

These facts guarantee the validity of the pharmacokinetic analysis of the present study.

Rate constants for the acetylation of sulfa-drugs[15] in man reported previously are 0.003—0.115/hr which are much smaller than those of AA shown in Table IX.

It is revealed that the urinary excretion of AA is not expressed by simple first order process. Similar phenomena have been observed with rabbits and rats by the authors of the present report besides the human subjects.

For the urinary excretion of acetaminophen glucuronide, Shibasaki, *et al.*[16] proposed a pharmacokinetic model shown in Chart 4, which contains one more exponential parameter other than the elimination rate constant of acetaminophen glucuronide. It is probable that the urinary excretion of AA is also well described by a similar model. Experiments and analysis of data are in progress and results will be reported.

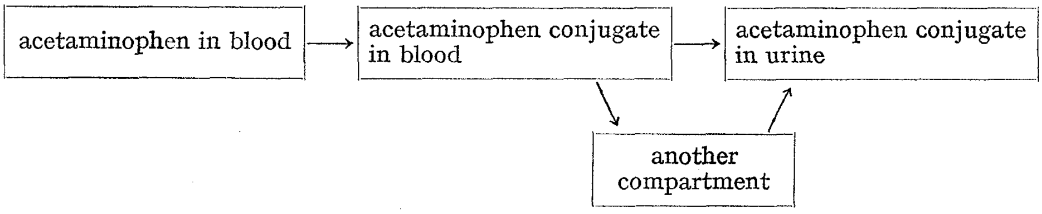

Chart 4

Appendix

(A) Estimation of the Excretion Rate Constant of NA from Cumulative Amounts of NA and AA excreted in Urine after Dosage of AA

If $X(t)$ and $Y(t)$ are cumulative amounts of AA and NA, respectively, excreted in urine and D_0 is the dose of AA, Eqs. 1a and 2a are obtained assuming first order rate processes for each of the steps of Chart 3.

$$L[X(t)] = x(s) = \frac{D_0 k_{13} k_{37}}{(s+k_{13})(s+K)s} \qquad \text{Eq. 1a}$$

$$L[Y(t)] = y(s) = \frac{D_0 k_{13} k_{34} k_{49}}{(s+k_{13})(s+K)(s+k_{49})s} \qquad \text{Eq. 2a}$$

where

$$K = k_{34} + k_{37} + k_{38}$$

Eqs. 1a and 2a are reduced to Eq. 3a.

$$y(s) = g(s)x(s) \qquad \text{Eq. 3a}$$

$$g(s) = \frac{k_{34} k_{49}}{k_{37}}\left(\frac{1}{s+k_{49}}\right) \qquad \text{Eq. 4a}$$

Inverse transform of Eq. 3a gives convolution (Eq. 5a).

$$Y(t) = \int_0^t X(\theta) G(t-\theta) d\theta \qquad \text{Eq. 5a}$$

where

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[15] K. Kakemi, T. Arita, and T. Koizumi, *Yakuzaigaku*, 25, 22 (1965).

[16] J. Shibasaki, R. Konishi, Y. Takeda, and T. Koizumi, *Chem. Pharm. Bull.* (Tokyo), 19, 1800 (1971).

$$\mathrm{G}(t) = L^{-1}[g(s)] = \frac{k_{34}k_{49}}{k_{37}}e^{-k_{49}t} \qquad \text{Eq. 6a}$$

Integration of Eq. 6a gives Eq. 7a.

$$\int_0^t \mathrm{G}(t)dt = \frac{k_{34}}{k_{37}}(1-e^{-k_{49}t}) = \bar{\mathrm{G}}(t) \qquad \text{Eq. 7a}$$

Rearrangement of Eq. 7a gives Eq. 8a and 9a.

$$\frac{k_{34}}{k_{37}} - \bar{\mathrm{G}}(t) = \frac{k_{34}}{k_{37}}e^{-k_{49}t} \qquad \text{Eq. 8a}$$

$$\log\left(\frac{k_{34}}{k_{37}} - \bar{\mathrm{G}}(t)\right) = \log\frac{k_{34}}{k_{37}} - \frac{k_{49}}{2.303}t \qquad \text{Eq. 9a}$$

The method of Umemura[10] gives $\bar{\mathrm{G}}(t)$ values directly, and k_{34}/k_{37} is calculated by Eq. 10a.

$$\frac{k_{34}}{k_{37}} = \frac{Y(\infty)}{X(\infty)} \qquad \text{Eq. 10a}$$

Semi-logarithmic plot of $k_{34}/k_{37}-\bar{\mathrm{G}}(t)$ against time t gives straight line and k_{49} is estimated from its slope.

(B) Estimation of the Elimination Rate Constant of AM from Cumulative Amount of AA after Oral Administration of AM and after Administration of AA

If $X(t)$ and $Y(t)$ are cumulative amounts of AA excreted in urine after oral administration of AA and AM respectively and Dx and Dy are respectively dose of AA and AM, then Eq. 11a and 12a are obtained.

$$L[X(t)] = x(s) = \frac{\mathrm{D}xk_{13}k_{37}}{(s+k_{13})(s+K)s} \qquad \text{Eq. 11a}$$

$$L[Y(t)] = y(s) = \frac{\mathrm{D}yk_{12}k_{23}k_{37}}{(s+k_{12})(s+K^{\circ})(s+K)s} \qquad \text{Eq. 12a}$$

where

$$K = k_{34} + k_{37} + k_{38} \text{ and } K^{\circ} = k_{23} + k_{25} + k_{26}$$

Eq. 11a and 12a are reduced to Eq. 13a.

$$y(s) = g(s)x(s) \qquad \text{Eq. 13a}$$

$$g(s) = \frac{\mathrm{D}yk_{12}(s+k_{13})k_{23}}{\mathrm{D}xk_{13}(s+k_{12})(s+K^{\circ})} \qquad \text{Eq. 14a}$$

Inverse transform of Eq. 13a gives convolution (Eq. 15a).

$$Y(t) = \int_0^t X(\theta)\mathrm{G}(t-\theta)d\theta \qquad \text{Eq. 15a}$$

where

$$\mathrm{G}(t) = L^{-1}[g(s)] = \frac{\mathrm{D}yk_{12}k_{23}}{\mathrm{D}xk_{13}(k_{12}-K^{\circ})}[(k_{13}-K^{\circ})e^{-K^{\circ}t}-(k_{13}-k_{12})e^{-k_{12}t}] \qquad \text{Eq. 16a}$$

Integration of Eq. 16a gives Eq. 17a.

$$\int_0^t \mathrm{G}(t)dt = \bar{\mathrm{G}}(t) =$$

$$\frac{\mathrm{D}yk_{12}k_{23}}{\mathrm{D}xk_{13}(k_{12}-K^{\circ})}\left[\frac{k_{13}-K^{\circ}}{K^{\circ}}(1-e^{-K^{\circ}t})-\frac{k_{13}-k_{12}}{k_{12}}(1-e^{-k_{12}t})\right] \qquad \text{Eq. 17a}$$

When $k_{12} \gg K^{\circ}$ and $k_{12}=k_{13}$, Eq. 18a is approximated.

$$\bar{G}(t) = \frac{Dy}{Dx}(1 - e^{-k^\circ t}) \tag*{Eq. 18a}$$

Rearrangement of Eq. 18a gives Eq. 19a and 20a.

$$\frac{Dy}{Dx} - \bar{G}(t) = \frac{Dy}{Dx} e^{-k^\circ t} \tag*{Eq. 19a}$$

$$\log\left(\frac{Dy}{Dx} - \bar{G}(t)\right) = \log\frac{Dy}{Dx} - \frac{K^\circ}{2.303} t \tag*{Eq. 20a}$$

Elimination rate constant of AM, K°, is estimated from the slope of straight line obtained when left hand side of Eq. 20a is plotted aginst time t. Because the absorption efficiency of AM and AA are not necessarily identical, ultimately excreted amount of AA after administration of AM and AA are used in place of doses, Dx and Dy.