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Urine Data Analysis for Pharmacokinetics of Acetanilide Hydroxylation in Man

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It was demonstrated that acetanilide hydroxylation in man is saturated by as small as 0.5 g dose. Subjects ingested low dose (100 mg) and high dose (500 mg) of acetaminophen and acetanilide. Total acetaminophen (free and conjugated) excreted in urine was determined. Ratio of amount excreted (RAE) between two dose levels was calculated. Saturation of pharmacokinetic process was verified by the value of regression coefficient of RAE to t.

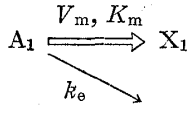
Saturation of pharmacokinetic steps causes unexpected accumulation of unchanged or metabolized drug with the repeated administration of the drug. Therefore, sufficient care is necessary not to overlook a saturable step in drug metabolism process. In the study of the previous paper,[2] the fate of acetanilide in rabbits was investigated, and it was revealed that the conversion of acetanilide to 4-hydroxyacetanilide (or acetaminophen) is saturated and that the elimination of acetanilide from the blood is delayed when excess of the drug is introduced into system circulation of the animal. Blood concentration time course was well expressed by Michaelis-Menten kinetics.

The purpose of the present paper is to see if hydroxylation of acetanilide is also saturable in man, as well as in rabbits, at ordinary dose range. In order to lessen such a burden to the subjects as venipuncture, we adhered to urine data analysis as we did in the previous study,[3] with minimum number of blood samples which were inevitable.

Theoretical

a) Linear Approximation of Saturable Step

Such a saturable step as defined by Eq. 1 and Eq. 2 is non-linear and Laplace transform[4] is of no use.



$$\frac{dA_1}{dt} = -\frac{V_m A_1}{K_m + A_1} - k_e A_1 \qquad \text{Eq. 1}$$

$$\frac{dX_1}{dt} = \frac{V_m A_1}{K_m + A_1} \qquad \text{Eq. 2}$$

Where $A_1 = A_0$, $X_1 = 0$ at $t = 0$.

1) Location: *a) 3190 Gofuku, Toyama 930, Japan; b) 1-14, Bunkyo-cho, Nagasaki 852, Japan.*
2) J. Shibasaki, T. Koizumi, and T. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1661 (1968).
3) T. Koizumi, M. Ueda, and S. Takada, *Chem. Pharm. Bull.* (Tokyo), **22**, 894 (1974).
4) J.G. Wagner, "Biopharmaceutics and Relevant Pharmacokinetics," Drug Intelligence Publications, Hamilton, Il., 1971, pp. 328—330.

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If we assume two-step-consecutive first order process which is expressed mathematically by Eq. 3 through 5, however, terminal straight line portion of X_1's "sigma minus plot"[5] coincides completely with that of X_2.

$$A_2 \xrightarrow{k_1} B \xrightarrow{k_2} X_2, \quad A_2 \xrightarrow{k_e}$$

$$\frac{dA_2}{dt} = -(k_1+k_e)A_2 \qquad \text{Eq. 3}$$

$$\frac{dB}{dt} = k_1A_2 - k_2B \qquad \text{Eq. 4}$$

$$\frac{dX_2}{dt} = k_2B \qquad \text{Eq. 5}$$

Where $A_2=A_0$, $B=X_2=0$ at $t=0$, and

$$k_1 = V_m/K_m, \qquad k_2 = (k_e+V_m/K_m)\frac{1}{1-e^{-X_1^\infty/K_m}}$$

Details of the derivation of k_1 and k_2 are discussed under Appendix I. When A_0/K_m approaches zero and the step (A_1 to X_1) becomes simple first order process, k_2 approaches infinity and the step (A_2 to X_2), too, becomes single first order process. From these facts, it is reasonable to approximate a saturable process by two-step-consecutive first order process. Such approximation permits us the use of Laplace transform and application of convolution equation[6] for recognition of saturation pharmacokinetics. When A_0/K_m is sufficiently large, the step (A_1 to X_1) is approximated with a zero order process. Such a case, however, is outside the scope of present discussion.

b) Ratio of Amount Excreted between Two Dose Levels (without Saturation)

A pharmacokinetic model is shown in Chart 1. D's and E's with subscript are the dose and time-dependent amount excreted, respectively. Arrows indicate the individual pharmacokinetic steps.

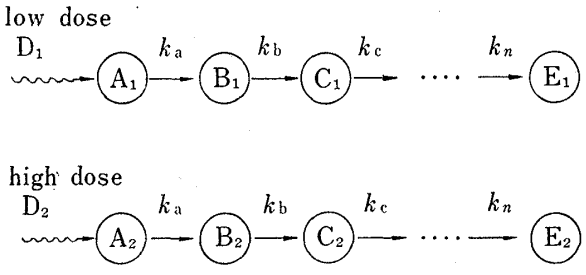

Chart 1

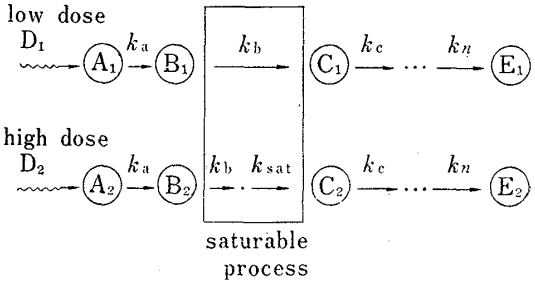

Chart 2

Laplace transform of $E_1(t)$ and $E_2(t)$ gives Eq. 6 and Eq. 7.

$$e_1(s) = D_1g_ag_b \cdots g_n \qquad \text{Eq. 6}$$

$$e_2(s) = D_2g_ag_b \cdots g_n \qquad \text{Eq. 7}$$

g's with subscript are transfer function of the respective pharmacokinetic steps, and s is a variable introduced by Laplace transform.

In this particular case, saturation does not occur and therefore, g's in Eq. 6 have one to one correspondence to those in Eq. 7. Therefore, we have Eq. 8.

5) B.K. Martin, *Brit. J. Pharmacol.*, **29**, 181 (1967).
6) G. Segre, *Ann. New York Acad. Sci.*, **57**, 918 (1962); M. Hanano, *Chem. Pharm. Bull.* (Tokyo), **15**, 994 (1967).

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$$e_2(s) = e_1(s)\frac{D_2}{D_1} \qquad \text{Eq. 8}$$

Inverse transform gives Eq. 9.

$$\text{RAE (ratio of amount excreted)} = \frac{E_2(t)}{E_1(t)} = \frac{D_2}{D_1} \qquad \text{Eq. 9}$$

c) Ratio of Amount Excreted between Two Dose Levels (with Saturation)

In Chart 2, saturable process is approximated by two-step-consecutive first order process (in rectangle).

D's and E's are the dose and the amount excreted, respectively. Laplace transform gives Eq. 10.

$$e_2(s) = \frac{D_2}{D_1}e_1(s)g_{sat} = \frac{D_2}{D_1}e_1(s)\frac{k_{sat}}{s+k_{sat}} \qquad \text{Eq. 10}$$

Inverse transform gives Eq. 11.

$$\begin{aligned} E_2(t) &= k_{sat}\frac{D_2}{D_1}\int_0^t E_1(\theta)\exp\left[-k_{sat}(t-\theta)\right]d\theta \\ &= \frac{D_2}{D_1}\left[E_1(t) - \exp\left(-k_{sat}t\right)\int_0^t \exp\left(k_{sat}\theta\right)\frac{dE_1}{d\theta}d\theta\right] \end{aligned} \qquad \text{Eq. 11}$$

Therefore, we have Eq. 12.

$$\text{RAE} = \frac{E_2(t)}{E_1(t)} = \frac{D_2}{D_1}\left[1 - \frac{\int_0^t \exp\left(k_{sat}\theta\right)\frac{dE_1}{d\theta}d\theta}{\exp\left(k_{sat}t\right)E_1(t)}\right] \qquad \text{Eq. 12}$$

Left hand side of Eq. 12 is zero at $t=0$, increases monotonously with the increase of t value, and asymptotically approaches to the dose ratio D_2/D_1.

In practice, the amount of unchanged drug or metabolites excreted in urine, $E_2(t)$, after administration of the test dose D_2 is determined periodically. In the same manner, $E_1(t)$ is determined with dose D_1, small enough not to cause saturation. Time dependency of RAE, *i.e.* $E_2(t)/E_1(t)$, is calculated. Saturation of pharmacokinetic steps is verified if regression coefficient[7] of RAE to t is significantly greater than zero.

Experimental

Material——Acetaminophen and acetanilide (the latter having been eliminated from J.P. VIII) used were J.P. grade as in the previous study.[2]

Assay——Blood concentration of acetanilide and total acetaminophen (free and conjugated) excreted in urine were estimated by the method of Brodie and Axelrod[8] with a slight modification.

Animal Study——Experiments with rabbits were carried out as described in the previous report.[2]

Human Study——Four adult male subjects apparently in good health ingested the drug solution in 100 ml water, 2 hours prior to breakfast after control urine sample had been taken. Following breakfast, the subjects were permitted to take foods and fluids as desired. Collection of total urinary output were made every one hour for 10 hours.

At interval of not less than seven days, each subject ingested low dose (100 mg) and high dose (300 mg for subject A and B, 500 mg for subject C and D) of acetaminophen and acetanilide in random order. For subject C and D, a few blood samples were taken from the upper arm vein by ordinary venipuncture.

Standard Meal——Two slices of buttered toast, a piece of processed cheese, a boiled egg, a cup of milk and a cup of black tea with sugar were used for the study on the effect of meal on drug absorption.

7) A. Sakuma, "Seibutsu Kentei Ho," Tokyo Daigaku Shuppan Kai, Tokyo, 1973, pp. 110—130.
8) B.B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **94**, 22 (1948).

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Results and Discussion

a) Rabbit Study

To test the applicability of the method described in the theoretical, studies were carried out with rabbits first, for which species it had been demonstrated that the acetanilide hydroxylation is saturated at 100 mg/kg dose level.[2)]

Low dose (50 mg/kg) and high dose (250 mg/kg) of acetaminophen and acetanilide were orally administered to a rabbit at interval not less than a week. Excreted amount of total acetaminophen (free and conjugated) were determined. Results are shown in Table I and Fig. 1.

TABLE I. Amount of Total Acetaminophen Excreted in Urine and the Ratio after Oral Administration of Low Dose and High Dose of Acetaminophen and Acetanilide to Rabbits

Time (hr)	Acetaminophen			Acetanilide		
	Low dose 100 mg	High dose 500 mg	RAE	Low dose 100 mg	High dose 500 mg	RAE
1	—	—	—	23.0	34.1	1.479
2	—	448.2	—	59.7	140.6	2.357
3	91.4	524.1	5.737	87.7	241.9	2.758
4	97.3	553.8	5.692	95.0	321.5	3.386
5	100.6	572.4	5.689	—	408.4	—
6	102.4	—	—	—	454.6	—
7	103.3	583.9	5.651	103.5	489.9	4.734
8	103.9	586.3	5.642	104.0	508.8	4.893
9	104.2	587.0	5.633	104.3	519.1	4.978
10	104.4	587.4	5.125	104.4	524.7	5.027
$S(x,x)$			17.2			21.2
$S(y,y)$			0.0057			5.9244
$S(x,y)$			−0.3017			11.1208
b(reg. coef.)[a)]			−0.0175			0.5246
S			0.00042			0.09075
$\sqrt{V_b}$			0.00286			0.03915
t_{cal}			6.1417			13.3986
$t(3,\ 0.05)=3.182$			signif.			signif.
$\sqrt{V_b'}$[b)]				0.0400		
t'_{cal}				13.552		
$t(4,\ 0.05)=2.776$				signif.		

a) Regression Coefficient was calculated with first 5 RAE values.
b) Because of heteroscedasticity, approximation of Cochran-Cox was adopted. (A. Sakuma, "Seibutsu Kentei Ho," Tokyo Daigaku Shuppan Kai, Tokyo, 1973, pp. 88—89).

Regression coefficient of RAE was almost zero for acetaminophen, whereas that was 0.52 for acetanilide. It is clear from these results that the saturation of pharmacokinetic process can be verified by the value of regression coefficient of RAE to t.

b) Effect of Meal on Gastrointestinal Absorption of Acetaminophen in Man

Prior to human study, effect of meal on acetaminophen absorption was investigated by ingesting the drug; (1) after overnight fasting and two hours before the standard meal, (2) in the midst of taking the standard meal, and (3) one hour after taking the standard meal. In each case, 100 mg of acetaminophen was given as the solution, and the excreted amount of total acetaminophen (free and conjugated) was determined. Results are shown in Table II, III and IV.

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Assuming that gastric empting time is shortest from empty stomach, RAE (regimen(2)/regimen(1), regimen(3)/regimen(1)) were calculated and compared. On regimen(2), regression coefficient was not significant for subject B and C. For subject A, however, that was significant. Zerotime shift of a half hour reduced the regression coefficient of subject A to no significant. On regimen (3), regression coefficient was significant for all the subjects, and 2.0, 0.5 and 0.6 hours of zerotime shift was necessary to make it no significant. These facts show that drug absorption started a few hours after the actual drug ingestion. Those period of time which were necessary to make regression coefficient no significant could be considered as the measure of gastric empting time.

To perform zerotime shift, in practice, urinary excretion data were first fitted to a multi-exponential equation by the method reported previously.[9]

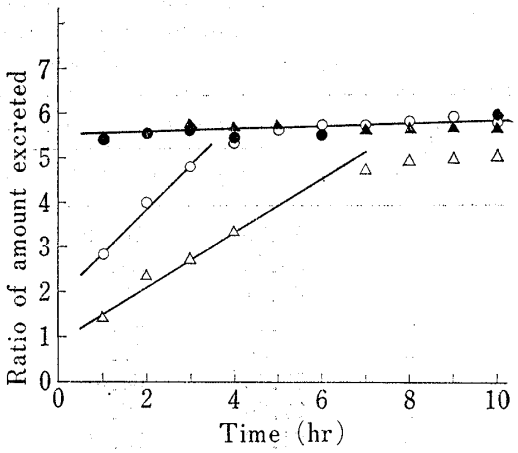

Fig. 1. Time Dependence of RAE

triangle···rabbit
circle···subject C
solid mark···acetaminophen
open mark···acetanilide

c) RAE of Acetaminophen and Acetanilide in Man

To four subjects, low dose and high dose of acetaminophen and acetanilide were administered, and total acetaminophen excreted in urine were determined periodically. Results are shown in Table V through VIII.

Table II. Amount of Total Acetaminophen Excreted in Urine after Oral Administration of Acetaminophen (100 mg) under Various Extent of Stomach Fill (Subject A)

Time (hr)	Amount excreted (mg)			Ratio of amount excreted			
	Regim(1)	Regim(2)	Regim(3)	(2)/(1)	(2)/(1)[a)]	(3)/(1)	(3)/(1)[b)]
1	10.99	6.53	0.23	0.5942	1.1374	0.0209	0.8681
2	29.80	19.95	1.86	0.6695	0.9034	0.0624	0.6493
3	45.02	33.34	9.54	0.7406	0.9207	0.2119	0.6777
4	55.78	48.98	19.35	0.8781	0.9772	0.3469	0.7444
5	64.69	60.12	30.51	0.9294	1.0138	0.4716	0.7800
6	70.22	70.19	41.52	0.9996	—	0.5913	—
7	75.29	78.62	50.46	1.0442	—	0.6702	—
8	79.33	85.07	57.38	1.0724	—	0.7233	—
9	82.08	89.78	61.92	1.0938	—	0.7544	—
10	84.34	94.00	64.76	1.1145	—	0.7678	—
$S(x,x)$				10.0	10.0	10.0	10.0
$S(y,y)$				0.0787	0.0347	0.1439	0.0301
$S(x,y)$				0.8790	−0.1734	1.1859	−0.0811
b (reg. coef.)[c)]				0.0879	−0.0173	0.1186	−0.0081
S				0.0014	0.0317	0.0033	0.0294
$\sqrt{V_b}$				0.0069	0.0325	0.0105	0.0313
t_{cal}				12.83	0.5335	11.34	0.2591
$t(3,\ 0.05)=3.182$				signif.	no sig.	signif.	no sig.

a) zerotime shift=0.5 hr
b) zerotime shift=2.0 hr
c) Regression coefficient was calculated with first 5 RAE values.

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

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Table III. Amount of Total Acetaminophen Excreted in Urine after Oral Administration of Acetaminophen (100 mg) under Various Extent of Stomach Fill (Subject B)

Time (hr)	Amount excreted (mg)			Ratio of amount excreted		
	Regim.(1)	Regim.(2)	Regim.(3)	(2)/(1)	(3)/(1)	(3)/(1)[a]
1	14.36	10.19	7.38	0.7096	0.5142	0.9060
2	30.53	24.99	21.89	0.8185	0.7170	1.0459
3	45.41	39.53	40.17	0.8705	0.8846	1.0625
4	57.74	49.55	54.57	0.8582	0.9451	1.0625
5	66.36	57.82	67.11	0.8713	1.0113	1.0811
6	73.15	64.63	76.86	0.8835	1.0507	—
7	77.26	69.30	83.61	0.8970	1.0822	—
8	81.24	73.38	88.98	0.9032	1.0953	—
9	84.24	76.78	93.69	0.9114	1.1122	—
10	87.01	79.83	97.04	0.9175	1.1153	—
$S(x,x)$				10.0	10.0	10.0
$S(y,y)$				0.0187	0.1604	0.0203
$S(x,y)$				0.3631	1.2223	0.3668
b (reg. coef.)[b]				0.0363	0.1222	0.0367
S				0.0055	0.0110	0.0069
$\sqrt{V_b}$				0.0135	0.0191	0.0152
t_{cal}				2.6845	6.3874	2.4211
$t(3,\ 0.05)=3.182$				no sig.	signif.	no sig.

a) zerotime shift=0.5 hr
b) Regression coefficient was calculated with first 5 RAE values.

Table IV. Amount of Total Acetaminophen Excreted in Urine after Oral Administration of Acetaminophen (100 mg) under Various Extent of Stomach Fill (Subject C)

Time (hr)	Amount excreted (mg)			Ratio of amount excreted		
	Regim.(1)	Regim.(2)	Regim.(3)	(2)/(1)	(3)/(1)	(3)/(1)[a]
1	4.85	5.44	1.13	1.1216	0.2330	1.1196
2	15.01	17.52	8.52	1.1672	0.5676	1.0813
3	25.33	30.94	21.24	1.2215	0.8385	1.0932
4	36.30	43.46	32.23	1.1972	0.8879	1.0435
5	42.13	51.72	41.45	1.2276	0.9839	1.0968
6	49.28	58.83	49.12	1.1938	0.9968	—
7	54.60	65.46	54.80	1.1989	1.0037	—
8	58.50	70.91	58.77	1.2121	1.0046	—
9	61.65	75.46	62.35	1.2240	1.0114	—
10	64.70	78.89	65.05	1.2193	1.0054	—
$S(x,x)$				10.0	10.0	10.0
$S(y,y)$				0.0077	0.3708	0.0032
$S(x,y)$				0.2420	1.8221	−0.0834
b (reg. coef.)[b]				0.0242	0.1822	−0.0083
S				0.00186	0.0388	0.00246
$\sqrt{V_b}$				0.00788	0.0360	0.00906
t_{cal}				3.0724	5.0659	0.9203
$t(3,\ 0.05)=3.182$				no sig.	signif.	no sig.

a) Zerotime shift=0.6 hr
b) Regression coefficient was calculated with first 5 RAE values.

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Table V. Amount of Total Acetaminophen Excreted inUrine and the Ratio after Oral Administration of Low Dose and High Dose of Acetaminophen and Acetanilide to Subject A

Time (hr)	Acetaminophen			Acetanilide		
	Low dose 100 mg	High dose 300 mg	RAE	Low dose 100 mg	High dose 300 mg	RAE
1	8.15	12.20	1.4969	2.12	6.52	3.0755
2	21.46	48.11	2.2418	12.36	26.55	2.1481
3	41.84	82.80	1.9790	27.22	54.73	2.0107
4	59.31	117.41	1.9796	41.27	87.33	2.1161
5	73.48	—	—	53.98	119.15	2.2073
6	84.62	162.02	1.9147	64.06	140.76	2.1973
7	92.77	184.93	1.9934	71.62	161.13	2.2498
8	100.42	198.26	1.9743	78.41	178.31	2.2741
9	105.43	207.30	1.9662	82.23	193.46	2.3527
10	—	213.13	—	87.32	203.48	2.3303
$S(x,x)$			14.8			10.0
$S(y,y)$			0.2896			0.7499
$S(x,y)$			0.5657			−1.7684
b (reg. coef.)[a]			0.0382			−0.1768
S			0.2680			0.4372
$\sqrt{V_b}$			0.0777			0.1207
t_{cal}			0.4920			1.4649
$t(3,\ 0.05)=3.182$			no sig.			no sig.

a) Regression coefficient was calculated with first 5 RAE values.

Table VI. Amount of Total Acetaminophen Excreted in Urine and the Ratio after Oral Administration of Low Dose and High Dose of Acetaminophen and Acetanilide to Subject B

Time (hr)	Acetaminophen			Acetanilide		
	Low dose 100 mg	High dose 300 mg	RAE	Low Dose 100 mg	High dose 300 mg	RAE
1	7.52	25.23	3.3551	4.84	9.86	2.0372
2	19.56	66.05	3.3768	14.06	30.02	2.1351
3	29.54	95.17	3.2217	25.47	56.58	2.2214
4	36.24	119.30	3.2919	36.40	83.10	2.2830
5	40.37	140.08	3.4699	46.45	109.60	2.3595
6	43.77	156.47	3.5748	54.63	129.46	2.3698
7	46.31	170.01	3.6711	62.62	148.08	2.3647
8	49.02	178.52	3.6418	68.79	161.47	2.3473
9	51.45	185.95	3.6142	73.48	173.06	2.3552
10	53.55	191.85	3.5826	79.43	184.32	2.3205
$S(x,x)$			10.0			10.0
$S(y,y)$			0.0347			0.0632
$S(x,y)$			0.1447			0.7925
b (reg. coef.)[a]			0.0145			0.0793
S			0.03262			0.00043
$\sqrt{V_b}$			0.03298			0.00381
t_{cal}			0.4398			20.83
$t(3,\ 0.05)=3.182$			no sig.			signif
$\sqrt{V_b}'$[b]				0.0332		
t_{cal}				1.9515		
$t(4,\ 0.05)=2.776$				no sig.		

a) Regression coefficient was calculated with first 5 RAE values.
b) Because of heteroscedasticity, approximation of Cochran-Cox was adopted. (A. Sakuma, "Seibutsu Kentei Ho," Tokyo Daigaku Shuppan Kai, Tokyo, 1973, pp. 88—89)

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Table VII. Amount of Total Acetaminophen Excreted in Urine and the Ratio after Oral Administration of Low Dose and High Dose of Acetaminophen and Acetanilide to Subject C

Time (hr)	Acetaminophen			Acetanilide		
	Low dose 100 mg	High dose 500 mg	RAE	Low dose 100 mg	High dose 500 mg	RAE
1	10.52	56.60	5.3802	5.03	14.41	2.8648
2	25.71	143.12	5.5667	14.02	55.47	3.9565
3	42.97	244.02	5.6788	23.26	111.57	4.7966
4	58.80	317.90	5.4065	33.74	185.49	5.4976
5	66.93	375.56	5.6112	43.60	253.76	5.8202
6	74.42	412.85	5.5476	52.34	302.14	5.7726
7	78.81	451.64	5.7307	60.02	345.56	5.7574
8	83.07	476.02	5.7303	66.27	387.97	5.8544
9	86.27	498.09	5.7736	71.07	422.26	5.9415
10	88.98	518.16	5.8233	74.72	447.03	5.9827
$S(x,x)$			10.0			10.0
$S(y,y)$			0.0678			5.7574
$S(x,y)$			0.3018			7.4519
b (reg. coef.)[a]			0.0302			0.7452
S			0.0587			0.2043
$\sqrt{V_b}$			0.0442			0.0825
t_{cal}			0.683			9.029
$t(3,\ 0.05)=3.182$			no sig.			signif.
$\sqrt{V_b'}$				0.0935		
t'_{cal}				7.65		
$t(6,\ 0.05)=2.447$				signif.		

a) Regression coefficient was calculated with first 5 RAE values.

Table VIII. Amount of Total Acetaminophen Excreted in Urine and The Ratio after Oral Administration of Low Dose and High Dose of Acetaminophen and Acetanilide to Subject D

Time (hr)	Acetaminophen			Acetanilide		
	Low dose 100 mg	High dose 500 mg	RAE	Low dose 100 mg	High dose 500 mg	RAE
1	—	17.03	—	3.17	5.80	1.8297
2	18.00	78.18	4.3433	16.04	36.75	2.2911
3	37.28	146.78	3.9372	38.78	77.86	2.0077
4	49.41	208.22	4.2141	52.12	138.21	2.6518
5	60.96	258.73	4.2443	69.44	217.97	3.1390
6	68.44	297.90	4.3527	84.70	292.02	3.4477
7	74.75	325.48	4.3542	91.76	345.78	3.7683
8	79.30	345.59	4.3580	98.42	389.73	3.9599
9	82.26	361.56	4.3953	104.18	421.37	4.0446
10	85.12	373.25	4.3850	109.25	442.08	4.0465
$S(x,x)$			10.0			10.0
$S(y,y)$			0.1134			1.0992
$S(x,y)$			0.3259			2.9793
b (reg. coef.)[a]			0.0326			0.2979
S			0.1028			0.2116
$\sqrt{V_b}$			0.0585			0.0840
t_{cal}			0.557			3.548
$t(3,\ 0.05)=3.182$			no sig.			signif.
$\sqrt{V_b'}$				0.1023		
t'_{cal}				2.592		
$t(6,\ 0.05)=2.447$				signif.		

a) Regression coefficient was calculated with first 5 RAE values.

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In all of the subjects, regression coefficient of RAE of acetaminophen was 0.01 to 0.04 and difference from zero was no significant, indicating that the saturation was not observed in acetaminophen pharmacokinetics at the dose level examined. Contrary to this, in subject C and D who ingested 500 mg dose (8.5 to 9.1 mg/kg), regression coefficient showed significant difference, indicating the saturation in acetanilide pharmacokinetics. While in subjects A and B who received 300 mg dose (5.5 to 6.0 mg/kg), difference was not significant and the existence of a saturable step was not confirmed at this dose level.

d) Blood Concentration of Acetanilide

On the basis of the results mentioned above, it is clear that pharmacokinetic steps of acetanilide are saturable at high dose level of 8.5 to 9.1 mg/kg, and that the saturable step is not other than the absorption and the hydroxylation of acetanilide, since acetaminophen showed no sign of saturation. Problem now is to discriminate the truely saturable step among these two steps. For this purpose, subjects C and D ingested another 100 mg and 500 mg dose of acetanilide, and blood concentration of unchanged drug was determined. Results are shown in Table IX.

Table IX. Blood Concentration Ratio between Two Dose Levels of Acetanilide in Man

Time (hr)	Subject C			Subject D		
	Low dose 100 mg (mg/dl)	High dose 500 mg (mg/dl)	Ratio	Low dose 100 mg (mg/dl)	High dose 500 mg (mg/dl)	Ratio
0.5	0.084	0.638	7.595	0.124	0.838	6.758
1.0	0.072	0.474	6.583	0.094	0.504	5.362

At 0.5 and 1.0 hour after dosage, blood concentration ratio (blood conc. with 500 mg dose/blood conc. with 100 mg dose) was greater than the dose ratio (500 mg/100 mg=5.0) in the both subjects, and the concentration ratio decreased as time proceeded. This sort of phenomenon does not occur if the absorption step is saturated (see Appendix II). Consequently, it is concluded that the hydroxylation process of acetanilide is saturated in man at high dose of 8.5 to 9.1 mg/kg.

Conclusion

On the basis of the facts described so far, it is clear that the hydroxylation process of acetanilide is saturated by as little as 0.5 g dose to man. Relatively high toxicity of this drug may be partly due to the occurance of saturation in metabolic process at such a low dose level.

Until recently, ethanol,[10] salicylate[11] and salicylamide[12] were the few drugs known to be metabolized in man by apparent zero order kinetics in the therapeutic dose range. Acetanilide is added to the group of these drugs.

10) H.W. Newman, R.H.L. Wilson, and E.J. Newman, *Science*, **116**, 328 (1952).
11) G. Levy, *J. Pharm. Sci.*, **54**, 959 (1965); E. Nelson, M. Hanano, and G. Levy, *J. Pharmacol. Exptl. Therap.*, **153**, 159 (1966).
12) G. Levy and T. Matsuzawa, *J. Pharmacol. Exptl. Therap.*, **156**, 285 (1967).

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Appendix

I. Derivation of k_1 and k_2

a) Terminal straight line portion of X_1's "sigma minus plot"

Separating the variables of Eq. 1, we have Eq. 1a, which gives Eq. 2a by integration.

$$k_e dt = -\frac{K_m + A_1}{A_1(K_m + V_m/k_e + A_1)} dA_1 \qquad \text{Eq. 1a}$$

$$k_e t = \frac{K_m}{K_m + V_m/k_e}\left[\log\frac{A_0}{A_1} + \frac{V_m/K_m}{k_e}\log\frac{A_0 + K_m + V_m/k_e}{A_1 + K_m + V_m/k_e}\right] \qquad \text{Eq. 2a}$$

Integration of Eq. 1 after substituting with Eq. 2 gives Eq. 3a.

$$X_1 = A_0 - A_1 - k_e\int_0^t A_1 dt \qquad \text{Eq. 3a}$$

Eq. 3a is transformed to Eq. 4a by integration by parts.

$$X_1 = A_0 - A_1 - k_e A_1 t - k_e\int_{A_1}^{A_e} t dA_1 \qquad \text{Eq. 4a}$$

Substituting the fourth term of Eq. 4a by Eq. 2a after integration, we have Eq. 5a.

$$X_1 = \frac{V_m}{k_e}\log\frac{A_0 + K_m + V_m/k_e}{A_1 + K_m + V_m/k_e} \qquad \text{Eq. 5a}$$

At $t=\infty$ we have Eq. 6a, since A_1 becomes zero.

$$X_1^{\infty} = \frac{V_m}{k_e}\log\frac{A_0 + K_m + V_m/k_e}{K_m + V_m/k_e} \qquad \text{Eq. 6a}$$

Consequently sigma minus value of X_1 is given by Eq. 7a.

$$\text{Sigma minus value of } X_1 = X_1^{\infty} - X_1 = \frac{V_m}{k_e}\log\frac{A_1 + K_m + V_m/k_e}{K_m + V_m/k_e}$$

$$= \frac{V_m}{k_e}\log(1+z) = \frac{V_m}{k_e}\left(z - \frac{1}{2}z^2 + \frac{1}{3}z^3 - \cdots\right) \qquad \text{Eq. 7a}$$

Where $z = A_1/(K_m + V_m/k_e)$.

When t is sufficiently large, A_1 becomes very small and Eq. 8a is obtained.

$$\log(X_1^{\infty} - X_1) = \log\frac{V_m/k_e}{K_m + V_m/k_e} + \log A_1 \qquad \text{Eq. 8a}$$

Substituting Eq. 2a and Eq. 6a into Eq. 8a, we have final Eq. 9a.

$$\log(X_1^{\infty} - X_1) = \log\frac{A_0 V_m/K_m}{k_e + V_m/K_m} + \frac{X_1^{\infty}}{K_m} - (k_e + V_m/K_m)t \qquad \text{Eq. 9a}$$

Eq. 9a defines the terminal straight line portion of X_1's sigma minus plot.

b) Sigma minus value of X_2

Simultaneous integration of Eq. 3 through 5 gives Eq. 10a.

$$X_2 = \frac{k_1 A_0}{k_e + k_1}\left[1 - \frac{k_2 e^{-(k_e+k_1)t}}{k_2 - (k_e + k_1)} - \frac{(k_e + k_1)e^{-k_2 t}}{(k_e + k_1) - k_2}\right] \qquad \text{Eq. 10a}$$

At $t=\infty$ we have Eq. 11a.

$$X_2 = \frac{k_1 A_0}{k_e + k_1} \qquad \text{Eq. 11a}$$

Therefore sigma minus value of X_2 is given by Eq. 12a.

$$X_2^{\infty} - X_2 = \frac{k_1 A_0}{k_e + k_1}\left[\frac{k_2 e^{-(k_e+k_1)t}}{k_2 - (k_e + k_1)} + \frac{(k_e + k_1)e^{-k_2 t}}{(k_e + k_1) - k_2}\right] \qquad \text{Eq. 12a}$$

Assuming that k_2 is greater than $(k_e + k_1)$ and t is sufficiently large, we have Eq. 13a.

$$\log(X_2^{\infty} - X_2) = \log\frac{k_1 A_0}{k_e + k_1} + \log\frac{k_2}{k_2 - (k_e + k_1)} - (k_e + k_1)t \qquad \text{Eq. 13a}$$

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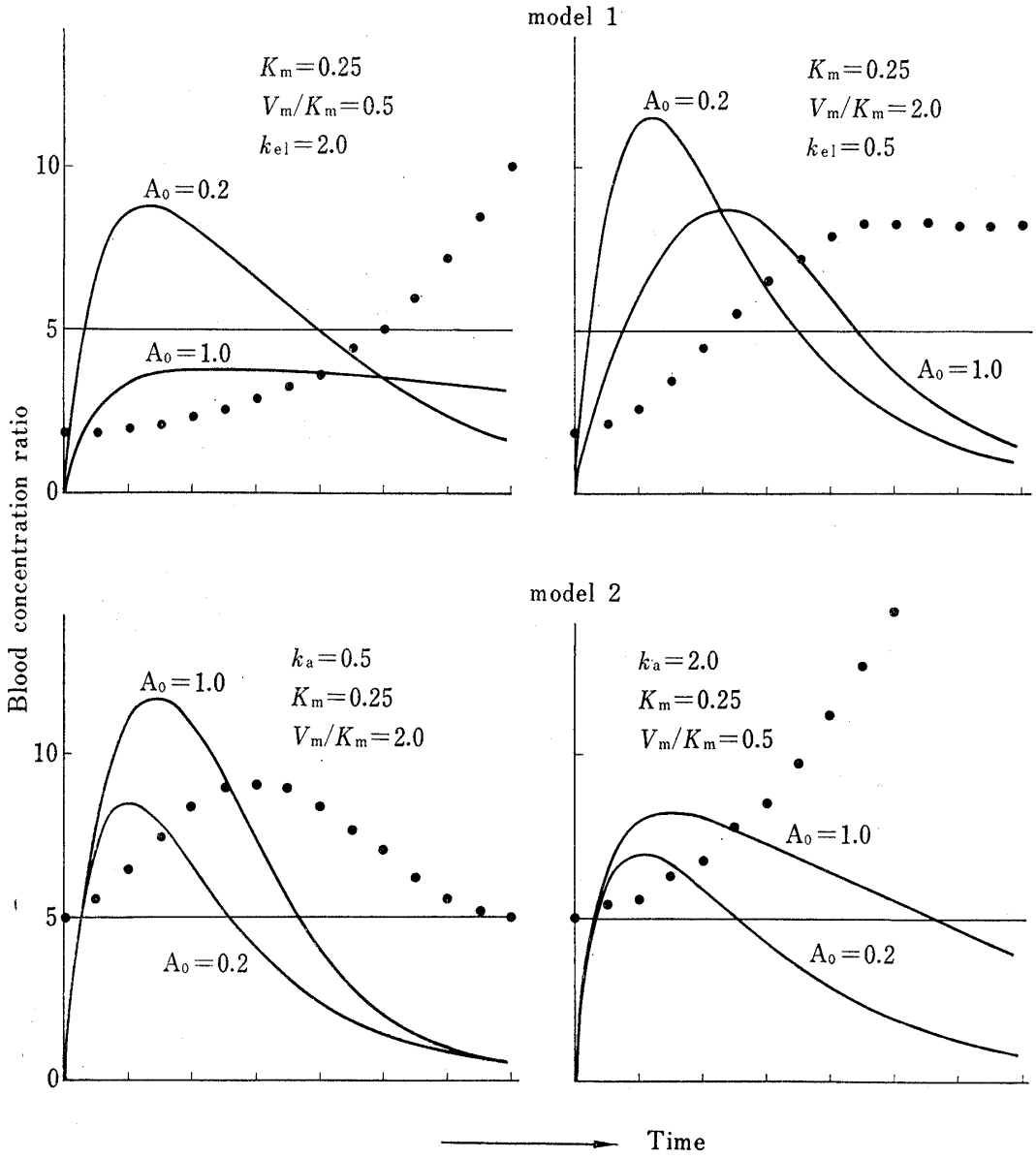

Fig. 2. Ratio of Blood Concentration with Two Dose Levels in Saturable Pharmacokinetic Models

smooth curve···normalized blood conc. (C/A_0)
dotted curve···ratio of blood conc. (C with $A_0=1.0$/C with Ao=0.2)
straight line···ratio of dose (1.0/0.2=5.0)

c) Evaluation of k_1 and k_2

Let Eq. 9a be equal to Eq. 13a, and we have Eq. 14a and 15a.

$$k_1 = V_m/K_m \qquad \text{Eq. 14a}$$

$$\frac{X_1^\infty}{K_m} = \log \frac{k_2}{k_2-(k_e+k_1)} \qquad \text{Eq. 15a}$$

Rearranging Eq. 15a for k_2, we have Eq. 16a.

$$k_2 = (k_e + V_m/K_m)\frac{1}{1-e^{-X_1^\infty/K_m}} \qquad \text{Eq. 16a}$$

II. Blood Concentration Ratio between Two Dose Levels in Saturable Pharmacokinetic Models

Eq. 17a through 20a define model 1 which has saturable step in the absorption process.

$$A \xrightarrow{V_m,\ K_m} B \xrightarrow{k_{el}}$$

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$$\frac{dA}{dt} = -\frac{V_m A}{K_m + A} \qquad \text{Eq. 17a}$$

$$\frac{dB}{dt} = \frac{V_m A}{K_m + A} - k_{el} B \qquad \text{Eq. 18a}$$

$$A = A_0,\ B = 0 \text{ at } t = 0 \qquad \text{Eq. 19a}$$

$$C = B/V_d \qquad \text{Eq. 20a}$$

Where A and B are the amount of drug at absorption site and in the body, respectively. V_d is the volume of distribution and C is blood concentration.

Eq. 19a through 22a define model 2 which has the saturable step in the elimination process.

$$A \xrightarrow{k_a} B \xRightarrow{V_m, K_m}$$

$$\frac{dA}{dt} = -k_a A \qquad \text{Eq. 21a}$$

$$\frac{dB}{dt} = k_a A - \frac{V_m B}{K_m + B} \qquad \text{Ea. 22a}$$

C/A_0 with two dose levels were computed by an analog computer and blood concentration ratio between two dose levels (blood conc. with higher dose/blood conc. with lower dose) was calculated with the various values of k_a, k_{el}, V_m and K_m. Results are shown in Fig. 2.

From Fig. 2, it is clear that for model 1 concentration ratio is less than the dose ratio (1.0/0.2=5.0) at small t, increases gradually and approaches a certain value. For model 2, however, the ratio of blood concentration once exceeds the final asymptotic value and approaches to it from higher value. And this is the case which was observed with pharmacokinetics of acetanilide in man.

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