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Mutual Inhibition in Hydroxylation of Acetanilide and N-Phenylurea in Rabbits[1]

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Blood concentration of unchanged N-phenylurea and the urinary excreted amount of its metabolites were determined periodically, after intravenous administration of N-phenylurea.

Blood concentration time course of N-pheylurea observed was well explained by a pharmacokinetic model that assumes Michaelis Menten equation for hydroxylation step and a first order process for the metabolism of N-phenylurea other than hydroxylation. This result is analogous to that of acetanilide reported previously.

In addition, elimination rate of acetanilide from the blood was affected and reduced by the simultaneous administration of N-phenylurea. It was proved that the hydroxylation process of these drugs was mutually inhibited.

Computation of the theoretical values were carried out by means of an analog computer.

Extensive studies on the drug biotransformation interaction in man were carried out by Levy, *et al.*[3] on salicylic acid and salicylamide,[3a] benzoic acid and salicylic acid,[3b] acetaminophen and salicylamide,[3c] and on acetaminophen and salicylic acid[3b] as well as on the capacity limited pharmacokinetics of salicylamide sulfate[4] and salicyluric acid[5] formation. All of these studies, however, treated the conjugation process of drug metabolism. In the previous paper[6] the authors of the present report have shown that the hydroxylation, the process that precedes the conjugation, in rabbits is capacity limited. The saturation effects were encountered when 30 to 100 mg/kg of acetanilide was given to rabbits, this amount being on the body weight basis ten times greater than the usual therapeutic dose of the drug. Since drug metabolism involves enzymic reaction, the saturation of the hydroxylation process is not surprising. Even expected is that a simultaneous administration of two or more compounds which share the common metabolic pathway will lead to mutual inhibition of the formation of respective hydroxyl metabolites.

An exploration of such a mutual pharmacokinetic effects of drug sharing the common metabolic route is highly pertinent. Informations of such drug biotransformation interaction may result in safer and more effective dosage regimen.

The purpose of the present study is to investigate the possibility of mutual inhibition of hydroxylation process by analyzing the blood data after simultaneous administration of acetanilide and N-phenylurea.

N-Phenylurea consists the partial chemical structure of an analgesic and antipyretic, Cynnapyrine, which was taken up in J.P.VI. Hydroxylation rate of this compound in rabbits

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2) Location: *a) 3190 Gofuku, Toyama, 930, Japan; b) 1–14 Bunkyocho, Nagasaki, 852, Japan.*
3) *a)* G. Levy and J.A. Procknal, *J. Pharm. Sci.*, **57**, 1330 (1968); *b)* L.P. Amsel and G. Levy, *ibid.*, **58**, 321 (1969); *c)* G. Levy and H. Yamada, *ibid.*, **60**, 215 (1971); *d)* G. Levy and C. Regardh, *ibid.*, **60**, 608 (1971).
4) G. Levy and T. Matsuzawa, *J. Pharm. Sci.*, **55**, 222 (1966).
5) G. Levy, *J. Pharm. Sci.*, **54**, 496, 959 (1965).
6) J. Shibasaki, T. Koizumi, and T. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1661 (1968).

was investigated by Bray, *et al.*[7] A study on the complete metabolic pathway is now in progress and the preliminary results[8] show that almost all of the excreted metabolites are converted to *p*-aminophenol and aniline by treatment with conc. HCl in an autoclave. Therefore metabolites of N-phenylurea which are converted ultimately to *p*-aminophenol are referred to as total hydroxyl metabolites and those that give aniline as total non-hydroxyl metabolites.

Theoretical

As is discussed in the appendix, two-compartment distribution and elimination model is well approximated by single compartment elimination model with shifted zero-time, provided that the distribution rate is sufficiently large compared with the elimination rate.

Therefore the following single compartment model was assumed to govern the metabolism of acetanilide and N-phenylurea after intravenous administration of these drugs and attainment of equilibrium between acetanilide and N-phenylurea in blood with these materials in the other fluid of distribution.

$$\text{Acetanilide} \overset{V_a,\,K_a}{\Longrightarrow} p\text{-Hydroxyacetanilide}$$

$$\text{N-Phenylurea} \overset{V_p,\,K_p}{\Longrightarrow} \text{Hydroxyl metabolites}$$

$$\text{N-Phenylurea} \xrightarrow{k_n} \text{Non-hydroxyl metabolites}$$

In the above scheme acetanilide and N-phenylurea were assumed to act mutually as competitive or non-competitive inhibitors of hydroxylation reaction. V's and K's with subscripts are Michaelis Menten kinetic constants in mg/dl/hr and mg/dl, respectively for the overall hydroxylation step. k_n is the first order rate constant in reciprocal hours for the metabolic process of N-phenylurea other than hydroxylation. If at any time (t), A is the concentration of acetanilide in blood in mg/dl and P that of N-phenylurea in the same unit, then the differential equations Eq. 1 through 4 describe the scheme shown.

(Elimination of acetanilide inhibited by N-phenylurea competitively)

$$\frac{dA}{dt} = -\frac{K_p V_a A}{K_a K_p + K_p A + K_a P} \qquad \text{Eq. 1}$$

(Elimination of N-phenylurea inhibited by acetanilide competitively)

$$\frac{dP}{dt} = -\frac{K_a V_p P}{K_a K_p + K_p A + K_a P} - k_n P \qquad \text{Eq. 2}$$

(Elimination of acetanilide inhibited by N-phenylurea non-competitively)

$$\frac{dA}{dt} = -\frac{K_p V_a A}{K_a K_p + K_p A + K_a P + A P} \qquad \text{Eq. 3}$$

(Elimination of N-phenylurea inhibited by acetanilide non-competitively)

$$\frac{dP}{dt} = -\frac{K_a V_p P}{K_a K_p + K_p A + K_a P + A P} - k_n P \qquad \text{Eq. 4}$$

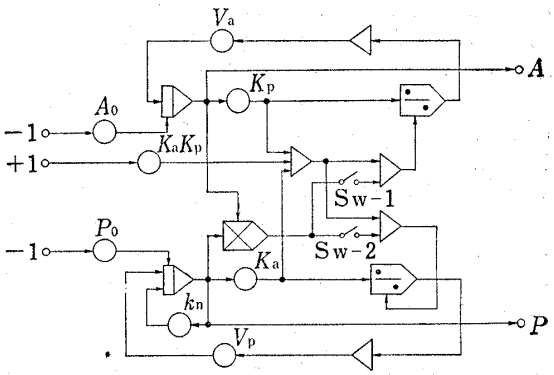

Fig. 1. Analog Computer Program for the Solution of Eq. 1 through 4

Switches are "off" for competitive inhibition model (Eq. 1 and 2) and "on" for non-competitive inhibition model (Eq. 3 and 4).

Derivation of Michaelis Menten equation in the presence of competitive or non-competitive inhibitor is found elsewhere.[9]

7) H.G. Bray, B.G. Humphris, W.V. Thorpe, K. White, and P.B. Woods, *Biochem. J.*, **52**, 412 (1952).
8) To be published.
9) H.B. Bull, "An Introduction to Physical Biochemistry," F.A. Davis Company, Philadelphia, Pa., 1964, pp. 373—376.

Eq. 1 through 4 are too complex to be solved for A and P explicitly. A and P *versus* time curve, however, can be obtained as the output of an analog computer[10] programmed as shown in Fig. 1.

Experimental

Animal——Unanesthetized male white rabbits weighing 2 to 3.5 kg were used. N-Phenylurea was dissolved in water to make 200 mg/30 ml solution and administered intravenously. As for acetaminophen, acetanilide and phenacetin, 200 mg/10 ml, 200 mg/50 ml and 100 mg/50 ml solution was given to rabbits, respectively. Blood specimens were taken (0.9 ml each) with one ml syringe containing 0.1 ml of 3.8% sodium citrate solution from ear vein. Urine collection was made through Nelaton's catheter inserted to the bladdar. Although food was withheld during experiment, water was given through the catheter passed to the stomach to keep constant urine output.

Drug——Acetanilide, acetaminophen and phenacetin used were J.P. grade. N-Phenylurea was prepared according to the procedure descrived in a literature.[11]

Analytical Method——Unchanged Acetanilide in Blood in the Presence of N-Phenylurea: The concentration of acetanilide in blood was estimated by the method of Brodie, *et al.*[12] after the separation of co-existing N-phenylurea by diazotation.[13]

One ml of blood sample (0.9 ml blood and 0.1 ml sodium citrate) was added to a glass stoppered centrifuge tube containing 9 ml of water. One ml of 30% trichloroacetic acid was added to the hemolysate. The tube was stoppered tightly, shaken vigorously and centrifuged. Five ml of the supernatant was transferred to a 50 ml test tube and 1 ml each of 1 N HCl and 2% $NaNO_2$[13] was added. After 10 minutes 25 ml of dichloroethane was added to the test tube, which was shaken for 30 minutes. (Five ml of the aqueous layer was used for the analysis of N-phenylurea as described in the next section.) Twenty ml of the organic solvent layer was transferred to a 25 ml test tube and dichloroethane was evaporated off on a boiling water bath. Five ml of 1 N HCl was added and heated for one and a half hours in an autoclave (121°, 1.0 kg/cm^2). Then one drop of phenolphthalein indicator was added and neutralized with 6 N NaOH. 1 N HCl was added dropwise until the mixture was in the acid side and 0.6 ml more. The volume was adjusted to 6 ml and 0.5 ml of 0.2% $NaNO_2$ was added. After 10 minutes, 0.5 ml of 1% ammonium sulfamate solution was added and the mixture was allowed to stand for 3 minutes. One ml of 50% sodium acetate solution and 0.5 ml of 0.2% N(1-naphthyl)ethylenediamine dihydrochloride was added. After 20 minutes, 1 ml of conc. HCl was added and the absorbance of the resulting dye was determined with a spectrophotometer at 550 mμ. A reagent blank run through the above procedure was used for zero setting.

Unchanged N-Phenylurea: To 5 ml of the aqueous layer obtained above 2 ml of 5% ammonium sulfamate solution was added. After 10 minutes 1 ml of 50% sodium acetate and 0.5 ml of 0.2% N(1-naphthyl)-ethylenediamine dihydrochloride were added. After 20 minutes, 1 ml of conc. HCl was added and the absorbance of the resulting dye was determined with a spectrophotometer at 550 mμ. A reagent blank run through the above procedure was used for zero setting.

Total Non-hydroxyl Metabolites in Urine: One ml of diluted urine was added to a 50 ml test tube containing 2 ml of conc. HCl. The aqueous phase was diluted with 2 ml of water. The mixture was heated in an autoclave (121°, 1.0 kg/cm^2) for one and a half hours. Five ml of 6 N NaOH was added and the mixture was cooled to room temperature. Twenty five ml of ether was added to the mixture and was shaken for 30 minutes. Twenty ml of the ether phase was transferred to another 50 ml test tube containing 5 ml of 0.1 N HCl, and was shaken for 30 minutes. Four ml of the acidic phase was transferred to a 25 ml test tube and 0.5 ml of 0.2% $NaNO_2$ was added. After 10 minutes, the assay was continued as described above under unchanged acetanilide.

Total Hydroxyl Metabolites in Urine: Total hydroxyl metabolites were converted to *p*-aminophenol in an autoclave and determined by the method of Brodie, *et al.*[12]

One ml of diluted urine was added to a 50 ml test tube containing 1 ml of conc. HCl and 1 ml of water. The mixture was heated in an autoclave (121°, 1.0 kg/cm^2) for 1.5 hours. Three ml of water and 5 g of K_2HPO_4 was added and mixed. Twenty five ml of ether was added and the mixture was shaken for 20 minutes. Twenty ml of ether phase was transferred to another 50 ml test tube containing 5 ml of 0.01 N HCl and was shaken for 10 minutes. Four ml of the acid phase was transferred to a 25 ml test tube and 1 ml of 1% phenol solution was added. Then 1 ml of freshly prepared sodium hypobromide solution was added. This solution was prepared by adding bromine water to 1 N sodium carbonate until the solution was slightly

10) Hitachi ALS-505E analog computer was used for the study of the present report.
11) F. Arnt, "Organic Syntheses," Coll. Vol. I, 2nd, ed. by A.H. Blatt, John Wiley and Sons, Inc., New York, N.Y., 1948, p. 453.
12) B.B. Brodie and J. Axelrod, *J. Pharmacol. Exptl. Therap.*, **94**, 22 (1948).
13) H. Ichibagase, S. Kojima, and M. Ichikawa, *Yakugaku Zasshi*, **84**, 707 (1964).

yellow. After 20 minutes the absorbance of the resulting dye was determined with a spectrophotometer at 620 mμ.

Result and Discussion

1) Elimination of Acetanilide after Intravenous Administration of Acetanilide alone

Blood concentration of acetanilide after intravenous administration of acetanilide are listed in Table I.

Table I. Blood Concentration (mg/dl) of Acetanilide after *i.v.* Administration of Acetanilide alone 300 mg to Rabbits

Rabbit Wt. kg time hr	F 3.7	I 2.8	V 3.2	P 2.5	Mean	S.D.
0.5	11.99	10.66	11.03	—	11.227	0.560
0.75	10.03	8.15	9.91	—	9.363	0.859
1.0	8.34	7.49	8.95	7.42	8.050	0.633
1.5	6.32	6.73	7.22	—	6.757	0.368
2.0	4.16	5.43	6.09	5.31	5.248	0.695
2.5	3.64	4.44	—	—	4.040	0.400
3.0	2.02	3.67	2.86	2.97	2.880	0.586
4.0	0.61	2.07	1.10	1.65	1.358	0.552
5.0	0.21	0.95	0.14	1.07	0.593	0.420

In the previous paper[6] it was shown by an analog computer that the elimination of acetanilide is well expressed with Michaelis Menten model after making proper zero-time shift to avoid complex mathematical treatments due to including the distribution process of the drug between blood and other fluid of distribution. In the study of the present paper, authors adhered to making zero-time shift and refrained from adopting two-compartment model to take the distribution process into account. For this, see discussion in the appendix.

Fit of the data of Table I to model 1 and 2 below was examined, after zero-time shift of one hour, by means of analog computer.

Model 1——Hydroxylation of acetanilide is assumed to proceed by first order kinetics.

$$A \xrightarrow{k_{el}}$$

If at anytime (t), A is the concentration in mg/dl of acetanilide in blood and k_{el} is the rate constant of hydroxylation in reciprocal hours, then the differential equation describing the scheme shown is

$$\frac{dA}{dt} = -k_{el}A \qquad \text{Eq. 5}$$

and initial condition is at $t=0$, $A=A_0$.

Model 2——Hydroxylation process is assumed to proceed by Michaelis Menten kinetics.

$$A \xRightarrow{V_a, K_a}$$

A bold arrow indicates that the step is other than first order process. V_a and K_a are Michaelis Menten kinetic constants in mg/dl/hr and mg/dl, respectively. The differential equation is

$$\frac{dA}{dt} = -\frac{V_a A}{K_a + A} \qquad \text{Eq. 6}$$

and initial condition is as above.

Compatibility of these models to the experimental data was examined by an analog computer, the program of which is given in insert of Fig. 2 and 3, and results are shown on respective figures.

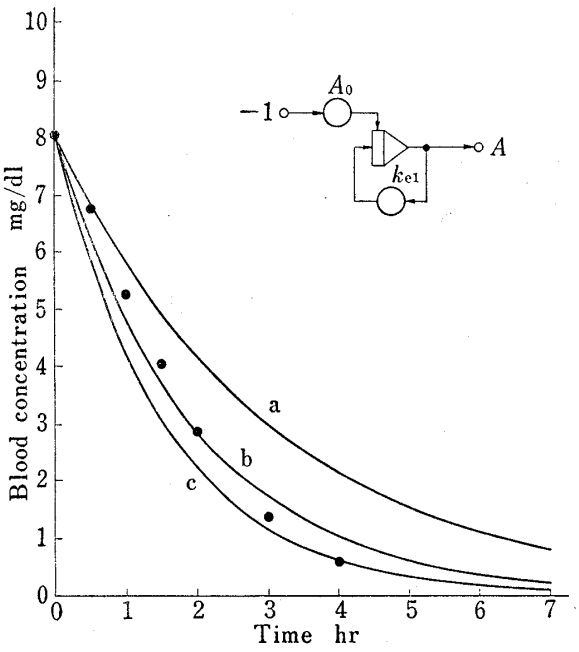

Fig. 2. Disagreement between Theoretical Blood Levels of Acetanilide Obtained by Model 1 with Various k_{el} Values and Observed Data

Plotted points are experimental mean values and solid lines are theoretical curves drawn by an analog computer programmed as the insert of the figure, where A_0=8.050 mg/dl, k_{el}=0.335 (curve a), 0.520 (curve b) and 0.649 (curve c) hr $^{-1}$

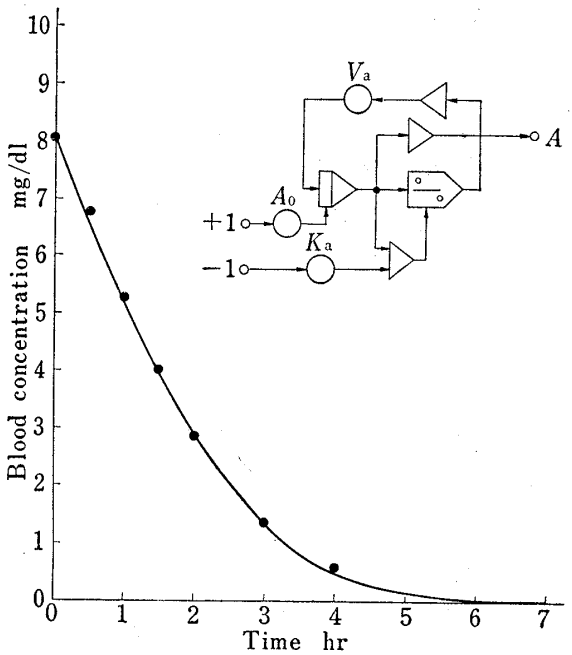

Fig. 3. Agreement between Theoretical Blood Level of Acetanilide Obtained by Model 2 and Observed Data

Plotted points are experimental mean values and solid line is a theoretical curve drawn by an analog computer programmed as the insert of the figure, where A_0=8.050 mg/dl, V_a=4.509 mg/dl/hr and K_a=3.773 mg/dl.

Although various values were tried for k_{el} on the computer setting looking for the fit to the experimental data (mean values), good agreement could not be found by model 1. On the other hand apparently good fit was obtained by model 2. It may be concluded that Michaelis Menten model is preferable to the first order mode for the description of acetanilide elimination from blood.

Best fit parameters are shown in Table II. For the purpose of comparison, parameters obtained in the previous paper[6] with individual rabbits are also shown in Table III, where V_a and K_a of the previous report have been divided by the respective V_d value to make dimension comparable.

Table II. Best Fit Parameters obtained by an Analog Computer

Model 2	A_0	8.050	mg/dl	Model 4	P_0	10.00	mg/dl
	V_a	4.509	mg/dl/hr		V_p	3.222	mg/dl/hr
	K_a	3.773	mg/dl		K_p	6.892	mg/dl
					k_n	0.0291	/hr

Table III. Best Fit Parameters Reported in the Previous Paper (Correspond to Model 2)

Rabbit	D	G	J	N	Q	W
Bdy. wt. kg	2.75	3.7	2.75	2.8	2.5	3.3
V_a mg/dl/hr	8.788	9.436	5.439	4.098	5.256	6.655
K_a mg/dl	5.187	5.564	3.208	2.418	3.098	3.925
k_2/hr	0.72	0.73	0.80	0.56	0.69	0.74
V_d ml	1361	1276	1618	1464	1446	1172

2) Elimination of N-Phenylurea after Intravenous Administration of N-Phenylurea alone

Blood concentration of N-phenylurea and excreted amount of the metabolites which give *p*-aminophenol on treatment with conc. HCl (total hydroxyl metabolites) and those that give aniline (total non-hydroxyl metabolites) after intravenous administration of N-phenylurea alone were determined and shown in Table IV.

Blood concentration of N-phenylurea was plotted on a logarithmic scale against time and is shown on Fig. 4.

Table IV. Blood Concentration (mg/dl) and Cumulative Amount (as mg of N-Phenylurea) of Total Hydroxyl Metabolites and Total Non-hydroxyl Metabolites Excreted in Urine after *i.v.* Administration of N-Phenylurea alone 200 mg to Rabbits

Time hr	C 2.8 kg	K 2.8 kg	B 3.1 kg		Mean	S.D.
Blood conc.						
0.5	12.33	12.69	10.09		11.703	1.150
1.0	9.93	9.76	9.96		9.883	0.088
2.0	8.24	6.98	7.79		7.670	0.521
3.0	5.87	5.63	6.71		6.070	0.463
4.0	3.77	4.48	5.69		4.647	0.793
5.0	2.75	3.06	4.13		3.313	0.591
6.0	1.33	0.86	2.89		1.693	0.868
7.0	0.68	0.35	1.86		0.963	0.648
8.0	0.31	0.24	1.22		0.590	0.446
Total hydroxyl metabolites						
1.0	0.86	3.38	3.33		2.523	1.176
2.0	17.05	16.04	13.24		15.443	1.612
3.0	33.74	33.99	32.89		33.540	0.471
4.0	55.00	51.84	49.91		52.250	2.098
5.0	75.52	66.83	64.59		68.980	4.714
6.0	92.57	83.95	82.90		86.473	4.332
7.0	111.41	101.86	98.91		104.060	5.335
8.0	120.33	118.42	113.11		117.287	3.053
9.0	131.77	125.54	—		128.655	3.115
10.0	138.05	—	135.20		136.625	1.425
				rest	34.0	
				total	170.6	
Total non-hydroxyl metabolites						
1.0	0.17	2.59	0.96		1.240	1.008
2.0	4.05	6.44	3.77		4.753	1.198
3.0	7.23	10.43	9.75		9.137	1.376
4.0	9.62	12.83	13.64		12.030	1.736
5.0	12.19	13.75	16.40		14.113	1.738
6.0	12.97	14.76	18.45		15.393	2.282
7.0	14.11	15.36	19.50		16.323	2.304
8.0	14.67	15.62	20.14		16.810	2.386
9.0	15.03	15.85	—		15.440	0.410
10.0	15.28	—	20.84		18.060	2.780
				rest	0.68	
				total	18.74	

$$\text{ratio} = \frac{170.6}{18.74} = 9.12$$

It is clear from Fig. 4 that the slope of the tangent line to the curve, which is the measure of the rate constant, increases with the decrease of the concentration. And the shape of the curve resembles to that of acetanilide elimination reported in the previous paper.[6]

Semi-logarithmic plot of urinary excretion rate of total hydroxyl and non-hydroxyl metabolites is shown on Fig. 5.

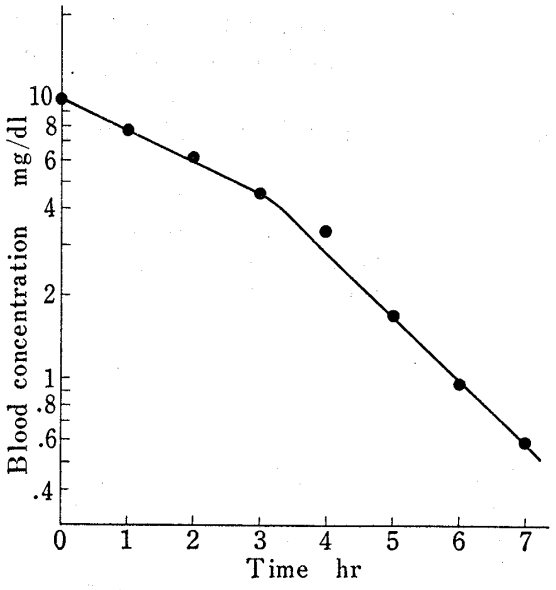

Fig. 4. Semilogarithmic Plot of experimentally Observed Blood Concentration of Unchanged N-Phenylurea after Dosage of N-Phenylurea 200 mg to Rabbits
Zerotime shift is one hour.

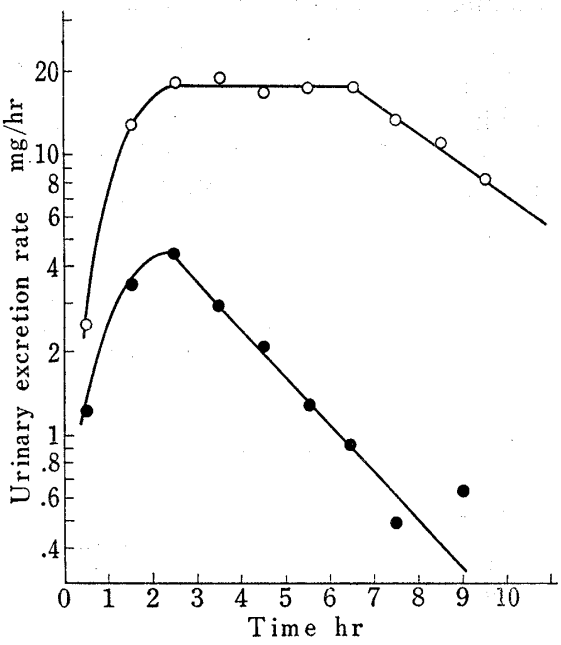

Fig. 5. Semilogarithmic Plot of Urinary Excretion Rate of Total Hydroxyl Metabolites (○) and Total Non-hydroxyl Metabolites (●) after Dosage of N-Phenylurea 200 mg to Rabbits

log-Rate plot of excreted total hydroxyl metabolites shows plateau at about 18 mg/hr, which suggests that the process that participate either formation or excretion of hydroxyl metabolite is capacity limited. From the terminal linear portion of Fig. 5, excretion rate constant and "rest" value, the amount of hydroxyl or non-hydroxyl metabolite which was to be excreted after 10 hours when the experiment was terminated, was calculated assuming that the excretion of the species continue to be first order, and listed in Table IV. The ratio of the amount of total hydroxyl metabolites ultimately excreted in urine to that of total non-hydroxyl metabolites was 9.12.

The amount of total hydroxyl and non-hydroxyl metabolites excreted in 6 hours after intravenous administration of 200, 100 and 50 mg of N-phenylurea are shown in Table V.

Table V. Cumulative Amount (as mg of N-Phenylurea) of Hydroxyl Body and Non-hydroxyl Body Excreted in 6 Hours after *i.v.* Dosage of N-Phenylurea 200, 100 and 50 mg to Rabbits

Rabbit	wt. kg	OH body mg	Non-OH mg	Ratio
200 mg dose				
CC	2.8	92.57	12.97	7.137
JJ	2.8	83.95	14.76	5.688
BB	3.1	82.90	18.45	4.493
mean		86.47	15.39	5.619
100 mg dose				
FF	3.4	78.87	5.68	13.886
JK	3.9	74.00	5.15	14.369
mean		76.44	5.42	14.103
50 mg dose				
DD	2.0	35.62	1.40	25.443
QQ	2.5	32.79	1.40	23.421
ST	2.7	30.37	1.53	19.850
mean		32.93	1.44	22.814

When compared the average values, amount of non-hydroxyl metabolites excreted in 6 hours is roughly proportional to the dose of N-phenylurea, on the other hand the excreted amount of hydroxyl metabolites is similar for 200 and 100 mg dose, indicating the saturation of hydroxylation process at about this dose range. In addition, the ratio of excreted hydroxyl metabolites to non-hydroxyl ones increases with decrease of the dose.

On the basis of these facts authors presumed that the hydroxylation process of N-phenylurea is capacity limited. And fit of the data in Table IV to model 3 and 4 described below was examined.

Model 3——Hydroxylation and formation of non-hydroxyl metabolites are assumed to proceed by first order kinetics.

$$P \xrightarrow{k_h} \text{(total hydroxyl metabolites)}$$
$$P \xrightarrow{k_n} \text{(total non-hydroxyl metabolites)}$$

If at anytime (t), P is the concentration in mg/dl of N-phenylurea in blood and k's with subscript are first order rate constants of the respective steps in reciprocal hours, then the differential equation describing the scheme is

$$\frac{dP}{dt} = -(k_h+k_n)P \qquad \text{Eq. 7}$$

and initial condition is at $t=0$, $P=P_0$.

Model 4——Hydroxylation step is assumed to proceed by Michaelis Menten kinetics and the other step by first order as below.

$$P \xrightarrow{V_p, K_p} X \text{ (total hydroxyl metabolites)}$$
$$P \xrightarrow{k_n} Y \text{ (total non-hydroxyl metabolites)}$$

V_p and K_p are Michaelis Menten kinetic constants in mg/dl/hr and mg/dl, respectively. If P and k_n are as defined on model 3, the differential equation is

$$\frac{dP}{dt} = -\frac{V_pP}{K_p+P} - k_nP \qquad \text{Eq. 8}$$

initial condition is the same as model 3.

Compatibility of these models to the experimental data was examined by an analog computer. The program of model 3 is essentially the same as that of model 1, where k_{el} is the sum of k_h and k_n. Result is shown on Fig. 6. The theoretical curve traced the first 4 points when k_h+k_n was set to 0.256 hr.$^{-1}$

For model 4, the analog computer was programmed as the insert of Fig. 7, and the result is shown in the figure.

The procedure looking for the values of parameters was as follows. At first k_n was set arbitrary then V_p and K_p were estimated so that the theoretical curve of P might trace the experimental data points as well as possible. After that, ultimate amount ratio of excreted total hydroxyl metabolites to that of non-hydroxyl metabolites (which was obtained as the ratio of output X to output Y of the program) was checked. If the ratio was other than the experimental value (that is 9.12), then k_n setting was corrected and repeated the whole procedure until the consistent values were obtained.

Although the theoretical curve does not trace the last 3 points, fit of model 4 is better than model 3. The best fit parameters are listed in Table II.

3) Effect of Other Drugs on the Hydroxylation of N-Phenylurea

(a) Acetaminophen——Two hundred mg of acetaminophen was given intravenously 3 hours after the administration of N-phenylurea 200 mg. Logarithm of blood concentration of unchanged N-phenylurea was plotted against time and is shown on Fig. 8a.

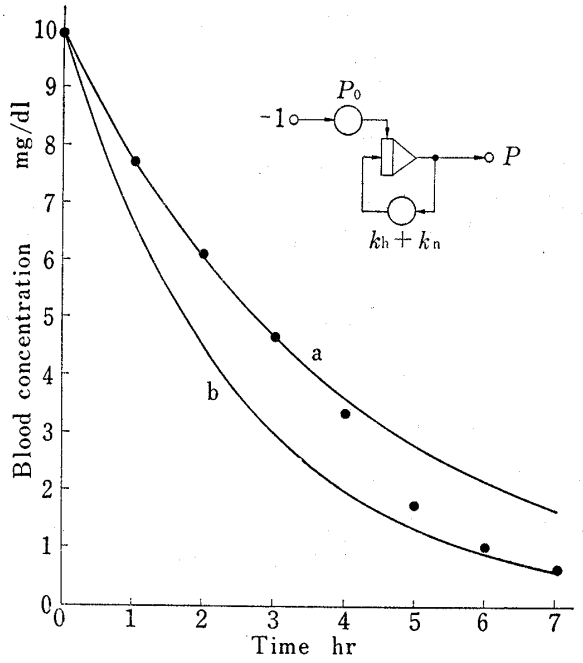

Fig. 6. Comparison of Observed N-Phenylurea Blood Level with Theoretical Values Calculated by Model 3

Plotted points are experimental values and solid lines are theoretical curves drawn by an analog computer programmed as the insert of the figure, where P_0=10.0 mg/dl, k_h+k_n=0.256 (curve a) and 0.403 (curve b) hr^{-1}

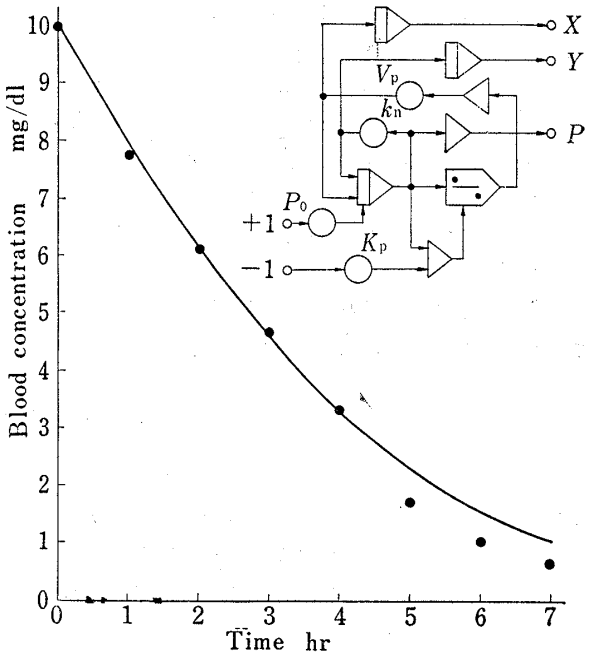

Fig. 7. Comparison of Observed N-Phenylurea Blood Concentration with Theoretical Values Calculated by Model 4

Plotted points are experimental values and solid line is a theoretical curve drawn by an analog computer programmed as the insert of the figure, where P_0=10.0 mg/dl V_p= 3.222 mg/dl/hr, K_p=6.892 mg/dl and k_n=0.0291 hr^{-1}

The plot shows continuously smooth curve and no sign of disturbance is observed around the time of acetaminophen injection. Acetaminophen gave no effect on the elimination of N-phenylurea.

(b) Acetanilide——Two hundred mg of acetanilide was given intravenously to rabbits 3 hours after the injection of N-phenylurea 200 mg. Blood concentration of unchanged N-phenylurea was plotted against time on a semi-logarithmic scale and is shown on Fig. 8b.

The plot is not smooth but two curves intersept at about the time of acetanilide administration. This result shows that the elimination rate of unchanged N-phenylurea from the blood was abruptly reduced by the acetanilide injection.

(c) Phenacetin——One hundred mg of phenacetin was given to rabbits intravenously 3 hours after N-phenylurea injection. Blood concentration of unchanged N-phenylurea was plotted against time on a semi-logarithmic scale and is shown on Fig. 8c.

The plot is continuously smooth curve as the case of acetaminophen and no effect of phenacetin on N-phenylurea elimination was observed.

De-ethylation of phenacetin is known as oxi-

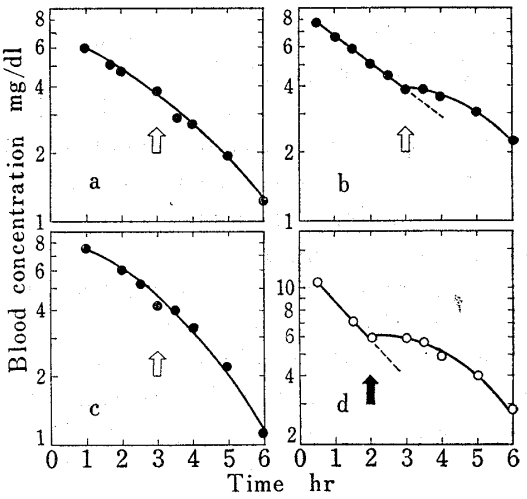

Fig. 8. Effect on the Elimination of N-Phenylurea Blood Concentration of *p*-Hydroxyacetanilide (a), Acetanilide (b) and Phenacetin (c), and Effect of N-Phenylurea on the Elimination of Acetanilide Blood Concentration (d)

Arrows indicate the time at which the second drug was administered.

dation process[14] and phenacetin was expected to reduce the hydroxylation rate of N-phenylurea. Nevertheless at the dose level of 100 mg no effect was observed, higher dosage was obstructed by low solubility of this drug.

4) Effect of N-Phenylurea on Acetanilide Elimination

Two hundred mg of N-phenylurea was given to rabbits 2 hours after the administration of acetanilide 200 mg. Blood concentration of acetanilide was plotted against time on a semilogarithmic scale and is shown on Fig. 8d.

The plot is not smooth but it is disturbed at about the time of N-phenylurea administration. This fact indicates that N-phenylurea has also influence on the elimination of acetanilide.

5) Elimination of Acetanilide and N-Phenylurea after Simultaneous Administration of Acetanilide and N-Phenylurea

Two hundred mg each of N-phenylurea and acetanilide was administered simultaneously to rabbits. Blood concentrations of acetanilide and N-phenylurea were determined separately and are shown in Table VI.

Table VI. Blood Concentration (mg/dl) of Acetanilide and N-Phenylurea after Simultaneous Administration of Acetanilide and N-Phenylurea 200 mg each to Rabbits

Time hr	A 2.3 kg	X 3.1 kg	M 2.8 kg	Mean	S.D.
Acetanilide					
0.5	6.42	7.74	6.94	7.033	0.543
1.0	6.16	5.50	—	5.830	0.330
1.5	5.37	5.01	5.53	5.303	0.218
2.0	—	4.26	4.76	4.510	0.250
2.5	3.56	3.88	3.94	3.793	0.167
3.0	2.28	3.54	—	2.910	0.630
4.0	1.86	—	1.22	1.540	0.320
5.0	—	1.26	—	1.260	—
6.0	0.40	1.01	—	0.705	0.305
8.0	—	0.44	0.18	0.310	0.130
N-Phenylurea					
0.5	10.50	10.89	10.77	10.720	0.163
1.0	9.96	10.02	10.32	10.100	0.157
1.5	9.49	9.37	9.74	9.533	0.154
2.0	—	8.80	8.91	8.855	0.055
2.5	8.74	7.84	7.83	8.137	0.427
3.0	7.52	8.58	—	8.050	0.530
4.0	6.64	—	7.00	6.820	0.180
5.0	—	5.75	—	5.75	—
6.0	4.54	4.92	—	4.730	0.190
8.0	1.96	2.53	1.86	2.117	0.295

Fit of model 5 through 7 below to the blood data of Table VI was compared.

Model 5——Hydroxylation of acetanilide and that of N-phenylurea are assumed to proceed by mutually independent Michaelis Menten kinetics. Differential equations describing this model are Eq. 6 and 8, and initial conditions are at $t=0$, $A=A_0$ and $P=P_0$.

Model 6——Hydroxylation of these drugs are assumed to proceed interdependently (competitively inhibited) as mentioned in theoretical. Differential equations describing this model are Eq. 1 and 2.

14) B.B. Brodie, *Ann. Rev. Biochem.*, **27**, 427 (1958).

Model 7——Hydroxylation of these drugs are assumed to proceed interdependently (non-competitively inhibited) as mentioned in theoretical. Differential equations describing this model are Eq. 3 and 4.

Compatibility of these models with the experimental data was examined by an analog computer. On computation, parameters were fixed to the values of Table II except for A_0, which was reduced to 6.0 mg/dl because of lower dose level. The results are shown on Fig. 9.

An examination of Fig. 9 shows that theoretical curve of model 6 is very close to the observed blood concentration of acetanilide and that of model 7 traces N-phenylurea blood concentration obtained. On the basis of these facts, it seems reasonable to conclude that when acetanilide and N-phenylurea are co-administered to rabbits, hydroxylation process of these drugs interacts mutually. Acetanilide elimination is inhibited competitively by N-phenylurea and hydroxylation of N-phenylurea is inhibited non-competitively by acetanilide, that is well expressed by Eq. 1 and 4 as shown on Fig. 10.

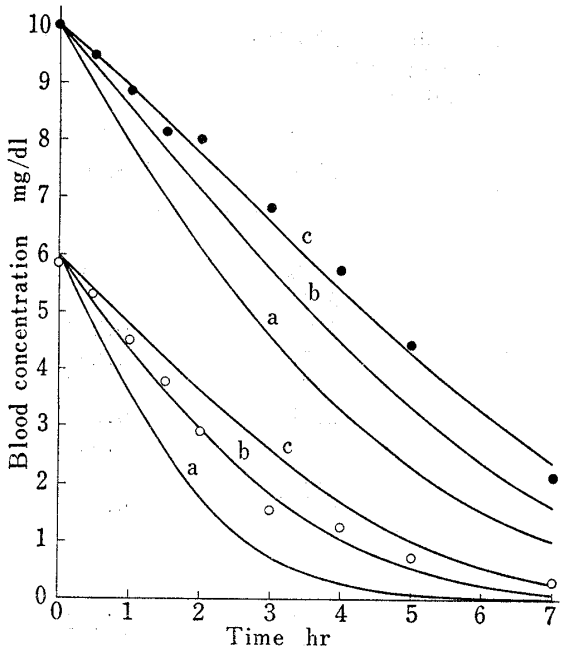

Fig. 9. Comparison of Observed Blood Concentration of Acetanilide (○) and N-Phenylurea (●) following Simultaneous Administration of These Drugs with Theoretical Curves drawn by an Analog Computer according to Model 5 (curve a), Model 6 (curve b) and Model 7 (curve c)

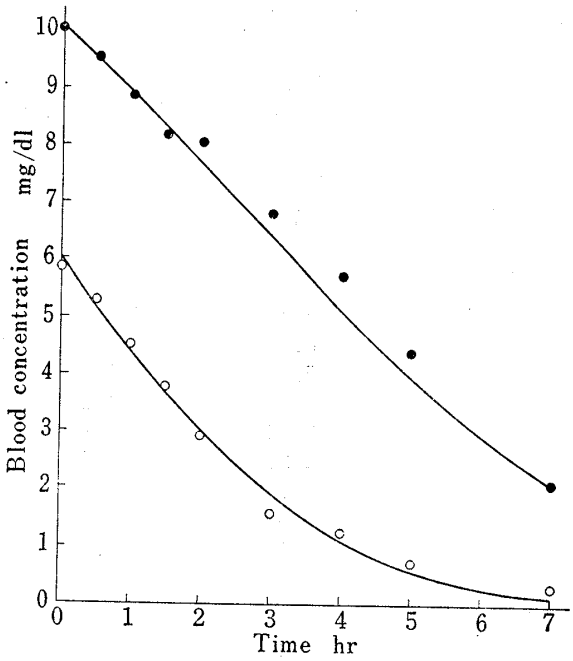

Fig. 10. Agreement between Observed Blood Concentration of Acetanilide (○) and N-Phenylurea (●) following Simultaneous Administration of These Drugs and Theoretical Curves drawn by an Analog Computer according to Eq. 1 and 4

Computer program is as shown on Fig. 1, where Sw-1 and Sw-2 are "off" and "on" respectively.

This conclusion supports the results of the previous paper[6] that the hydroxylation process of acetanilide in rabbits is capacity limited and saturable.

The most important finding of this study is the mutual inhibition in hydroxylation process of drug biotransformation. If hydroxylation of aliphatic side chain and de-alkylation of O- and N-alkyl group, which are the most familiar metabolic route, are the same type of reaction as reported by Brodie,[14] then it is probable that the interaction in drug metabolism might occur with high possibility when two or more drugs are combined in a single formulation, or even when they are given in separate dosage form at the same time. For safer and more effective dosage schedule, these mutual inhibitory effect of combined drugs should carefully be taken into account at every stage of dynamic behavior of drugs in the living body.

Appendix

Approximation of Two Compartment Rapid Distribution Model by Single Compartment with shifted Zero-time Model

The adoption of two compartment system indicated in the diagram below to simulate the distribution and elimination of the drug leads to the following differential equations.

$$C_t \underset{k_1}{\overset{k_2}{\rightleftharpoons}} C_p \xrightarrow{V, K}$$

$$\frac{dC_p}{dt} = -k_1C_p + k_2C_t - \frac{VC_p}{K+C_p} \qquad \text{Eq. 1a}$$

$$\frac{dC_t}{dt} = k_1C_p - k_2C_t \qquad \text{Eq. 2a}$$

at $t=0$, $C_p = C_p^\circ$ and $C_t = 0$.

Where C_p and C_t are the concentration at any time t in mg/dl of the drug in blood and in tissue respectively. k_1 and k_2 are the first order rate constants of distribution in reciprocal hours and V and K are Michaelis Menten kinetic constants in mg/dl/hr and mg/dl, respectively.

It is difficult to solve these equations explicitly for C_p and C_t, therefore attempts are made to obtain an approximate value of C_p.

a) **Approach from Rapid Distribution and Slow Elimination Assumption**——Assuming that V is zero, Eq. 1a and 2a give Eq. 3a.

$$C_p = \frac{k_1C_p^\circ}{k_1+k_2}e^{-(k_1+k_2)t} + \frac{k_2C_p^\circ}{k_1+k_2} \qquad \text{Eq. 3a}$$

The meaning of Eq. 3a is that the equilibrium between C_p and C_t proceed with first order rate process, whose rate constant is equal to k_1+k_2, and the equilibration ratio, C_t/C_p, is equal to k_1/k_2.

Consequently, it may be assumed, when the elimination rate parameter, V, is small enough (but not zero) compared with distribution rate constants (k_1, k_2), that the values of C_p and C_t approach to dynamic equilibrium, where $C_t/C_p = k_1/k_2$ or

$$\frac{dC_t}{dt} = \frac{k_1}{k_2}\frac{dC_p}{dt} \qquad \text{Eq. 4a}$$

and that the attainment of the equilibration is exponential process with rate constant equal to k_1+k_2.

On the other hand, sum of Eq. 1a and 2a gives Eq. 5a.

$$\frac{dC_p}{dt} + \frac{dC_t}{dt} = -\frac{VC_p}{K+C_p} \qquad \text{Eq. 5a}$$

Substitution of Eq. 4a into Eq. 5a and rearrangement gives Eq. 6a.

$$\frac{dC_p}{dt} = -\frac{k_2}{k_1+k_2}\frac{VC_p}{K+C_p} \qquad \text{Eq. 6a}$$

If the solution of Eq. 6a is $C_p = Y(t)$, then $Y(t)$ satisfies Eq. 7a. Where Y_0 is the value of $Y(t)$ at $t=0$.

$$\frac{k_2V}{k_1+k_2}t = K\{\ln Y_0 - \ln Y(t)\} + Y_0 - Y(t) \qquad \text{Eq. 7a}$$

$Y(t)$ above expresses the time dependency of C_p after the dynamic equilibrium between C_p and C_t has been achieved. As the consequence, Eq. 8a approximates C_p that satisfies Eq. 1a and 2a, provided that k_1 and k_2 are sufficiently large compared with V/K. Where X_0 is a constant.

$$C_p = X_0 e^{-(k_1+k_2)t} + Y(t) \qquad \text{Eq. 8a}$$

When t is relatively small, the first term of Eq. 8a is more significant and the equation mainly expresses the distribution of the drug (pre-equilibrative phase). When t is large and the first term is relatively insignificant, it expresses the elimination (post-equilibrative phase).

Comparing Eq. 3a and 8a, it is clear that the second term is a constant and time independent when V is zero but that it is time dependent if V is not zero.

b) Approach from Zero Order and First Order Approximation for Elimination Step—— When C_p is sufficiently large compared with K value, elimination rate of C_p (the third term in the right hand side of Eq. 1a) is approximated by V and Eq. 1a becomes Eq. 9a, which is solved simultaneously with Eq. 2a for C_p and Eq. 10a is obtained.

$$\frac{dC_p}{dt} = -k_1 C_p + k_2 C_t - V \qquad \text{Eq. 9a}$$

$$C_p = X_0 e^{-(k_1+k_2)t} + Y_0 - \frac{k_2 V}{k_1+k_2} t \qquad \text{Eq. 10a}$$

$$X_0 = \frac{k_1 C_p^\circ}{k_1+k_2} + \frac{k_1 V}{(k_1+k_2)^2}$$

$$Y_0 = \frac{k_2 C_p^\circ}{k_1+k_2} - \frac{k_1 V}{(k_1+k_2)^2}$$

Incidentally solution of single compartment (zero order elimination) model (Eq. 11a) is given by Eq. 12a.

$$\frac{dC_p}{dt} = -V \qquad \text{Eq. 11a}$$

$$C_p = C_p^\circ - Vt \qquad \text{Eq. 12a}$$

On the other hand, when C_p is sufficiently small compared with K value, Eq. 1a is approximated by Eq. 13a.

$$\frac{dC_p}{dt} = -k_1 C_p + k_2 C_t - \frac{V}{K} C_p \qquad \text{Eq. 13a}$$

Eq. 2a and 13a are solved simultaneously for C_p and Eq. 14a is obtained.

$$C_p = X_0 e^{-\alpha t} + Y_0 e^{-\beta t} \qquad \text{Eq. 14a}$$

$$X_0 = \frac{k_2-\alpha}{\beta-\alpha} C_p^\circ, \qquad Y_0 = \frac{k_2-\beta}{\alpha-\beta} C_p^\circ$$

$$\alpha = \frac{1}{2}(b+\sqrt{b^2-4c}), \qquad \beta = \frac{1}{2}(b-\sqrt{b^2-4c})$$

$$b = k_1 + k_2 + \frac{V}{K}, \qquad c = k_2 \frac{V}{K}$$

Assuming that V/K is negligibly small compared with k_1 and k_2, α and β are approximated as below.

$$\alpha = k_1 + k_2, \qquad \beta = \frac{k_2}{k_1+k_2} \frac{V}{K}$$

Incidentally solution of single compartment (first order elimination) model (Eq. 15a) is given by Eq. 16a.

$$\frac{dC_p}{dt} = -\frac{V}{K} C_p \qquad \text{Eq. 15a}$$

$$C_p = C_p^\circ e^{-\frac{V}{K}t} \qquad \text{Eq. 16a}$$

Respective comparison of Eq. 10a with Eq. 12a and Eq. 14a with Eq. 16a suggests the following rule at the both extreme cases ($C_p \gg K$ and $C_p \ll K$). Solutions of two compartment (distribution and elimination) models, Eq. 10a for $C_p \gg K$ and Eq. 14a for $C_p \ll K$, consist of two terms. One is an exponential term of which exponent is $(k_1+k_2)t$, and the other term is the solution of the single compartment (elimination) model, Eq. 12a for $C_p \gg K$ and Eq. 16a for $C_p \ll K$, rate constant of which is multiplied by $k_2/(k_1+k_2)$. If the rule just mentioned is assumed to hold for C_p value comparable with K, as well as at the extreme cases, then Eq. 8a results.

c) **Evaluation of X_0 and Y_0**——X_0 and Y_0 of Eq. 8a are evaluated from initial conditions.

$$X_0 + Y_0 = C_p^\circ \qquad \text{Eq. 17a}$$

$$(k_1+k_2)X_0 + \frac{k_2}{k_1+k_2}\frac{VY_0}{K+Y_0} = k_1C_p^\circ + \frac{VC_p^\circ}{K+C_p^\circ} \qquad \text{Eq. 18a}$$

Rearrangement of Eq. 17a gives Eq. 19a.

$$X_0 = C_p^\circ - Y_0 \qquad \text{Eq. 19a}$$

Substituting Eq. 19a into Eq. 18a, Eq. 20a is obtained.

$$(k_1+k_2)Y_0 - \frac{V^\circ Y_0}{K+Y_0} = R_0 \qquad \text{Eq. 20a}$$

$$R_0 = k_2C_p^\circ - \frac{VC_p^\circ}{K+C_p^\circ}, \qquad V^\circ = \frac{k_2}{k_1+k_2}V$$

Eq. 20a is solved for Y_0 and Eq. 21a is obtained.

$$Y_0 = \frac{(V^\circ+R_0-k^\circ K)+\sqrt{(V^\circ+R_0-k^\circ K)^2+4k^\circ KR_0}}{2k^\circ} \qquad \text{Eq. 21a}$$

$$k^\circ = k_1 + k_2$$

When C_p° is sufficiently large compared with K, Eq. 21a is approximated by much simpler Eq. 22a.

$$Y_0 = \frac{(V^\circ+R_0-k^\circ K)+(V^\circ+R_0+k^\circ K)}{2k^\circ} = \frac{V^\circ}{k^\circ} + \frac{R_0}{k^\circ} \qquad \text{Eq. 22a}$$

Consequently X_0 and Y_0 are expressed as follows;

$$X_0 = \frac{k_1}{k_1+k_2}C_p^\circ - \frac{k_2V}{(k_1+k_2)^2} + \frac{VC_p^\circ}{(k_1+k_2)(K+C_p^\circ)} \qquad \text{Eq. 23a}$$

$$Y_0 = \frac{k_2}{k_1+k_2}C_p^\circ + \frac{k_2V}{(k_1+k_2)^2} - \frac{VC_p^0}{(k_1+k_2)(K+C_p^\circ)} \qquad \text{Eq. 24a}$$

d) **Comparison of the Analog Computer Solution of Eq. 1a and 2a with Eq. 7a and 8a**——Blood concentration of acetanilide after intravenous injection of acetanilide 300 mg to rabbit J is listed in Table VII. Non-linear least square fit of the data of Table VII to Eq. 1a and 2a was carried out by the method of Atkins[15] on a digital computer.[16] Rate and other constants obtained are listed in Table VIII.

Using the values listed in Table VIII, comparison of Eq. 1a and 2a with Eq. 7a and 8a was carried out by an analog computer. The program and results are shown on Fig. 11 and 12.

It is clear from Fig. 12 that the both curves are almost identical and approximation of Eq. 1a and 2a (curve a) by Eq. 7a and 8a (curve b) is excellent for the values of Table VIII.

15) G.L. Atkins, *Biochim. Biophys. Acta*, **252**, 405 (1971).
16) Computer program written in FORTRAN was kindly supplied by Kanji Takada, M.S., Kyoto University, who had received it personally from Dr. Atkins.

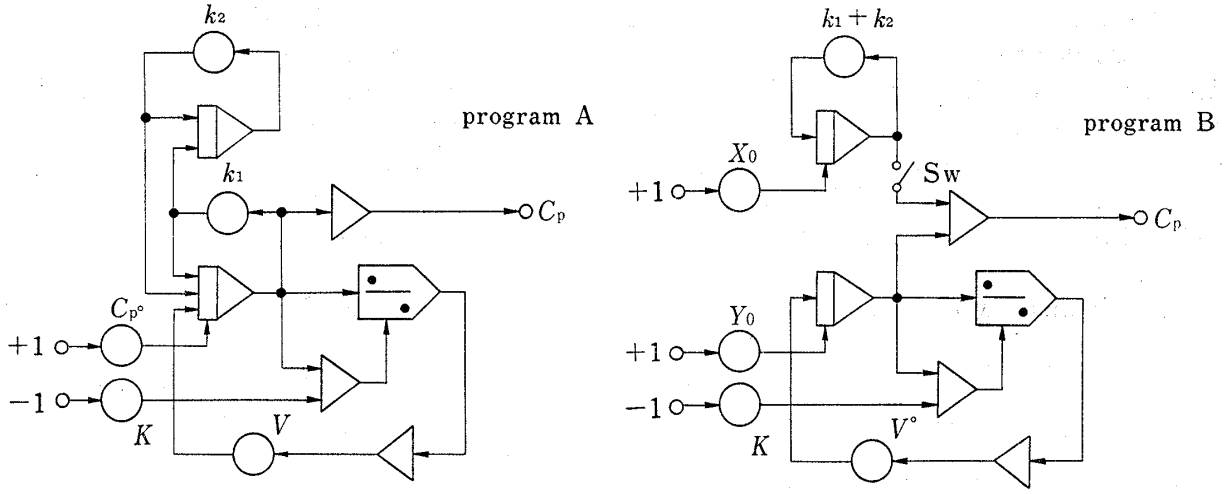

Fig. 11. Analog Computer Program for the Solution of Eq. 1a and 2a (Program A), of Eq. 7a and 8a (Program B with Sw "on") and of Eq. 6a (Program B with Sw "off")

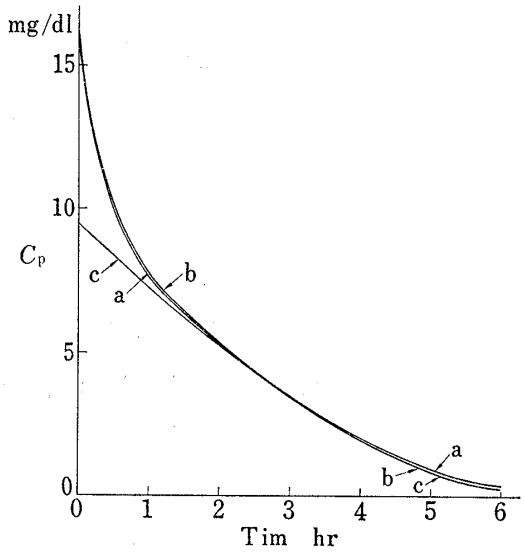

Fig. 12. Comparison of Theoretical Values Calculated by Eq. 1a and 2a (curve a) with Those Calculated by Eq. 7a and 8a (curve b) and by Eq. 6a (curve c)

Curves are output of an analog computer programmed as shown on Fig. 11, where C_p°=16.034 mg/dl, k_1=1.081 hr,$^{-1}$ k_2=1.735 hr,$^{-1}$ V=3.969 mg/dl/hr, K=1.498 mg/dl, V°=2.445 mg/dl/hr, X_0=6.578 mg/dl and Y_0=9.456 mg/dl.

TABLE VII. Blood Concentration (mg/dl) of Acetanilide after *i.v.* Administration of Acetanilide 300 mg to Rabbit J (2.8 kg)

Time (min)	Blood conc. (mg/dl)	Time (min)	Blood conc. (mg/dl)
5	14.50	122	5.25
10	10.90	153	4.25
33	10.45	182	3.50
46	7.95	241	2.00
64	7.30	300	0.95
95	6.55		

TABLE VIII. Computer Calculated Constants with Standard Errors

A_0 (mg/dl)	16.03361±0.84240
k_1 (/hr)	1.08125±0.34248
k_2 (/hr)	1.73455±0.91447
V (mg/dl/hr)	3.96892±2.25175
K (mg/dl)	1.49818±3.05855

Since Eq. 8a consists of exponential term and single compartment term, C_p is even simulated by the single compartment term alone, if zero-time is shifted so that the exponential term becomes negligibly small. Theoretical curve of the single compartment term alone, which corresponds to Eq. 6a and is drawn by program B of Fig. 11 with Sw at "off" position, is also shown in Fig. 12 (curve c).