[Chem. Pharm. Bull. 21(8)1754—1763(1973)]

UDC 547.495.2.9 : 615.31.033.076.9

Drug Absorption, Metabolism, and Excretion. IX.[1] Some New Aspects of Pharmacokinetics on Tolbutamide in Rabbits[2]

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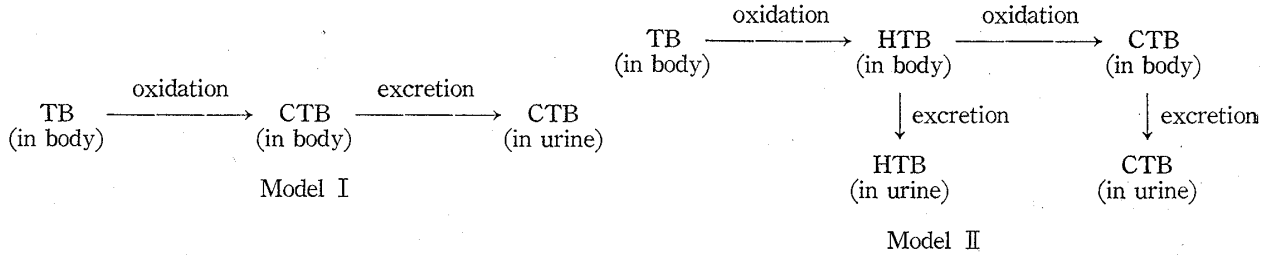
(Received January 16, 1973)

Pharmacokinetics on the metabolism and excretion of tolbutamide (TB) following the rapid intravenous injection of TB was investigated in rabbits based on the kinetic model where TB is first oxidized to 1-butyl-3-*p*-hydroxymethylphenylsulfonylurea (HTB), which is secondly converted to 1-butyl-3-*p*-carboxyphenylsulfonylurea (CTB) and both the metabolites are excreted in urine. It was found that binding of TB to plasma proteins and/or blood cells could not be neglected and the excretion of HTB formed in body would be well interpreted by assuming an intervenient compartment between blood and urine.

Furthermore, pharmacokinetics of HTB was investigated after the administration of HTB to rabbits. It became clear that the amount of CTB formed in body after the administration of HTB was far smaller than that formed in body after the administration of TB indicating that the assumption that a metabolite administered *per se* would behave in the same manner as the metabolite formed in body from its precursor is not always free from criticism, though it has been undoubtedly employed to many pharmacokinetic studies of drugs. On the contrary, it was suggested that CTB administered *per se* would behave samely in body as CTB formed through metabolic reaction from TB or HTB by the experiment in which rabbits were given CTB intravenously.

Nelson and O'Reilly[4] have reported pharmacokinetic studies on tolbutamide (TB) in man in which two-step consecutive first order kinetic model (Model I) was employed considering 1-butyl-3-*p*-carboxyphenylsulfonylurea (CTB) as an only metabolite. Büttner and Portwich[5] and Portwich, *et al.*[6] have also reported pharmacokinetics on TB based on the same model. Later, it has become clear that a considerable amount of 1-butyl-3-*p*-hydroxymethylphenylsulfonylurea (HTB) is also excreted in addition to CTB in urine of man, rabbit and rat receiving TB.[7] According to the recent findings of Schulz and Schmidt[8] that HTB has a markedly



1) Part VIII: J. Shibasaki, R. Konishi, T. Ueki, and T. Morishita, *Chem. Pharm. Bull.* (Tokyo), **21**, 1747 (1973).
2) A part of this work was presented at the 91st Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, April 1971.
3) Location: *1–14 Bunkyo-machi, Nagasaki.*
4) E. Nelson and I. O'Reilly, *J. Pharmacol. Exptl. Therap.*, **132**, 103 (1961).
5) H. Büttner and F. Portwhich, *Antimicrob. Agents Chemother.*, **1965**, 177.
6) F. Portwich, H. Büttner, and H. W. Hansen, *Verh. Deut. Ges. Inn. Med.*, **72**, 769 (1967).
7) J. Tagg, D.M. Yasuda, M. Tanabe, and C. Mitoma, *Biochem. Pharmacol.*, **16**, 143 (1967); R.C. Thomas and G.J. Ikeda, *J. Med. Chem.*, **9**, 507 (1966).
8) E. Schulz and F.H. Schmidt, *Klin. Wochschr.*, **48**, 759 (1970).

hypoglycemic effect in man contrary to CTB, HTB is considered to be pharmacologically of more importance than CTB. Therefore, standing on the new point of view represented by Model II where TB is first oxidized to HTB, which is subsequently converted to CTB and both the metabolites are excreted in urine, the pharmacokinetics of TB in rabbits dosed with TB intravenously was studied. Further, the pharmacokinetics of HTB and CTB in rabbits after each administration were also investigated.

Experimental

Animal Experiments——Male albino rabbits weighing 1.9—4.3 kg were used. Each dose of TB, HTB, and CTB was dissolved in a slight excess of NaOH solution and made up to 20 ml with purified water for administration. TB (150 mg/kg) was given by rapid intravenous injection from ear vein and also by oral and peritoneal routes. CTB (150 mg/kg) was given by rapid intravenous injection. HTB was given by rapid intravenous injection (50, 150 mg/kg) and by oral and peritoneal routes (150 mg/kg). HTB was also given by logarithmic rate-infusion to ear vein using Natsume KN type infusion pump equipped with a series of gears generating various infusion rates. Logarithmic rate-infusion was carried out at first-order rate ($0.2\ hr^{-1}$) for 8 hr during which it was intended that the total dose amounted to about 150 mg/kg. Food was withheld during the experiments although water was given through stomach tube frequently to keep the urine flow constant. Blood specimens were taken with a syringe containing 3.8% sodium citrate solution from ear vein. Blood collections after TB administration were made hourly and those after HTB and CTB administration were made at appropriate intervals as frequently as possible till about 3 hr after the administration as the latter two compounds were removed rapidly from blood. Urine collections were made hourly or at longer intervals through Nelaton's catheter inserted into bladder.

Drugs——TB was the gift of Japan Höchst Co., Ltd. HTB and CTB were synthesized in the manner established by the authors.[1)]

Analytical Methods——The methods reported by the authors[1)] were employed.

Thin-Layer Chromatography——Silica gel HF_{254} according to Stahl was spread about 250 μ thick and activated at 110° for 1.5 hr. The solvent system was BuOH–EtOH–1/15 M phosphate buffer (pH 7.0) reported by Wittenhagen, *et al.*[9)] The compounds were revealed as dark spots against fluorescent background under ultraviolet light (253.7 mμ). *Rf* values of the compounds were as follows: TB, 0.90; HTB, 0.81; CTB, 0.45. Without any pretreatment, urine specimens were spotted on the plates.

Computer Analysis——An analog computer (Hitachi ALS 505) and X–Y recorder (Watanabe WX 431) were used.

Result and Discussion

Pharmacokinetic Investigation on the Data following the Administration of TB

The blood levels of unchanged TB after intravenous dosage of 150 mg/kg TB are presented in Table I.

Table I. The Blood Levels of TB after Intravenous Administration of TB (150 mg/kg) to Rabbits

Rabbit and dose (mg)	Blood levels (mg %) Time of blood collection (hr after administration)													Elimination rate constants[a)] by graphical method (hr^{-1})
	0.5	1.0	1.5	2.0	3.0	4.0	5.0	6.0	7.0	8.0	10.0	12.0	24.0	
TB-B, 285	—	23.0	—	17.3	16.6	16.5	14.0	—	12.0	—	—	—	—	0.09
TB-D, 525	45.0	37.5	—	28.8	27.9	24.3	20.0	20.0	18.8	15.1	14.7	9.7	3.0	0.11
TB-F, 570	—	29.8	—	23.0	18.4	13.9	11.4	9.2	8.2	7.3	—	—	—	0.20
TB-O, 450	30.1	20.4	16.4	14.5	12.1	10.1	7.2	7.2	5.3	4.8	—	—	—	0.19
TB-R, 420	—	39.2	—	32.3	25.7	22.7	18.5	17.7	14.7	12.8	—	—	—	0.14
Mean														0.15

a) Obtained from the slopes of the linear portions of logarithmic plots of the blood levels of TB.

9) G. Wittenhagen, G. Mohnike, and W. Langenbeck, *Z. Physiol. Chem.*, **316**, 157 (1959).

When the blood levels in Table I are plotted on a logarithmic scale against time, the resulting graphs, an example of which is presented in Fig. 1, show the tendencies of the two-exponential curves but can be roughly regarded as linear later than about 2 hr after the administration. The rate constants obtained from the slopes of the linear portions are also listed in Table I, the mean value being 0.15 hr^{-1}. Wittenhagen, *et al.*[9] have estimated the blood levels of TB at 0.5, 1.0, and 4.0 hr after the intravenous dosage of 250 mg/kg TB in rabbits. By plotting these data similarly, the value of about 0.1 hr^{-1} was obtained, which is approximately in accord with those of the present works.

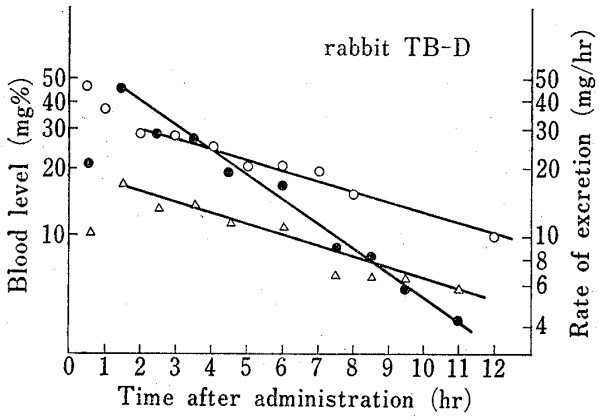


Fig. 1. Logarithmic Plots of the Blood Levels of TB (–○–) and Log 'rate' plots of the Excretion of HTB (–△–) and CTB (–●–) after Intravenous Administration of TB (150 mg/kg) to a Rabbit

The amounts of HTB and CTB in urine specimens collected at various times after the intravenous dosage of 150 mg/kg TB are given in Table II indicating that the total output of CTB is appreciably larger than that of HTB. Moreover, the data suggest that the ratio of hourly output of CTB to that of HTB is considerably larger than unity at the earlier periods after the administration of TB and then gradually decreasing, which is possibly due to smaller rate of excretion of HTB than CTB.

Table II. The Amounts of HTB and CTB excreted in Urine of Rabbits after Intravenous Administration of TB (150 mg/kg)

Rabbit and dose (mg)		Amounts of HTB and CTB in each urine specimen (mg equivalent of TB) Time of urine collection (hr after administration) 1	2	3	4	5	6	7	8	9	10	12	24	Total	Rate constants[a] by graphical method (hr^{-1})
TB-B 285	HTB	4.3	8.0	6.7	5.9	4.0	5.1	5.6	—	6.8	—	—	28.7	75.1	0.13
	CTB	9.4	23.5	15.3	11.3	7.3	4.8	5.1	—	6.5	—	—	7.5	90.7	0.33
TB-C 525	HTB	8.8	23.8	25.2	20.3	15.2	12.0	9.3	8.0	6.2	—	—	41.6	170.4	0.22
	CTB	14.8	42.0	41.7	27.2	19.2	12.6	10.0	7.5	5.0	—	—	15.8	195.8	0.31
TB-D 525	HTB	10.8	17.5	13.1	13.7	11.3	—	22.3	6.8	6.5	6.2	11.8	28.4	148.4	0.14
	CTB	21.0	46.5	28.7	27.2	19.0	—	33.7	8.9	8.0	6.8	8.2	12.6	220.6	0.23
TB-R 420	HTB	13.9	14.1	13.9	9.9	—	13.1	—	10.0	—	—	—	26.6	101.5	0.24
	CTB	37.9	46.2	31.6	21.1	—	23.7	—	11.5	—	—	—	14.7	186.7	0.39
Mean	HTB														0.18
	CTB														0.32

a) Obtained from the slopes of the linear portions of log 'rate' plots of HTB and CTB excreted in urine. Compare with the elimination rate constants of TB from the blood, which are shown in Table I.

The excretion rates of HTB and CTB (mg/hr) are plotted on a logarithmic scale against the mid points of time intervals,[10] a typical example of which is also presented in Fig. 1. The log 'rate' plots of both the metabolites have their maximum values at 1.5 hr plots and then appear to become roughly linear. The slope of log 'rate' plots of CTB is much steeper than that of logarithmic plots of the blood levels of TB while the slopes of HTB and TB are not significantly different. An analog computer was used to examine the fit of Model II to

10) Hereafter, this kind of plots will be termed "log 'rate' plots."

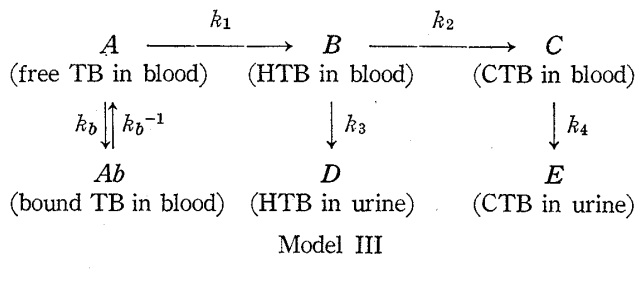
the observed data of the blood levels of TB and the amounts of HTB and CTB excreted. Consequently, it was found that when the parameters were set to fit the observed blood levels of TB, the calculated amount of CTB became far less than the observed values especially at the earlier times after the administration and on the contrary, when the parameters were set to fit the observed amount of CTB, the decline of the calculated blood levels of TB became much steeper than that of the observed blood levels of TB. Generally, the phenomenon that the excretion of a drug or its metabolites proceeds more rapidly than the decreasing of the blood levels of the parent drug is rather strange, because the excretion, which is the final step of drug behavior in body, should be rate-limited by the slowest step among the overall processes from the administration to the excretion. A similar situation has been reported by Funk[11] with some strongly protein-bound sulfanilamides. He has measured the plasma levels of the sulfanilamides and the amount of unchanged drugs excreted in urine of rat receiving the drugs intravenously and found that the excretion proceeds more rapidly than the fall of the plasma concentrations of the drugs. Further, he has suggested the effect of protein-binding on the observed phenomenon. It has been well established that TB are strongly bound to serum proteins.[12] The authors deduced that the observed trend that the excretion of CTB proceeds more rapidly than the fall of the blood levels of TB would be due to binding of TB with plasma proteins and/or blood cells and further, the blood levels of TB determined in the present work might be the sum of free and bound TB. Thus, Model III[13] was presented, in which TB injected intravenously is bound to plasma proteins and/or blood cells reversibly and only free TB is metabolized to HTB. In this publication, alphabetic letters in the model such as A, B, C, Ab, *etc.* mean not only the designation of each compartment but also the amount of the compound in each compartment and k's are all first-order rate constants.

When the volume of distribution is assumed to be identical with free and bound TB, the blood levels of free ($\bar{A}$) and total ($\overline{A+Ab}$) TB according to Model III may be expressed respectively:

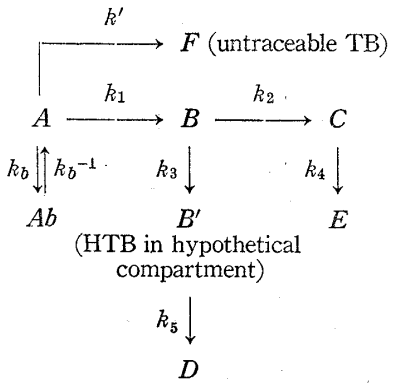
$$\bar{A} = A/Vd$$

$$\overline{A+Ab} = (A+Ab)/Vd$$

where Vd is the volume of distribution.



Model III



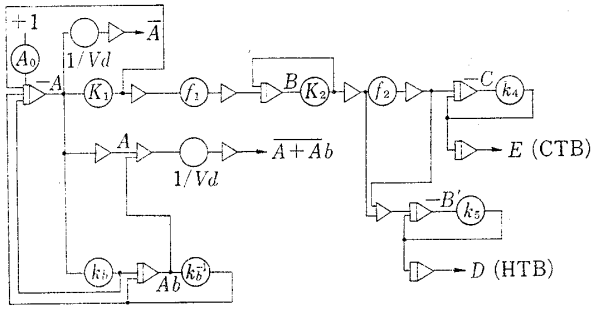
Model IV

The fit of Model III was examined to the experimentally observed blood levels of TB which are now regarded as $\overline{A+Ab}$ and also to the amounts of HTB (D) and CTB (E) excreted in urine. The examination by an analog computer indicated that Model III gave good agree-

11) K.F. Funk, *Acta Biol. Med. Germ.*, **25**, 151 (1970).
12) For example: J. Judis, *J. Pharm. Sci.*, **61**, 89 (1972).
13) Application of the reversible first-order kinetics to the binding of TB in blood was found to be adequate for interpreting the present experimental data, although it is well known that the Langmuir isotherm is more reasonable expression for the drug-protein interactions.

ment for $\overline{A+Ab}$ and E but could not give satisfactory agreement for D. The observed D showed the tendencies incresing much slower than the calculated D, which was anticipated from the results in Table II as mentioned previously. In order to improve the fit of Model III to D, a new compartment B' was introduced for HTB between B and D and further, k_3 was assigned to the rate constant for the transfer of HTB from B to B' and k_5 to that from B' to D. Here, the compartment B' is quite hypothetical and introduced only for the convenience of kinetical interpretations. The similar compartment as B' for HTB was formerly assumed by some of the present authors for the excretion of conjugates of N-acetyl-*p*-aminophenol.[14] Generally, there might be some compounds of which excretion from blood to urine would obey more complicated kinetics than single-step first-order process. In the next place, since the sum of HTB and CTB recovered in urine was considerably less than the dose and no metabolite other than HTB and CTB was detected in urine by thin-layer chromatography, one more competitive route derived from compartment A and a resulting compartment F were added to Model III. F represents TB eliminated by the routes other than the urinary excretion and/or fixed in tissues, but the details remain questionable. Further, k' was assigned to the rate constant for the transfer from A to F. Making these modifications on Model III, Model IV was finally proposed, by which satisfactorily good agreement between the calculated and observed data were obtained.

The calculation of the theoretical values according to Model IV was carried out by means of an analog computer. The differential equations and the computer program corresponding to Model IV are shown in Fig. 2. A typical example of good agreement of the theoretical curves according to Model IV and the experimentally observed data relating to $\overline{A+Ab}$, D,



$$\frac{dA}{dt} = -(k_b+K_1)A + k_b^{-1}Ab$$

$$\frac{dAb}{dt} = k_bA - k_b^{-1}Ab$$

$$\frac{dB}{dt} = k_1A - K_2B$$

$$\frac{dB'}{dt} = k_3B - k_5B'$$

$$\frac{dD}{dt} = k_5B'$$

$$\frac{dC}{dt} = k_2B - k_4C$$

$$\frac{dE}{dt} = k_4C$$

where $K_1=k_1+k'$, $K_2=k_2+k_3$, $f_1=k_1/K_1$, $f_2=k_2/K_2$,
$\bar{A}=A/Vd$, $\overline{A+Ab}=(A+Ab)/Vd$, A_0=Dose

Fig. 2. Analog Computer Program and Differential Equations Corresponding to Model IV

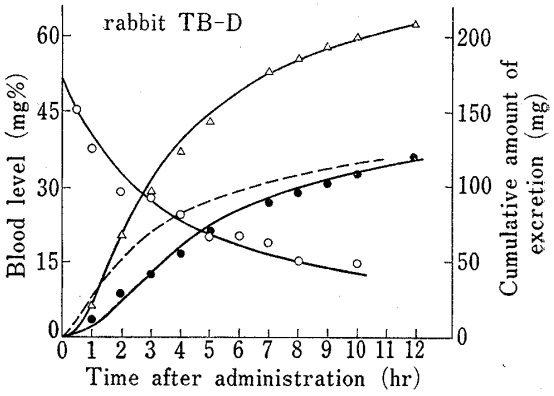


Fig. 3. Agreement between Theoretical Curves according to Model IV drawn by an Analog Computer and Experimental Data following Intravenous Administration of TB

Solid lines are theoretical values and dotted line is those of excreted HTB according to Model IV without hypothetical compartment B'. Plotted points are experimental values; sum of free and protein-bound TB in blood (○), HTB in urine (●), CTB in urine (△).

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

Table III. Rate and Other Constants on Interpreting TB Pharmacokinetics according to Model IV determined by an Analog Computer

Rabbit and dose (mg)	Rate constant (hr^{-1})								Volume of distribution for TB (dl)
	k_b	k_b^{-1}	k_1	k'	k_2	k_3	k_4	k_5	
TB-B, 285	0.20	0.03	0.22	0.06	2.47	1.64	1.60	0.37	9.36
TB-D, 525	0.14	0.13	0.21	0.05	2.97	1.72	1.83	0.71	10.30
TB-R, 420	0.46	0.34	0.38	0.10	2.67	1.16	2.36	0.99	7.10

and E is shown in Fig. 3 and the values of the parameters involved in Model IV determined by an analog computer are listed in Table III.

Pharmacokinetic Investigation on the Data following the Administration of HTB and CTB

Nelson and O'Reilly[4] determined the excretion rate constant of CTB by the experiment in which CTB was dosed intravenously and assigned it to the rate constant for the excretion of CTB formed in body from TB. In general, when the pharmacokinetics of drugs are studied in which the processes of metabolism are involved, supplementary studies following the administration of the metabolites *per se* are often carried out[15] expecting that the metabolites administered *per se* behave in the same manner as those formed in body from the precursors.

(1) **HTB Administration**——The blood levels of HTB at various times and the amounts of HTB and CTB in urine collected at various times after the intravenous dosage of 150 mg/kg HTB are listed in Table IV and V, respectively.

Table IV. The Blood Levels of HTB after Intravenous Administration of HTB (150 mg/kg) to Rabbits

Rabbit and dose (mg)	Blood levels (mg%) Time of blood collection (hr after administration)									Elimination rate constants[a] by graphical method (hr^{-1})
	0.17	0.33	0.50	0.67	0.83	1.00	1.25	1.33	1.66	
HTB-A, 420	19.7	13.8	8.8	5.5	2.6	—	1.8	—	—	2.8
HTB-B, 525	13.4	9.8	4.6	3.1	1.8	0.8	—	—	—	2.8
HTB-D, 375	19.1	12.0	8.4	6.2	4.7	3.9	—	2.9	0.7	2.0
HTB-K, 405	16.9	9.4	6.5	6.0	5.6	—	—	1.3	—	2.0
Mean										2.4

a) Obtained from the slopes of the linear portions of logarithmic plots of the blood levels of HTB.

Table V. The Amounts of HTB and CTB excreted in Urine of Rabbits after Intravenous Administration of HTB (150 mg/kg)

Rabbit and dose (mg)		Amounts of HTB and CTB in each urine specimen (mg equivalent of HTB) Time of urine collection (hr after administration)									Rate constants[a] by graphical method (hr^{-1})
		1	2	3	4	5	6	7	24	Total	
HTB-A 420	HTB	181.0	45.1	14.2	4.2	2.1	0	0	0	246.6	1.2
	CTB	21.1	13.0	1.9	0.8	0	0	0	0	36.8	1.2
HTB-B 525	HTB	301.5	29.4	5.4	1.6	0	0	0	0	337.9	2.0
	CTB	35.4	10.5	0.8	0	0	0	0	0	46.7	2.3
HTB-K 405	HTB	38.0	89.4[b]	44.7	28.4	15.0	13.6	7.5	0	236.6	0.5
	CTB	10.1	24.6[b]	5.1	1.5	0	0	0	0	41.3	1.5
Mean	HTB										1.2
	CTB										1.7

a) Obtained from the slopes of log 'rate' plots of HTB and CTB excreted in urine. Compare with the elimination rate constants of HTB from the blood, which are shown in Table IV.
b) Maximum values were observed. Details are given in the text.

It is clear by the comparison of Table II and V that the amount of CTB excreted is small when HTB is administered, while it is large when TB is administered. Since it was suspected that 150 mg/kg dose of HTB by rapid intravenous injection caused temporary high level of HTB in blood and consequently the saturation of the oxidative process of HTB to CTB occurred, the following experiments were carried out expecting to exclude the possible occurrence of the saturation, in which the doses and the administration methods of HTB were varied. When rabbits were given 50 mg/kg HTB by rapid intravenous injection or 150 mg/kg HTB by oral and peritoneal routes, the excretion of CTB was as low as in the case given 150 mg/kg HTB by rapid intravenous injection. Further, when rabbits were given about 150 mg/kg HTB by logarithmic rate infusion at the rate of 0.2 hr^{-1}, which is approximately corresponding to the elimination rate of the blood levels of TB after the rapid intravenous administration of 150 mg/kg TB, it was found unexpectedly that the excretion of CTB became negligibly small. Accordingly, it may be concluded that whenever HTB is administered, the amount of CTB excreted is far less than HTB regardless of the doses and the administration methods indicating that HTB administered *per se* is less susceptible to the oxidation to CTB than HTB formed in body from TB. McDaniel, *et al.*[16] reported that CTB was formed from the oxidation of HTB catalyzed by alcohol dehydrogenase and aldehyde dehydrogenase present in 9000 x*g* supernatant fraction of rat liver. According to their studies, HTB given *per se* would be difficult to diffuse to the site where these enzymes are located while HTB formed in body from TB could easily diffuse to the site. Here, it is demonstrated that the assumption that the metabolites administered *per se* will behave in the same manner as the same metabolites formed in body from the precursors is not always reasonable, though it has been undoubtedly employed in the pharmacokinetic studies. The comparison of recoveries of HTB and CTB in urine after the respective administration of TB and HTB is shown in Table VI.

Table VI. Comparison of Recoveries of HTB and CTB excreted in Urine after Administration of TB and HTB to Rabbits

Compound	Administration		No. of rabbits	Mean of 24 hr total recovery (% of dose)	Mean of fraction excreted as (% of recovery)	
	Dose (mg/kg)	Route			HTB	CTB
TB	150	rapid *i.v.*	5	67.8	42.0	58.0
TB	150	*p.o.*	3	76.8	48.6	51.4
TB	150	*i.p.*	3	73.0	43.1	56.9
HTB	150	rapid *i.v.*	4	67.0	87.6	12.4
HTB	50	rapid *i.v.*	3	76.3	78.3	21.7
HTB	150	log *i.v.*[a]	3	84.8	98.2	1.8
HTB	150	*p.o.*	2	81.2	70.5	29.5
HTB	150	*i.p.*	1	79.9	77.2	22.8

a) Dosed about 150 mg/kg HTB by logarithmic rate-infusion at the rate of 0.2 hr^{-1} for 8 hr.

Typical examples of logarithmic plots of the blood levels of HTB and log 'rate' plots of HTB and CTB excreted in urine *versus* times after the intravenous dosage of HTB are shown in Fig. 4. The elimination rate of HTB from blood after the dosage of HTB is much faster than that of TB after the dosage of TB, which is accord with the findings of Schulz and Schmidt[8] that the serum half-life of HTB is less than one-tenth that of TB in man. The log 'rate' plots of HTB and CTB excreted in urine are roughly regarded as linear from 0.5 hr

15) For example: A.H. Beckett and G. T. Tucker, *J. Pharm. Pharmacol.*, **20**, 173 (1968); G.A. Portmann, E.W. McChesney, H. Stander, and W.E. Moore, *J. Pharm. Sci.*, **55**, 72 (1966).
16) H.G. McDaniel, H. Podgainy, and R. Bresser, *J. Pharmacol. Exptl. Therap.*, **167**, 91 (1968).

plot except one example (HTB-K), which gives maximum values at 1.5 hr plot and then appears to decrease linearly. Both the log 'rate' plots are decreasing more slowly than the plots of the HTB blood levels suggesting that introducing protein binding of HTB into kinetic model would be unnecessary.

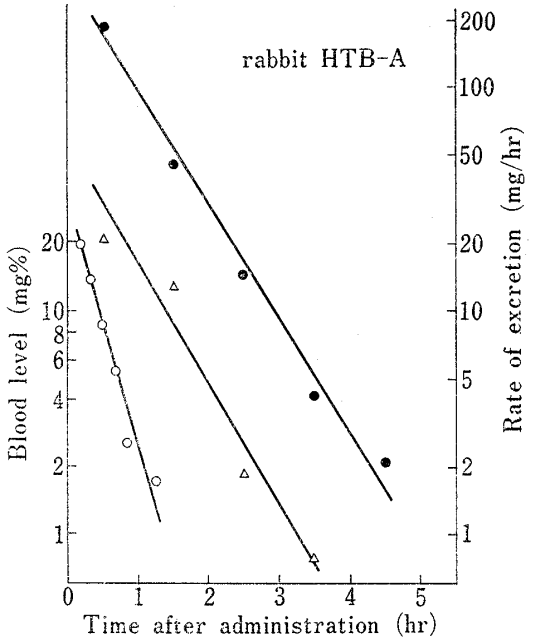


Fig. 4. Logarithmic Plots of the Blood Levels of HTB (-○-) and Log 'rate' plots of the Excretion of HTB (-●-) and CTB (-△-) after Intravenous Administration of HTB (150 mg/kg) to a Rabbit

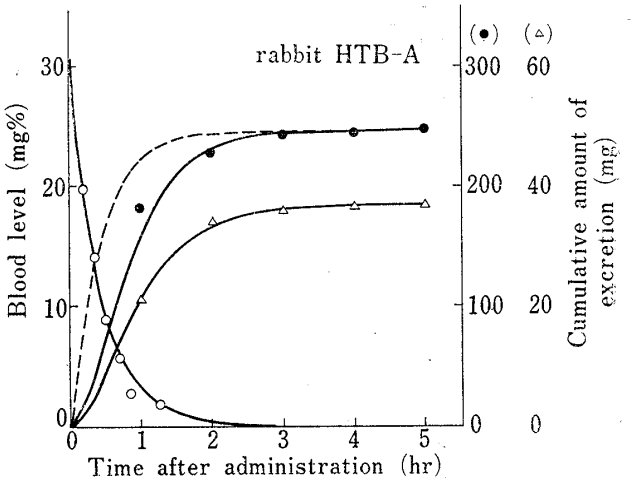
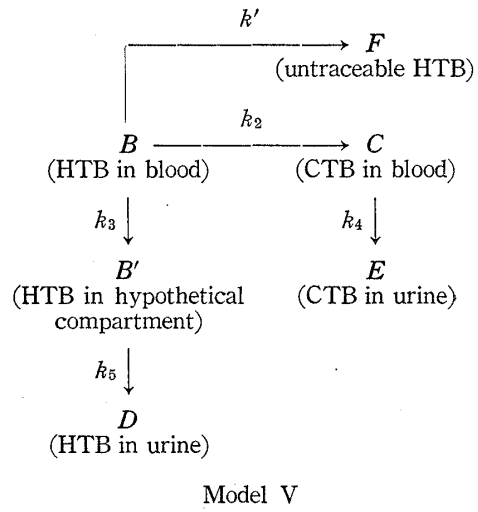


Fig. 5. Agreement between Theoretical Curves according to Model V drawn by an Analog Computer and Experimental Data following Intravenous Administration of HTB

Solid lines are theoretical values and dotted line is those of excreted HTB according to Model V without hypothetical compartment B'. Plotted points are experimental values; HTB in blood (○), HTB in urine (●), CTB in urine (△).

Searching good agreement of the observed data and the theoretical values relating to the blood levels of HTB and the amounts of HTB and CTB excreted in urine, Model V was found to be suitable where the hypothetical compartment B' and competitive process derived from compartment B for untraceable HTB were also added as in the case of TB pharmacokinetics. Another support for the necessity of compartment B' would be provided by the fact that the peak was seen in the log 'rate' plots of HTB by rabbit HTB-K.

A typical example of good agreement of the theoretical curves according to Model V and the experimentally observed data relating to the blood levels of HTB ($\bar{B}$) and the amounts of HTB (D) and CTB (E) excreted in urine is shown in Fig. 5 and the values of the parameters involved in Model V determined by an analog computer are listed in Table VII.



Model V

(2) CTB Administration——The blood levels of CTB at various times and the amount of CTB in urine specimens collected at various times after the intravenous dosage of 150 mg/kg CTB are listed in Table VIII and IX, respectively.

Typical examples of logarithmic plots of the blood levels of CTB and log 'rate' plots of CTB excreted in urine *versus* times after the intravenous dosage of CTB are shown in Fig. 6. Since the logarithmic plots of the blood levels of CTB and the log 'rate' plots of CTB excreted

Table VII. Rate and Other Constants on Interpreting HTB Pharmacokinetics according to Model V determined by an Analog Computer

Rabbit and dose (mg)	Rate constant (hr^{-1})					Volume of distribution for HTB (dl)
	k_2	k_3	k'	k_4	k_5	
HTB-A, 420	0.21	1.34	0.76	1.66	2.17	14.20
HTB-B, 525	0.28	2.02	0.85	2.62	3.42	24.53
HTB-K, 405	0.17	0.97	0.52	1.61	0.55	18.21

Table VIII. The Blood Levels of CTB after Intravenous Administration of CTB (150 mg/kg) to Rabbits

Rabbit and dose (mg)		Time after administration and blood levels of CTB ($\bar{C}$)								Elimination rate constants[a] by graphical method (hr^{-1})	Volume of distribution[b] for CTB (dl)
CTB-A 510	Time (hr)	0.13	0.25	0.58	1.00					2.0	15.0
	$\bar{C}$ (mg%)	26.5	20.8	10.9	4.6						
CTB-B 615	Time (hr)	0.17	0.33	0.67	0.83	1.08	1.33	1.90	2.00	2.1	10.2
	$\bar{C}$ (mg%)	29.5	19.6	15.2	8.1	6.6	4.5	1.1	0.7		
CTB-C 510	Time (hr)	0.19	0.30	0.80	1.03	1.08	1.40			3.1	17.6
	$\bar{C}$ (mg%)	19.0	15.3	2.3	1.7	1.1	0.6				

a) Obtained from the slopes of logarithmic plots of the blood levels of CTB.
b) Obtained by dividing the dose by the blood level at zero time, which is determined by extrapolating logarithmic plots of the blood levels of CTB to zero time.

Table IX. The Amount of CTB excreted in Urine of Rabbits after Intravenous Administration of CTB (150 mg/kg)

Rabbit and dose (mg)	Amount of CTB in each urine specimen						Rate constants[a] by graphical method (hr^{-1})
	Time of urine collection (hr after administration)						
	1	2	3	4	24	Total	
CTB-A 510	455.9	35.6	8.6	1.1	0	501.2	2.0
CTB-B 615	486.6	64.9	6.6	1.5	0	559.6	2.1
CTB-C 510	388.2	74.1	6.7	0	0	469.0	2.1

a) Obtained from the slopes of log 'rate' plots of CTB excreted in urine. Compare with the elimination rate constants of CTB from the blood, which are shown in Table VIII.

in urine are roughly regarded as linear and approximately parallel as shown in Fig. 6, the process that CTB injected intravenously is excreted unchanged in urine would be simulated by single-step first-order kinetic model presented as Model VI. The values of the rate constants obtained from these graphs are listed in Table VIII and IX, respectively. These values are roughly in accord with k_4 in Table III and VII suggesting that CTB administered *per se* would behave samely in body as CTB formed through metabolic reaction from TB or HTB.

By the present works, it becomes obvious that the excretion patterns of CTB are essentially identical whether it is formed from TB or HTB or administered *per se* while the oxidation patterns of HTB depend upon largely whether it is formed from TB or given *per se*. It might be deduced, therefore, that the behavior of a metabolite which is formed in body from its precursor and susceptible to further metabolism could not always be duplicated by

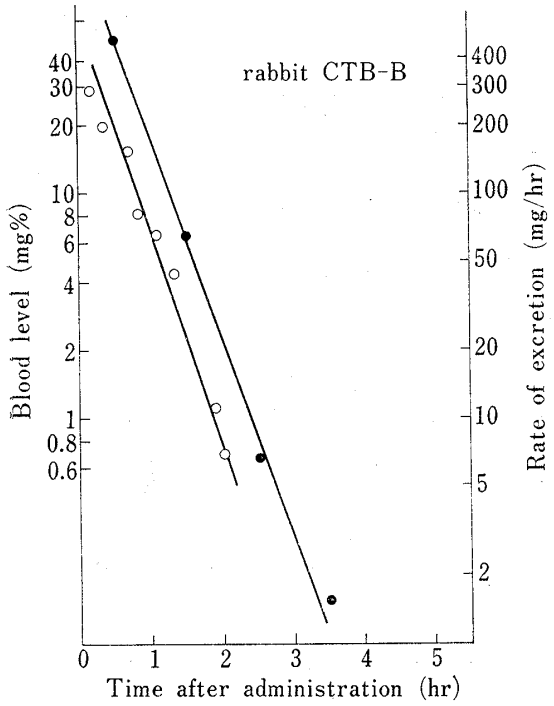


Fig. 6. Logarithmic Plots of the Blood Levels of CTB (–○–) and Log 'rate' plots of the Excretion of CTB (–●–) after Intravenous Administration of CTB (150 mg/kg) to a Rabbit

$$C \xrightarrow{k_4} B$$

(CTB in blood) (CTB in urine)

Model VI

the same metabolite which is given *per se*, but this assumption remains speculative in the absence of more extensive experimental data. It is well known that the pharmacological activity of a drug may to some extent depend on the blood level of its diffusible form which is not bound to proteins. Wishinsky, *et al.*[17] showed that the addition of salicylate to TB-containing serum will increase the proportion of the unbound TB, and they suggested that this might to some degree explain the increase by salicylate of hypoglycemic effect of sulfonylureas which is observed by Stowers, *et al.*[18] With these facts in mind, it seems clinically of importance to investigate the detailed kinetic characteristics of protein-binding of TB and those affected by the simultaneous administration of other drugs.

Acknowlegement The authors are indebted to J. Shirabe, M. Kiriyama, N. Shoya, K. Kitaoka, and M. Rikitake for their technical assistances.

17) H. Wishinsky, E.J. Glasser, and S. Perkal, *Diabetes*, **11**, suppl., 18 (1962).
18) J.M. Stowers, L.W. Constable, and R.B. Hunter, *Ann. N. Y. Acad. Sci.*, **74**, 689 (1959).