Chem. Pharm. Bull. 19(5) 881—891 (1971)

UDC 547.853.3.09 : 615.31'7.03.076.9

Studies on the Metabolic Fate and the Pharmacokinetics of 5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) in Man. V.[1] Pharmacokinetics of BCP in Man and in Rabbit following Intravenous Administration of BCPNa

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(Received July 6, 1970)

In order to support the application of the model 1[1] to simulate the behavior of 5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) in man, further studies were carried out independently in man and in rabbits.

The human plasma concentration and urinary excretion after the intravenous administration of BCPNa in a clinical test were simulated well by the model 1 with the same order of values for the rate constants as in the oral administration.

Then, using rabbits, BCP in organs and tissues along with the plasma concentration and urinary excretion were determined and compared with the simulated values by the model 1.

After correction for the mass balance, the tissue amounts of BCP were simulated fairly well.

These results indicated the applicability of the model 1 both in man and in rabbits.

In order to simulate the behavior of 5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) in man after its oral administration, the model 1 was applied well as described in the previous paper.[1] Generally speaking, however, in a simulation on biological data, when the number of obtainable data are less than the number of parameters to be determined, validity of a model is not confirmative.[3] Therefore, to support the application of the model 1 in man, a study was carried out on the behavior of BCP after BCPNa was administered intravenously (*i.v.*) to man.

Furthermore, to prove the distribution of BCP in tissues, the tissue amounts of BCP were determined in rabbits, instead of man, after the *i.v.* administration of BCPNa along with the plasma concentration and urinary excretion. If the tissue amount of BCP was also simulated well by the model, the more dependable the model would be.

Experimental

Studies on the *i.v.* Administration of BCPNa to Man——1) Human Subjects: Administration were made to the subjects after the surgical operations. The following subjects were excluded. a) Subjects who had diseases in liver or kidney. b) Subjects with hypoproteinemia. c) Subjects who bled seriously in the operation. d) Subjects with anemia. e) Subjects who took other drugs which would interfere the determination of BCP and its metabolites. Age, body weight, bleeding volume in the operation and the volume of the infused nutrient solution are listed in Table I.

2) Administration of BCPNa and Collection of the Samples: After the operation, 20 ml of the solution of BCPNa (400 mg as BCP) were administered (*i.v.*) within a few minutes for the first time, then every 24 hours for 5 days. Blood samples (3 ml) were drawn at 0, 0.5, 1.0, 1.5, 2.0, 4.0, 8.0, 12.0 hours after the

1) Part IV: T. Yashiki, T. Matsuzawa, M. Yamada, T. Kondo, Y. Uda, Z. Hokazono and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 869 (1971).

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3) Y. Araki, *Farumashia*, **4**, 559 (1968).

Table I. Age, Body Weight of the Human Subject, Bleeding Volume at the Surgical Operation and the Volume of the Infused Nutrient Solution

No.	Age	Sex	Body weight (kg)	Bleeding volume (ml)	Nutrient solution (liter/day)
1′	22	♂	49	550	2.0—3.0
2′	28	♀	44	170	1.6—2.0
3′	22	♂	54	272	1.5—2.5
4′	37	♀	48	352	1.0—3.0
5′	55	♀	35.5	130	1.0—2.6

administration in the first day. From the second day, the blood samples were drawn just before the next administration and after 24 hours of the 5th administration (6th day), the last sample was drawn. Urines were collected at 0, 4, 8, 12 hours after the administration in the first day. From the second day, the urines were collected until the next administration and after 24 hours of the 5th administration (6th day) the last sample was collected.

3) Determination of BCP and Its Metabolites: BCP in the plasma, and total BCP, a metabolite II[4] in the urine were determined by ultraviolet (UV) absorption method.[5] The metabolite IV[6] was determined by gas–liquid chromatography (GLC).[7] The pattern of the metabolites in a urine was checked also by GLC.[8]

4) Analogue Computer: MELCOM (EA-7304) was used in this analysis.

Studies on the *i.v.* Administration of BCPNa to Rabbits——1) Rabbits, Administration Dose and Route: Male subjects weighing 2.8—3.2 kg were used. BCPNa was dissolved in saline and 10 ml of the solution was injected from the auricular vein within a few minutes. The dose was 50 mg of BCP per kg.

2) Determination of BCP in the Tissues: The determination method by Mima, *et al.*[9] was used with slight modifications. To 2 g of an organ or a tissue of a rabbit (brain, lung, spleen, liver, kidney, adrenal glands, stomach, intestine, fat and muscle), was added 10 ml of water and homogenized with a homogenizer[10] in an ice–water. To 3 ml of the homogenate, was added 3 ml of 1N HCl and BCP was extracted with 25 ml of heptane containing 1% iso-amylalcohol by shaking it for 25 min. After centrifugation, 20 ml of the heptane layer was transferred to another centrifuge tube and was shaken with 10 ml of 0.1M borax solution for 20 min. From the absorbance at 271 mμ of the borax solution, the concentration of BCP was determined.[5] Blank values were obtained by the same procedures on the organs or tissues of an intact rabbit. In the cases of brain and heart, pure heptane were used to avoid the turbidities in the borax solution. When the concentration of BCP was low in the sample, the sample weight was increased. At the addition of equal volume of 0.5, 1, 2 or 6N HCl to the homogenate, almost the same determined value of BCP was obtained and no interaction of BCP found in the urine[5] was observed in this case. The recoveries of the determination on different organs or tissues were within 95.2—99.3%, though 92.2% from heart and 80.3% from brain when 125 μg of BCP were added to the blank samples.

3) Extirpation of the Organs or Tissues: In a short period after the administration, plasma concentration of BCP in a tissue was so high that the blood should be removed to measure the BCP in the tissue correctly. After a rabbit was anaesthetized with urethan, saline was circulated from the abdominal vein to bleed out just before the sacrifice, then the organs and tissues were taken out.

Result and Discussion

I. Simulation of the Behavior of BCP after *i.v.* Administration of BCPNa to Man

Plasma Concentration and Urinary Excretion——As shown in Fig. 1, the plasma concentration decreased rapidly in 4 hours then decreased slowly following apparent first order kinetics in the first day, which suggested apparently two compartmental model was applicable for the analysis. The average half-life calculated from the slopes of the second straight lines

4) 1-Cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine.
5) T. Yashiki, T. Kondo, Y. Uda and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 478 (1971).
6) 5-*n*-Butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine.
7) T. Yashiki, Y. Uda, T. Kondo and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 487 (1971).
8) T. Yashiki, T. Matsuzawa, T. Kondo, Y. Uda, T. Shima, H. Mima, S. Senda and H. Izumi, *Chem. Pharm. Bull.* (Tokyo), **19**, 468 (1971).
9) H. Mima, T. Matsuzawa, K. Terada and T. Kondo, *Takeda Kenkyusho Nempo*, **24**, 9 (1965).
10) Ultra Turax (Janke & Kunkel).

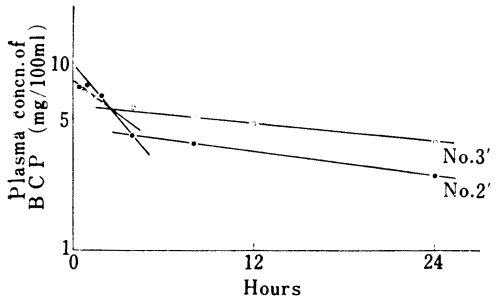

Fig. 1. Semilogarithmic Plot of the Plasma Concentration of BCP after the *i. v.* Admini stration of BCPNa (400 mg as BCP) to Man

No. 2', 3': the experimental number of the subject

TABLE II. Apparent Biological Half-Life ($t_{1/2}$) and Distribution Volume (V_d) of BCP after the *i.v.* Administration of BCP Na (400 mg as BCP) to Man

No.	$t_{1/2}$ (hours)	V_d(liter)
1'	29.9	12.1
2'	28.4	8.9
3'	41.2	7.2
4'	20.9	7.4
5'	48.6	6.1
Average	33.8±11.1[a)]	8.3±2.3[a)]

$t_{1/2}$ and Vd were obtained from the slope and the intercept of the 2nd straight line in Fig. 1 respectively.
a) standard deviation

coincided well with that obtained in the oral administration of BCP to healthy males, *i.e.*, 33.6±1.3 hours.[1)] Apparent distribution volumes obtained by extraporating the second straight lines were also shown in Table II.

After the successive administration, plasma concentration increased with days to reach the equilibrium values in 4 days (Fig. 2, Table III). The average equilibrium plasma concentration was 8.1±2.1 mg/100 ml which was almost the same as 7.0 mg/100 ml obtained when 400 mg BCP per day (divided into 3 doses) was administered orally to a man of 55 kg.[1)]

After the first administration, total BCP and II were excreted 18.6±7.2 (S.D.)%, 15.2±10.6% of dose respectively in 24 hours (Table IV), which were more than the excreted amounts in the oral administration (9.7±0.6%, 6.2±1.2%).[1)] The metabolite IV were excreted only trace in three subjects out of five and the average excreted amount was less than that in the oral administration (3.5±1.2%).[1)]

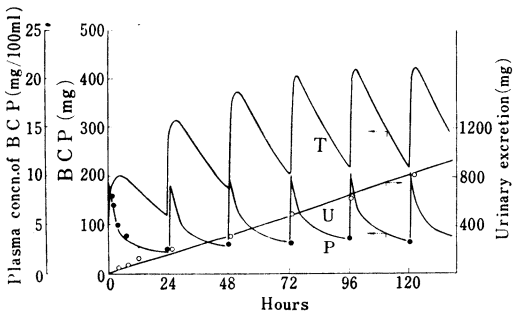

Fig. 2. Simulation of the Plasma Concentration of BCP (P), Urinary Excretion of Total BCP and Its Metabolites (U) and Tissue Amount of BCP (T) after the Successive *i.v.* Administration of BCPNa (400 mg as BCP) to Man (No. 2', Model 1)

——: simulation curve, ●,○: experimental data

After the successive administration, the cumulative excreted amount increased almost linearly with days as shown in Fig. 2, though the excretion in the first day was faster than that in the following day.

GLC showed an abnormal metabolic pattern in the two subjects, *i.e.*, the peak of unknown 2[8)] was larger than that of II which was the main metabolite in the oral administration and the peak of IV was very small. On this abnormality in the metabolism, several causes were considered such as due to the inherent individual difference, individual disease or tothe dosage form.

Model for the Simulation——For analysis, the model 1[1)] was also applied except the absorption process. Namely, BCPNa is *i.v.* administered into the plasma water, where a fraction is bound to plasma protein, transferred to tissue and excreted in the urine as unchanged or the metabolites proportionally to the plasma water concentration (Fig. 3).

In simulation of the administration process, the whole dose (400 mg as BCP) could not be loaded into the plasma water compartment instantly in this model 1, owing to the voltage

Table III. Plasma Concentrations, Tissue Amounts of BCP in Equilibria after the Successive *i.v.* Administration[a] of BCP Na (400 mg as BCP) to Man

No.	1st day		1—6 days			
	Tissue amount of BCP (mg)	Hr[b]	Plasma concn. of[c] BCP (mg/100 ml)	Day[b]	Tissue amount[c] of BCP (mg)	Day[b]
1′	220	5	5.40	4	298	5
2′	205	5	6.75	4	325	5
3′	220	6	9.33	4	550	5
4′	240	5	8.50	4	568	5
5′	240	4	10.70	4	528	5
Average	225±15[d]		8.14±2.09[d]		453.8±131[d]	

a) Administration was made once a day.
b) the period to be in equilibrium
c) The value in equilibrium is expressed by the average of the maximum and the minimum values in a day.
d) standard deviation

Table IV. Urinary Excretion in 24 hours after the *i.v.* Administration of BCPNa (400 mg of BCP) to Man

No.	Total BCP (%)	II (%)	IV (%)
1′	24.7	29.6	—
2′	25.6	16.9	6.0
3′	8.4	19.3	3.8
4′	19.8	1.9[a]	—
5′	14.3	8.2[a]	—
Average	18.6±7.2[b]	15.2±10.6[b]	2.0±2.8[b]

—: trace
a) Abnormal gas-chromatogram was observed, *i.e.*, the peak of the unknown 2 was larger than the peak of II.[8]
b) standard deviation
II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

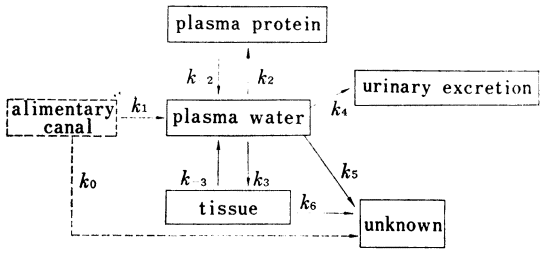

Fig. 3. Model 1 to Simulate the Behavior of BCP in Man and in Rabbits

For *i.v.* administration, alimentary canal compartment is unnecessary, however, it is considered conveniently in calculation to avoid the overload of the computer. The values for k_0, k_6 are assumed to be zero.

overload of the computer. Therefore as an approximation, the apparent saturation amount (13 mg/100 ml× volume of plasma) was loaded in the plasma protein compartment and the rest of the dose was loaded in the plasma water compartment as the initial values.

The following assumptions were also made as done in the oral administration.[1] i) The concentration in a compartment is homogeneous. ii) The binding of BCP to plasma albumin is a second order process, *i.e.*, first order with respect to the plasma water concentration of BCP and first order with respect to the concentration of unbound plasma albumin. iii) The binding of BCP to plasma albumin is saturated apparently when the plasma concentration reaches to 13 mg/100 ml. The initial concentration of the plasma albumin is expressed by the binding equivalence of BCP,

i.e., 13 mg/100 ml. iv) Other transfer-processes of BCP in a body follow first order kinetics. v) Urinary excretion of unchanged total BCP, II and IV follow parallel first order kinetics. The rates of the metabolism and of excretion are not separated in this analysis. vi) The excretion rates of the unknown are proportional to the concentration of BCP in the plasma water.

The same mass equations and block diagram[1] were used as in the oral administration except the absorption process from the alimentary canal.

Simulation of the Plasma Concentration, Tissue Amount of BCP and Urinary Excretion—The individual plasma concentration and urinary excretion in the successive administration were simulated within the experimental variation with the rate constants shown in Table V, though in the first day, both the calculated values were slightly larger than the experimental values in the early stage (0—6 hours) after the administration (Fig. 4).

As this discrepancy might be due to the approximation made in the administration process, the values of k_2 and k_{-2} were multiplied by 4 and 6 respectively. By this treatment, a little improvement in the fittings was observed. However, considering the number and variation of the experimental data, the simulation could still be made without the treatment.

From Table V, the rates of binding of BCP to plasma albumin and of distribution to tissue were high, whereas the rate of release from tissue was low, which was the same trend as in the oral administration.

Table V. Rate Constants for Simulation of the Behavior of BCP after the *i.v.* Administration of BCPNa (400 mg as BCP) to Man

k's[a]	1′	2′	3′	4′	5′	Average±S.D.
k_2	0.45	0.45	0.45	0.45	0.45	0.45
k_{-2}	0.60	0.60	0.60	0.60	0.60	0.60
k_3	42.5	21.5	27.5	35.0	20.0	29.3 ±9.4
k_{-3}	0.215	0.13	0.25	0.235	0.225	0.211±0.048
k_4	6.50	2.00	1.00	1.40	0.90	2.36 ±2.35
	(10.00)[b]	(5.00)	(2.25)	(2.08)	(1.40)	(4.15 ±3.55)
k_5	5.00	5.00	1.75	2.68	1.85	3.26 ±1.63
	(1.50)	(2.00)	(0.50)	(2.00)	(1.35)	(1.47 ±0.62)

a) The dimensions of k's are (hr^{-1}) except k_2(liter·mg^{-1}·hr^{-1}).
b) The values in parentheses stand for the rate constants to simulate the excretion in the first day after the administration.

Similarly as before, considering the data obtained in the first day after the initial administration as the data in the single administration, the amounts of BCP in plasma were calculated until the amounts decreased to zero and the areas under the curves were estimated gravimetrically. The average value was 191.5±56.2 (S.D.) mg. Though rigorous comparison of the area with those obtained in the oral administration (200.0±8.5 mg, 540.7±48.1 mg in 300 and 900 mg of dose respectively[1]) could not be made, for the experiments were carried out on different subjects, this average value was consistent with the assumption made in the oral administration that the absorption of BCP was complete.

In simulation of the urinary excretion, two kinds of values were obtained for k_4 and k_5. The values in parentheses were for the excretion in the first day and the other values were in the following days (Table V). The high excretion rate in the first day might be mainly due to the change in the physiological functions of a subject by the surgical operation.

As shown in Table III, the maximum tissue amounts of BCP were found in 4—6 hours in the first day, and the average value was a little larger than that in the single oral administration (185 mg in a man of 55 kg). After the successive administration, the maximum tissue

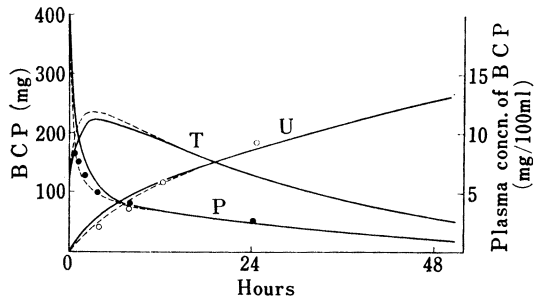

Fig. 4. Simulation of the Plasma Concentration of BCP (P), Urinary Excretion of Total BCP and Its Metabolites (U) and Tissue Amount of BCP (T) after the Initial *i.v.* Administration of BCPNa (400 mg as BCP) to Man (No. 2′ Model 1)

The data obtained in the first day after the initial administration was considered as those in the single administration.
—: $k_2=0.45$ liter·mg^{-1}·hr^{-1}, $k_{-2}=0.60$ hr^{-1}, $k_{-3}=21.5$ hr^{-1}, $k_{-3}=0.13$ hr^{-1}, $k_4=5.0$ hr^{-1}, $k_5=2.0$ hr^{-1}
---: $k_2=1.8$ liter·mg^{-1}·hr^{-1}, $k_{-2}=3.60$ hr^{-1}, $k_3=20.0$ hr^{-1}, $k_{-3}=0.225$ hr^{-1}, $k_4=2.75$ hr^{-1}, $k_5=1.0$ hr^{-1}

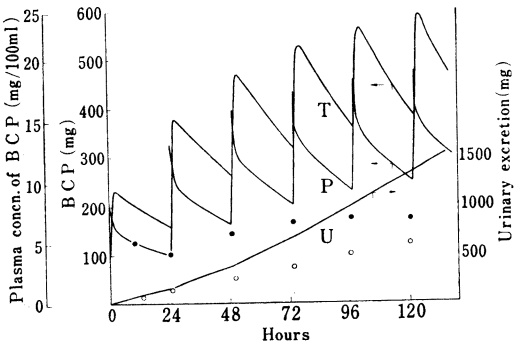

Fig. 6. Simulation of the Plasma Concentration of BCP (P), Urinary Excretion of Total BCP and Its Metabolites (U) and Tissue Amount of BCP (T) after the Successive *i.v.* Administration of BCPNa (400 mg as BCP) to Man (No. 3′ Another model)

$k_{12}=1.58$ hr^{-1}, $k_{21}=1.01$ hr^{-1}, $k_{13}=0.05$ hr^{-1}
—: simulation curve, ●,○: experimental data

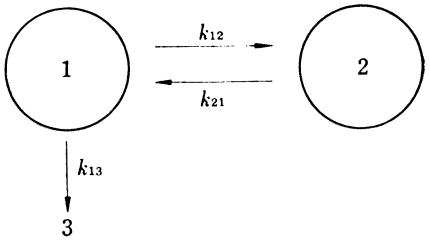

Fig. 5. Another Model Tested for Simulation of the Behavior of BCP after the *i.v.* Administration of BCPNa (400 mg as BCP) to Man

1: central compartment
2: peripheral compartment
3: excretion as total BCP and its metabolites in the urine
k's: first order rate constants for the transference

amounts were found in more than 4 days and the average equilibrium value was almost the same as that in the successive oral administration (400 mg in a man of 55 kg).

The result that each rate constant has the same order of value as that in the oral administration supports the application of the model 1 to simulate the behavior of BCP in man also in the *i.v.* administration.

Simulation by Another Model——Curve fittings were also tried by another model shown in Fig. 5. The plasma concentration and urinary excretion in the first day were also simulated well by the model. After the successive administration, however, the calculated plasma concentration increased with days, which did not coincide with the experimental data (Fig. 6). Thus, the model was not applicable to simulate the behavior of BCP after the *i.v.* administration of BCPNa to man.

II. Studies on the *i.v.* Administration of BCPNa to Rabbits

On the distribution of BCP to tissues, the following experimental results were known. a) BCP in tissues were measured by UV absorption method 3 hours after the *i.v.* administration of BCPNa to rabbits. In liver, lung, kidney, heart, brain, spleen, adrenal glands, fat and muscle, BCP were distributed.[9] b) After successive administration of BCPNa, the administration was ceased. Eight to ten days after the cessation, BCP in tissues were only trace and the accumulation was not observed in rabbits.[11] c) Autoradiograms were taken

11) T. Matsuzaki, T. Kondo and H. Mima, "unpublished data".

periodically after 2-^{14}C-BCP were administered orally to mice. After 5 minutes, BCP was found in stomach and liver and after 1 hr, in heart, kidney and liver besides stomach and intestine.[12] d) In the synovia, about half of the plasma concentration of BCP was determined after the oral administration of the drug to man.[13] e) Besides liver, kidney and muscle, the uptake of BCP was observed in *Anulus fibrosus* after its oral administration to rats. Similar uptake was found also in man.[14]

These results suggested the readiness for distribution of BCP into tissue compartment. However, these data were taken semi-quantitatively or at arbitrary times.

In order to analyze the time course of the tissue amount of BCP, the drug in tissues were determined quantitatively and periodically in rabbits along with the plasma concentration and urinary excretion after the *i.v.* administration.

Application of the Model 1 to the Data on Rabbits——First, for simulation of the behavior of BCP in rabbits, a preliminary examination was carried out on the applicability of the model 1 to the published data,[9] in which the plasma concentration after the oral administration of 20, 50, 100, 200, 300 and 500 mg of BCP and the urinary excretion at 200 mg of dose were shown graphically. Also, the apparent saturation plasma concentration was shown to be *ca.* 30 mg/100 ml of BCP experimentally.

The data were for the oral administration, and *i.v.* administration was made in this experiment expecting the good distribution of BCP into tissues. The examination on the applicability of the model 1 to the data (*p.o.*), however, would also give a direction for the applicability of the model to the data (*i.v.*) as in the human case.

For analysis by the model 1, the same assumptions were made as done in man except a few modifications described below. The average biological constants were assumed as follows.

body weight: 2.75 kg
blood volume: 148 ml (5.4% of the body weight)[15]
plasma volume: 67 ml (45% of the blood volume)
plasma albumin: 2.9 g$=4.26\times10^{-2}$ mmole (4.3% of the plasma volume,[16] mol. wt.$=6.8\times10^{4}$,[17])

On the binding of BCP to the plasma protein in rabbits, 7.5×10^{-2} mmole of BCP (1.7 mole BCP per mole of plasma albumin) was in the plasma at the apparent saturation.[18]

If Karush's equation[19] was applied, all the binding sites of the primary association constant ($K_1=1.08\times10^{5}$ M^{-1}, $n_1=1.02$) and 10% of the second sites ($K_2=1.35\times10^{3}$ M^{-1}, $n_2=6.98$) were bound at the apparent saturation. Therefore, two compartments for the plasma protein should be considered to simulate the binding of BCP to the albumin.

In the analysis, however, a rough approximation was made that BCP were bound to plasma albumin only at one type of sites which were saturable at the plasma concentration of 30 mg/100 ml, because a more elaborate model does not always give the more precise result owing to the limitations of obtainable data comparing the number of parameters to be determined, especially when the variation of the data is significant.

12) S. Kikuchi and J. Suzuoki, "unpublished data".
13) K. Nakabayashi and K. Kato, "Clinical Literature on Paramidin®," Takeda Chem. Ind. Ltd., 1968, p 20.
14) J. Suzuki, S. Inoue, H. Tsuji, M. Mitsuhashi, I. Kosaki, K. Murayama and T. Mitsuhashi, "Clinical Literature on Paramidin®," Takeda Chem. Ind. Ltd., 1968, p. 25.
15) "Seibutsugaku Jiten" ed. by T. Yamada, F. Maekawa, F. Egami, R. Yasugi, Iwanami Shoten, Tokyo, 1960, p. 277.
16) "Handbook of Biological Data" ed. by W.S. Spector, Division of Biology and Agriculture, The National Academy of Sciences, The National Research Council, W.B. Saunders Co., Philadelphia and London, 1961.
17) F.W. Putnam, "The Plasma Protein" Vol. 1, Academic Press, N.Y., London, 1960, p. 169.
18) BCP: 300 μg/ml$\times$67 ml$=$20.1 mg$=7.5\times10^{-2}$ mmole. BCP/Plasma albumin$=7.5\times10^{-2}$ mmole/4.26×10^{-2} mmole$=$1.7.
19) K. Kakemi, T. Arita, M. Hashi and K. Sumii, *Yakugaku Zasshi*, **86**, 739 (1966); F. Karush, *J. Am. Chem. Soc.*, **72**, 2705 (1950).

The initial concentration of the plasma albumin was expressed by the binding equivalence of BCP, *i.e.*, 30 mg/100 ml.

The GLC of the chloroform extract from the urine of rabbits showed the peak of BCP, but those of the human metabolites, II and IV were very small. Hence the urinary excretion actually stood for the excretion of total BCP in this case.

Curve fittings of the plasma concentrations and urinary excretions following the oral administration of 200 mg of BCP were tried as both the data were reported on the same rabbit at this dose. Though the variation of the individual data was significant, the tendencies of the time courses were simulated well.

Simulations were also made at the doses of 20, 50, 100 and 500 mg of BCP with the rate constants estimated at 200 mg of dose. The calculated curves were within the variation of

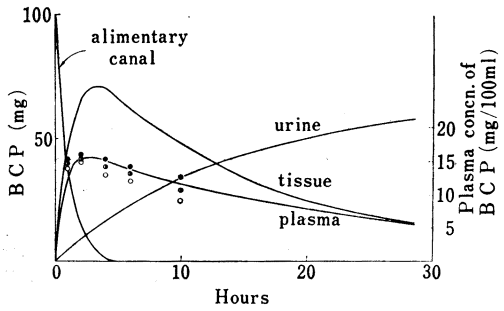

Fig. 7. Simulation of the Plasma Concentration, Urinary Excretion and Tissue Amount of BCP after Oral Administration of 100 mg of BCP to Rabbits (Model 1, Body Weight: 2.75 kg)

k_1=2.5 hr^{-1}, k_2=0.16 liter·mg^{-1}·hr^{-1}, k_{-2}=0.55 hr^{-1}, k_3=227 hr^{-1}, k_{-3}=0.68 hr^{-1}, k_4=17.6 hr^{-1}, k_5=7.6 hr^{-1}
●,◑,○: plasma concentrations on different rabbits

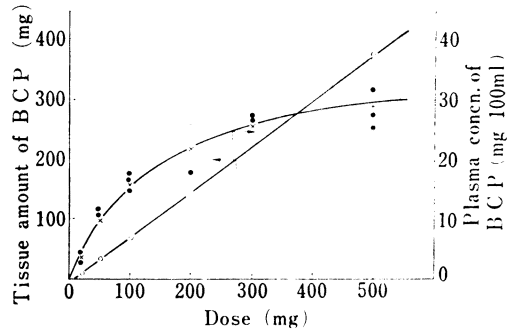

Fig. 8. Relation between Dose and the Maximum Value of Plasma Concentration or Tissue Amount of BCP after the Oral Administration of BCP to Rabbits (Model 1, Body Weight: 2.75 kg)

×,○: calculated value for the plasma concentration and tissue amount of BCP respectively
●: experimental values for the plasma concentration on different rabbits[9]

TABLE VI. Distribution of BCP in the Organs and Tissues of Rabbits (mg/kg) after the *i.v.* Administration of BCPNa (50 mg as BCP per kg)

Organ or tissue	Hours					
	3	3	10	15	20	24
(Plama)	223.6	228.2	153.9	63.4	56.7	44.4
Liver	12.3	10.6	7.5	6.4	1.9	4.7
Lung	12.1	11.5	5.3	8.5	0.6	1.2
Kidney	15.2	43.8	19.2	9.7	10.8	5.9
Heart	8.4	13.9	6.2	6.4	0	3.5
Brain	16.7	4.6	9.5	8.0	7.6	14.1
Spleen	10.4	13.9	10.3	33.1	5.6	8.2
Adrenal glands	12.1	15.4	16.6	—	1.1	25.1
Muscle	6.8	7.7	7.5	5.6	0.8	3.2
Lumbodorsal fat	4.0	12.3	3.8	1.8	1.1	1.2
Stomach	9.9	9.9	6.4	—	2.3	—
Intestine	6.6	6.6	9.7	—	0.2	—
Rabbit (kg)	3.20	2.97	3.00	2.86	3.03	2 98

—: not determined

individual data (*cf.* Fig. 7). The characteristic behavior was simulated well that the maximum plasma concentration of BCP did not increase linearly with dose but approached an asymptotic value, whereas the maximum tissue amount of BCP increased linearly with dose (Fig. 8). These results suggested the applicability of the model 1 also to simulate the behavior of BCP in rabbits.

Determination of the BCP in Organs or Tissues of Rabbits——In the model 1, tissue compartment includes the whole organs, tissues and body fluids except the alimentary canal and plasma as described previously.[1] In this experiment, however, BCP were determined in brain, heart, lung, liver, kidney, spleen, adrenal glands, muscle, fat, stomach and intestine of rabbits.

As shown in Table VI, BCP in tissues decreased with time, but it could be detected even after 24 hours of the administration. The plasma concentration also decreased rapidly and showed 4.4 mg/100ml after 24 hours. In the urine, *ca.* 70% of dose was excreted in 15—20 hours as unchanged total BCP (Table VII).

Table VII. BCP in Plasma, Tissue and Urinary Excretion after the *i.v.* Administration of BCPNa (50 mg as BCP per kg) to Rabbits

No.	Hr	Body Wt. (kg)	Dose (mg)	I Plasma BCP (%)	II[a] Urinary excretion (%)	III[b] Tissue BCP (%)	I+II+III (%)	(I+II+III×2.4) (%)
1	3	3.20	160	12.1	19.8	21.0	52.9	82.4
2	1.5	2.97	148.5	14.0	—			
	3			12.3	12.8	23.2	48.3	80.8
3	1.5	3.00	150	13.8	—			
	3			11.4	—			
	4.5			—	16.0			
	6			7.2	—			
	10			8.3	30.5	17.5	56.3	80.8
4	15	2.86	143	3.4	70.9	14.2	88.5	108.5
5	1.5	3.03	151.5	10.4	12.0			
	3			11.4	—			
	3.5			—	29.3			
	6			7.7	46.2			
	20			3.1	71.0	4.4	78.5	84.6
6	24	2.98	149	2.4	—	9.8	—	—

a) Urinary excretion stands for the total BCP excreted in the urine.
b) the expectation of tissue amount of BCP
%: percent of dose —: not determined

The average BCP in tissue was calculated by the mean value of the contents in organs and tissues whose weights were measurable, *i.e.*, brain, heart, lung, liver, kidney, spleen, adrenal glands, stomach and intestine. From the average BCP in tissue, the expectation of tissue amount of BCP in a rabbit was defined as follows.

$$\text{Tissue amount of BCP (Expectation)} = \frac{\sum(W_i \times C_i)}{\sum W_i} \times \frac{W_t^{20)}}{D} \times 100\ (\%)$$

W_i: weight of the organ or tissue i (kg)
C_i : concentration of BCP in the organ or tissue i (mg/kg)
W_t: body weight of a rabbit (kg)
D : dose of BCP (mg)

Total weight of the tissues in which BCP were determined, however, corresponded only 10% of the body weight. On the other hand, the sum of BCP in plasma, tissue and total BCP

20) Comparing the body weight, weight of the plasma is so light to be negligible.

in urine increased as the time elapsed after the administration (Table VII). This fact suggested there remained organs, tissues or body fluids in which undetermined BCP were distributed in high concentration and the BCP was excreted in the urine with time. In order to keep the sum constant independently on the time elapsed, the tissue amount of BCP (expectation) was multiplied by 2.4 as the correction factor, which made the sum almost constant throughout 24 hours, *i.e.*, 87.4% of dose.[21] This value was almost coincident with the total BCP excreted in the urine (79.5±5.9(SD)%)[11] after the successive *i.v.* administration of BCP Na (50 mg/kg as BCP) to five rabbits.

Comparison of the Calculated Tissue Amounts of BCP with the Experimental Values——For the administration, BCPNa was injected intravenously within a few minutes. However, to simulate the condition by the model 1, the whole dose could not be loaded into the plasma water in the short period owing to the voltage overload of the computer. Therefore, an approximation was made that in the plasma protein compartment, the saturation amount (20 mg), in the plasma water, 5 mg and in the alimentary canal, 115 mg of BCP were loaded as the initial values and the absorption rate was increased by five times of that in the oral administration.

In Fig. 9, the simulafon curves with the absorption rate constants (k_1), 12.5 and 2.5 hr^{-1} were presented. In the two case, the simulation curves showed little differences only within 4 hours after the administration. Comparing with the experimental values, both the calculated plasma concentrations were lower in the early stage (1.5—6.0 hours) and showed no delay in decrease of the concentration. Thus the effects of the approximation in the administration were not observed with the set of rate constants, which might be due to the sampling schedule (from 1.5 hours on plasma, urine and from 3 hours on tissue), the rapid absorption of BCP from the alimentary canal and to the variation of the data.

The obtained tissue amount of BCP (expectation) were smaller than the calculated amounts, but the corrected amounts were simulated fairly well, though the fluctuations of the data were large as the experiments were carried out on different rabbits.

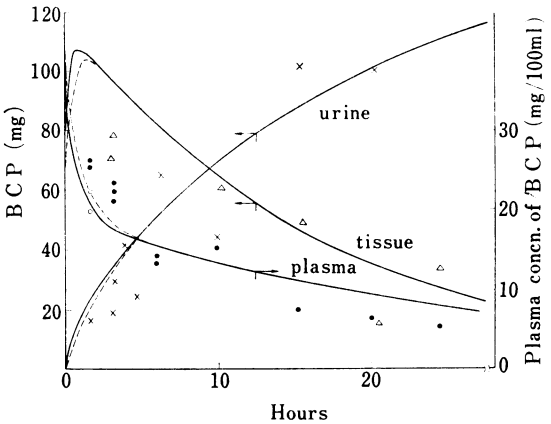

Fig. 9. Simulation of the Tissue Amount of BCP (△), Plasma Concentration (●) and Urinary Excretion (×) after the *i.v.* Administration of BCP-Na (50 mg as BCP per kg) to Rabbits (Model 1, 2.8 kg)

—: k_1=12.5 hr^{-1}, k_2=0.16 liter·mg^{-1}·hr^{-1}, k_{-2}=0.525 hr^{-1}, k_3=227 hr^{-1}, k_{-3}=0.677 hr^{-1}, k_4=25.0 hr^{-1}, k_5=0.75 hr^{-1}

---: k_1=2.5 hr^{-1}, The values of other k's were the same as shown above.

tissue amount of BCP: the expectation of tissue amount of BCP ×2.4

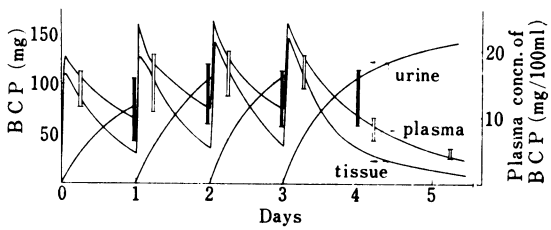

Fig. 10. Simulation of the Plasma Concentration, Urinary Excretion and Tissue Amount of BCP after the Successive *i.v.* Administration of BCP-Na (50 mg as BCP per kg) to Rabbits (Model 1, Body Weight: 2.75 kg, n=5)

administration: once a day

□: The range of the plasma concentrations determined.[11]

■: The range of the urinary excretions determined.[11]

21) The average value of the sum corrected, which were shown in the last column of the Table VII: [BCP in plasma and urine plus (tissue amount of BCP (expectation) × 2.4)].

Simulation was also tried on the experimental data in the successive administration of BCPNa to rabbits[11] with the rate constants determined in the single administration. According to the experimental conditions, administrations were made once a day and the determinations were made 6 hours after the each administration on plasma concentration and every 24 hours on the urinary excretion.

As shown in Fig. 10, the equilibrium plasma concentration and tissue amount of BCP were attained in 3 days. After cessation of the administration, 62.5% of BCP was eliminated in 1 day and 95% in 3 days from the plasma. The calculated curves were well within the experimental variation.

These results indicated the model 1 was also applicable to simulate the behavior of BCP in rabbits. The fact that the tissue amounts of BCP (expectation) were simulated fairly well by the model 1 after the correction on the experimental values, implied the calculated tissue amounts of BCP were not fictitious, but had close relation (linear) to the observed tissue amounts of BCP (expectation).

Acknowledgement The authors are grateful to Dr. S. Tatsuoka, General Manager, Mr. J. Manaka, Research Manager and Dr. T. Fujisawa, Deputy Research Manager, Research and Development Division, Takeda Chem. Ind. Ltd., for their permission of these series of investigations and continuous encouragement. Thanks are due to Mr. Z. Hokazono for his co-operation in calculation with the analogue computer.