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

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In order to elucidate the behavior of BCP in man, plasma concentration of BCP and urinary excretion of total BCP and its metabolites were determined periodically after its oral administration.

By analysis of the data with an analogue computer, the characteristic behavior of BCP was found that the maximum tissue amount of BCP increased almost linearly with dose both in the single and successive administration, whereas the maximum plasma concentration did not increase linearly with dose and approached an asymptotic value.

This behavior of BCP was considered due to its high degree of binding property to plasma albumin which was saturated at the plasma concentration of 13 mg/100 ml, kinetical conditions and to the dosage range in which the maximum plasma water concentration of BCP increased almost linearly with the dose given.

It was also suggested that by increasing the dose to a reasonable extent, increased clinical effect might be expected, though the maximum plasma concentration approached a limiting value.

In the previous papers, the metabolic fate of a non-steroidal anti-inflammatory drug, BCP[4] was investigated in man to find two hydroxylated compounds, *i.e.*, 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine (II) and 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine (IV) as the urinary metabolites[5] and the determination procedures for BCP and its metabolites in urines were established.[1,6]

In this study, plasma concentration of BCP, urinary excretion of unchanged BCP and its metabolites were determined following oral administration of BCP crystals or capsules to healthy male adults. The data were analyzed by an analogue computer to elucidate the kinetics of BCP in man.

1) Part III: T. Yashiki, Y. Uda, T. Kondo and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 487 (1971).
2) This work was presented at the 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
3) Location: *Higashiyodogawa-ku, Osaka.*
4) S. Senda, H. Izumi and H. Fujimura, *Arzneimittel-Forsch.*, **17**, 1519 (1967).
5) Part I: T. Yashiki, T. Matsuzawa, T. Kondo, Y. Uda, T. Shima, H. Mima, S. Senda and H. Izumi, *Chem. Pharm. Bull.* (Tokyo), **19**, 468 (1971).
6) Part II: T. Yashiki, T. Kondo, Y. Uda and H. Mima, *Chem, Pharm. Bull.* (Tokyo), **19**, 478 (1971).

Experimental

Oral Administration of 300, 600 and 900 mg of BCP——A healthy male (No. 1. age: 32, weight: 62 kg) ingested BCP crystals (less than 2 mm in diameter) 1 hr after light breakfast with *ca.* 200 ml of water. Blood samples were drawn at 0, 2, 4, 6, 8, 24, 48, and 72 hours following the oral administration of 300, 600 and 900 mg of dose. The urines (pH 5.4—7.5) were collected at 0, 2, 4, 6, 8, 10 hours, then every 24 hours until the excretion of the drug and the metabolites were completed. At least, three weeks later, another dose was given.

Oral Administration of 300 and 400 mg of BCP——A healthy male (No. 2. age: 24, weight: 60 kg) ingested BCP crystals (less than 2 mm in diameter) 1 hr after light breakfast with *ca.* 200 ml of water. The urine samples (pH 5.6—7.5) were collected at 0, 2, 4, 8, 10 hours, then every 24 hours until 96 hours after the administration.

Oral Administration of 300 and 900 mg of BCP——Three healthy males (No. 3, 4, 5. age: 26, 29 and 36, weight: 84, 69, and 62 kg respectively) ingested BCP capsules (300 mg per capsule) 1 hr after light breakfast with *ca.* 200 ml of water. Blood samples were drawn at 0, 2, 4, 6, 8 or 10, 24, 48, and 72 hours and the urines were collected at 0, 2, 4, 6, 8, or 10 hours after the administration. The pH's of the urines were within 5.1—7.7.

Determination of BCP and Its Metabolites——Concentrations of BCP in plasma[7,8] and total BCP in urine were determined by ultraviolet (UV) absorption method.[6] The metabolites in urines were determined by UV absorption[6] method and/or by gas-liquid chromatography.[1]

Analogue Computer——An analogue computer (MELCOM EA-7304) was used for the analysis.

Result

1. Urinary Excretion of BCP and Its Metabolites

Cumulative excretion curves following oral administration of single doses of 300, 600 and 900 mg of BCP crystals show that unchanged BCP and its metabolites were excreted for almost 200 hours. One of the excretion curves at 600 mg of dose is shown in Fig. 1. Almost the same percents of total excretions were observed in the dosage range of 300 to 900 mg (Table I). The average total excretion corresponded to *ca.* 50% (48.4%) of dose (25.0% as total BCP, 17.3% as II and 6.1% as IV).

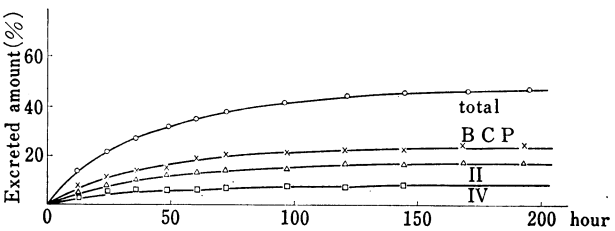

Fig. 1. Urinary Excretion of Total BCP and Its Metabolites II and IV after Oral Administration of 600 mg of BCP Crystals to a Man (Subject 1, 62 kg)

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine
The amounts of the metabolites were expressed by the BCP equivalent values.

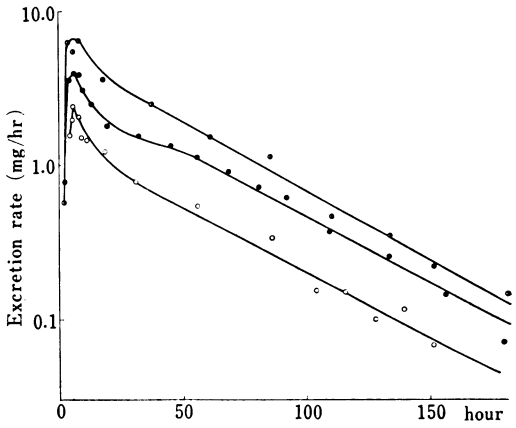

Fig. 2. Urinary Excretion Rate of Total BCP after the Oral Administration of 300, 600 and 900 mg of BCP to a Man (Subject 1, 62 kg)

dose: 900 mg (◑), 600 mg (●), 300 mg (○) of BCP
From the excreted amounts of total BCP in a certain period after the administration of 300, 600 and 900 mg of the drug, the excretion rates (mg/hr) were calculated and no correction for smoothing was made.

7) H. Mima, T. Matsuzawa, K. Terada and T. Kondo, *Takeda Kenkyusho Nempo*, **24**, 9 (1965).
8) H. Mima, T. Matsuzaki, K. Okutani and M. Hattori, *Takeda Kenkyusho Nempo*, **26**, 32 (1967).

The excretion rate of total BCP or its metabolites showed the maximum in 4—8 hours after the administration, which did not change by the dose (300—900 mg). Then, the rate decreased rapidly in *ca.* 20 hours (1st decrease) and decreased more slowly following apparent first order kinetics (2nd decrease). Fig. 2 shows the excretion rate of total BCP.

The apparent biological half-lives of BCP calculated from the slopes of the second decrease were almost the same in values, though the dose changed by three times as shown in Table II.

Table I. Relation between Dose and the Excreted Amounts after Oral Adiministration of BCP to Man

Subject	Dose (mg)	Total[a] BCP (%)	II (%)[b]	IV (%)[b]	Total (%)[c]
1	900	26.2	17.4	5.6	49.2
	600	24.0	17.4	7.2	48.6
	300	26.3	21.2	7.5	55.0
2	400	22.8	15.5	6.0	44.3
	300	25.7	15.0	4.0	44.7
Average ± S.D.		25.0 ± 1.5	17.3 ± 2.4	6.1 ± 1.4	48.4 ± 4.3

a) sum of free BCP the BCP with some kind of interaction[6]
b) The excreted amounts were expressed by the BCP equivalents.
c) Total excreted amounts (total BCP and its metabolites II and IV) were determined until the excretion were completed for the subject 1 and were estimated from the data obtained in 96 hours with the analogue computer for the subject 2.
II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

Table II. Apparent Biological Half-Life of BCP in Man

Subject 1		Subject 2	
Dose (mg)	$t_{1/2}$ (hours)[a]	Dose (mg)	$t_{1/2}$ (hours)[a]
900	33.2	400	35.0
600	31.9	300	34.7
300	33.2		

a) $t_{1/2}$ was calculated from the slope of the 2nd decrease in the excretion rate after the oral administration.

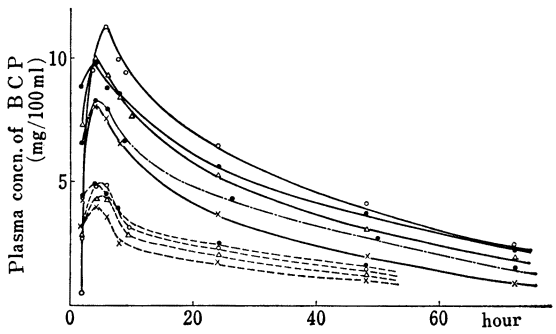

Fig. 3. Plasma Concentrations of BCP after the Oral Administration of 300, 600 and 900 mg of BCP to Men
subject: 1 (●), 3(×), 4(○), 5(△)
dose: 900 mg (—), 600 mg (—), 300 mg (---)

2. Plasma Concentration of BCP

Plasma concentrations of BCP following oral administration of the drug are shown in Fig. 3. The maximum concentrations were observed in 4—6 hours and even 72 hours after the administration, BCP could be determined in plasma.

By plotting the plasma concentrations of BCP on the semilogarithmic axis, it was found that the curve following the maximum value could be represented as the sum of, at least, two exponentials.

A feature of the plasma concentration curves is that the maximum concentration does not increase proportionally as the dose increases from 300 to 900 mg, but shows a tendency to approach an asymptotic value.

This phenomenon had been also observed in rabbits[7] and in man[8] during the clinical tests, *i.e.*, the constant plasma concentrations attained after the successive oral administration lay between 7—10 mg/100 ml (determined before breakfast)[9] in spite of the increase of the dose from 300 to 1200 mg per day.

The average plasma concentrations of BCP determined at 3.00 p.m.[9] in the 4th and 8th days were 9.9±1.0 mg (S.D.)/100 ml at 900 mg of dose ($n=2$), 12.8±1.5 mg/100 ml at 1200 mg ($n=7$) and 12.9 mg/100 ml at 1800 mg ($n=1$) and the plasma concentration was apparently saturated at the doses more than 1200 mg.

The plasma concentration at the saturation must be different for each human subject, but the experimental measurement of the individual concentration was difficult. Moreover, as the standard deviation observed was relatively small, the asymptotic value was assumed to be 13 mg/100 ml of BCP for every human subject in this study.

Brodie, *et al.* also reported the same saturation phenomenon with phenylbutazone in man.[10]

On the other hand, Kakemi, Arita, *et al.* investigated the binding of BCP to bovine and human serum albumin *in vitro* and they found BCP was bound at two types of sites on bovine serum albumin according to the Karush's equation.[11]

If the binding relation of BCP to bovine serum albumin *in vitro* is also held in human plasma albumin *in vivo*, 8 moles of BCP can be bound per mole of albumin at the saturation.

At the apparent saturation in plasma concentration (13 mg/100 ml), however, the calculated mole ratio of BCP to the albumin is 0.85[12] and 85% of the binding sites of the primary association constant ($K_1=1.08\times10^5$ M^{-1}, $n_1=1.02$) on the albumin are bound with BCP. Therefore, only primary sites were considered for the binding of BCP in man.

$$\frac{r}{(A)}=\frac{n_1\cdot K_1}{1+K_1(A)}+\frac{n_2\cdot K_2}{1+K_2(A)}$$

$n_1=1.02$, $n_2=6.98$
$K_1=1.08\times10^5$M^{-1}, $K_2=1.35\times10^3$M^{-1}
r: number of moles of BCP bound per mole of protein
n_i: number of binding sites in the i'th type of sites per protein
K_i: intrinsic association constant for the binding of BCP by sites in the i'th type
A: concentration of free BCP in equilibrium

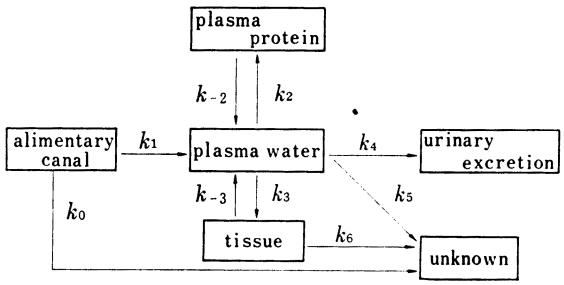

Fig. 4. A Model to Simulate the Behavior of BCP in Man after the Oral Administration (for the Model 1, $k_0=k_6=0$)

The dimensions of the rate constants, k's are (hr^{-1}) except k_2 (liter·mg^{-1}·hr^{-1}).

9) The plasma concentration determined before breakfast corresponds to the minimum value and that at 3.00 p.m. corresponds to the second maximum in a day as shown in Fig. 12.
10) J. J. Burns, R.K. Rose, T. Chenkin, A. Goldman, A. Schulert and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **109**, 346 (1953).
11) K. Kakemi, T. Arita, M. Hashi and K. Sumii, *Yakugaku Zasshi*, **86**, 739 (1966).
12) For the calculation, a man of 55 kg in the body weight is assumed. BCP 13 mg/100 ml=0.488 mM (mol. wt. of BCP=266.35). BCP in plasma=1.22 mmole (plasma volume=2.5 liter[13]). Albumin in plasma =1.45 mmole (3.8% of plasma volume,[11] mol. wt. of albumin=6.56×10^4[14]). BCP bound per mole of albumin=1.22/1.45=0.85. The amount of unbound BCP in plasma is neglected as it is assumed to be only a few percents.
13) B. Harrow and A. Mazur, "Text book of Biochemistry," translated by T. Nishimura, Gihodo, Tokyo, 1964, p. 191.
14) B. W. Low, *J. Am. Chem. Soc.*, **74**, 4830 (1952).

Several papers dealt with the influences of protein binding in plasma on the behavior of drugs *in vivo*.[15-18]

3. The Model for Simulation of the Behavior of BCP in Man (Model 1)

Orally administered BCP is absorbed from the alimentary canal to plasma water, then a fraction is bound to plasma albumin, distributed into tissue and eliminated by excretion as unchanged or metabolites (Fig. 4).

The transfer of BCP to tissue or excretion in urine are assumed to be carried out proportionally to the concentration in plasma water, where a minor fraction of plasma BCP exists as unbound, diffusible species.

Anatomically, the tissue compartment is not clear in this model, and the whole body except plasma and alimentary canal can be included.

For the analysis by this model 1 with the analogue computer, the following assumptions were also made. i) The concentration in a compartment is homogeneous. ii) Orally administered BCP is absorbed completely from the alimentary canal ($k_0=0$). iii) The binding of BCP to albumin is a second order process, *i.e.*, first order with respect to the plasma water concentration of BCP, first order with respect to the concentration of unbound plasma albumin. iv) Other transfer-processes of BCP in a body (absorption, distribution, release and excre-

$$\frac{dm_a}{dt} = -(k_0+k_1)m_a$$

$$\frac{dm_w}{dt} = k_1 m_a - \frac{k_2 m_w m_p}{v_{pl}} - (k_3+k_4+k_5)m_w + k_{-2}m_{pb} + k_{-3}m_t$$

$$\frac{dm_{pb}}{dt} = \frac{k_2 m_w m_p}{v_{pl}} - k_{-2}m_{pb}$$

$$\frac{dm_t}{dt} = k_3 m_w - (k_{-3}+k_6)m_t$$

$$\frac{dm_e}{dt} = k_4 m_w$$

$$\frac{dm_u}{dt} = k_0 m_a + k_5 m_w + k_6 m_t$$

$$\frac{dm_p{}^{a)}}{dt} = -\frac{dm_{pb}}{dt}$$

m_a: amount of BCP in alimentary canal
m_w: amount of BCP in plasma water
$m_p{}^{a)}$: amount of unbound plasma albumin
m_{pb}: amount of BCP bound to plasma protein
m_t: amount of BCP in tissue
m_e: amount of BCP and its metabolites excreted in urine
m_u: amount of unknown
v_{pl}: volume of plasma

a) Initial amount of plasma albumin is expressed by the binding equivalence of BCP (13 mg/100ml × v_{pl}).

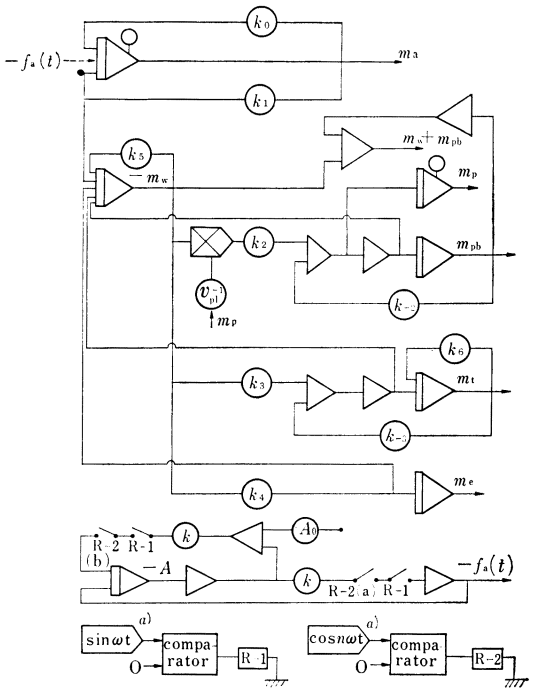

Fig. 5. Analogue Computer Program for Simulation of the Behavior of BCP in Man

$f_a(t)$: successive administration of BCP according to the function of time; R-1, R-2: electronic switch; A_0: each administration dose; k=10.0
a) function generators for the administration schedule

15) E. Krüger-Thiemer, W. Diller and P. Bünger, *Antimicrob. Ag. Chemother.*, **1965**, 183.
16) B.B. Brodie, *Proc. Roy. Soc. Med.*, **58**, 946 (1965).
17) M.C. Meyer and D.E. Guttman, *J. Pharm. Sci.*, **57**, 895 (1968).
18) J.M. Thorp, "Absorption and Distribution of Drugs," ed. by T.B. Binns, E. & S. Livingston Ltd., Edinburgh, London, 1964, p. 64.

tion, *etc.*) follow first order kinetics. v) The binding of BCP to 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 of BCP. vi) Urinary excretions of unchanged BCP, II and IV follow parallel first order kinetics. The rate of metabolism and that of excretion are not separated in this analysis. vii) As described before, *ca.* 50% of dose are excreted as known substances. In the model 1, the excretion of the unknown is directly proportional to the concentration of BCP in plasma water ($k_6=0$). viii) The following biological constants for a man are assumed. Blood volume: 80.1 ml/kg,[13] plasma volume: 45.3 ml/kg,[13] albumin: 3.8% of plasma volume,[11] body weight: 55 kg unless specified.

4. Mass Equations and the Block Diagram

Mass equations and the block diagram describing the model 1 are as shown in the previous page (Fig. 5).

5. Rate Constants for the Model 1

The rate constants were obtained by try and error for individual subject at each dose of BCP. For the asymptotic amount of BCP in plasma, different saturation amount of BCP in plasma (13 mg/100 ml × volume of plasma) was set for each subject according to his body weigt, as the apparent saturation concentration was assumed to be 13 mg/100 ml. Table III shows the rate constants with which calculated curves of plasma concentration and urinary excretion fitted well the experimental data (Fig. 6—8).

It can be seen that the binding of BCP to plasma albumin and distribution to tissue are very fast, whereas the release of BCP from tissue is a slow process.

TABLE III. The Rate Constants obtained from Individual Subject at Each Dosage of BCP (*p.o.*)

Dose (mg)	300	400	600	900
cases	5	1	1	4[a]
k_1[b]	0.500	0.500	0.500	0.500
k_2	0.425	0.425	0.425	0.425
k_{-2}	0.450	0.450	0.450	0.450
k_3	17.64 ±0.39[c]	17.80	17.80	17.80
k_{-3}	0.107±0.019	0.125	0.125	0.149±0.080
k_4	2.78 ±0.72	2.20	2.40	2.26 ±1.54
k_5	3.52 ±0.39	3.25	3.25	4.00 ±1.06

a) Abnormal metabolic pattern was observed by gas-liquid chromatography in one case.
b) The dimensions of k's are (hr^{-1}) except k_2 (liter · mg^{-1} · hr^{-1}). The values of the rate constants were obtained by try and error at first to simulate the plasma concentration and urinary excretion, then simulations could be made by changing the values of the rate constants (k_3, k_{-3}, k_4, k_5) and leaving the values of k_1, k_2, and k_{-2} unchanged.
c) average value and the standard deviation

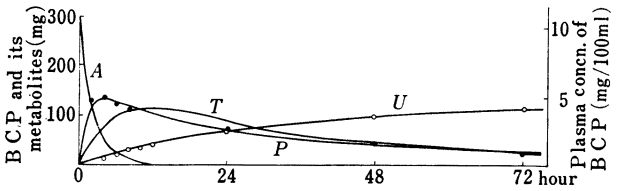

Fig. 6. Simulation Curves of Plasma Concentration (P), Tissue Amount (T), Alimentary Canal Amount (A) of BCP and Urinary Excretion (U) of BCP and Its Metabolites after Oral Administration of 300 mg of BCP to a Man (Subject 1, 62 kg, Model 1)

urinary excretion: total excreted amount of total BCP and BCP equivalents of the metabolites II and IV
$k_1=0.50$ hr^{-1}, $k_2=0.425$ liter · mg^{-1} · hr^{-1}, $k_{-2}=0.45$ hr^{-1}, $k_3=17.80$ hr^{-1}, $k_{-3}=0.125$ hr^{-1}, $k_4=2.65$ hr^{-1}, $k_5=3.25$ hr^{-1}

Each rate constant did not deviate significantly from the average, though the experiments were carried out on different subjects.

6. Simulation of the Plasma and Plasma Water Concentrations

The calculated plasma concentrations fitted well the experimental data (Fig. 6—9). The maximum plasma concentration did not increase proportionally to the dose but approached a limiting value, also the maximum appeared after almost the same period of the administration indifferently to the dose and the decrease in the absorption rate constant could not be detected with increase of the dose.

The areas under the plasma concentration curves measured by weight method increased almost proportionally to the dose, which suggested the ratio of the absorption amount to the dose did not change much with dose (Table IV).

The calculated plasma water concentrations correspond only 1.0—3.5% of plasma concentrations which indicates a large portion of BCP in plasma is bound to the albumin (Fig. 10). There is apparently a linear relation between dose and the maximum plasma water concentration in the dosage range of 300—900 mg, though the apparent straight line does not pass the origin (Fig. 9).

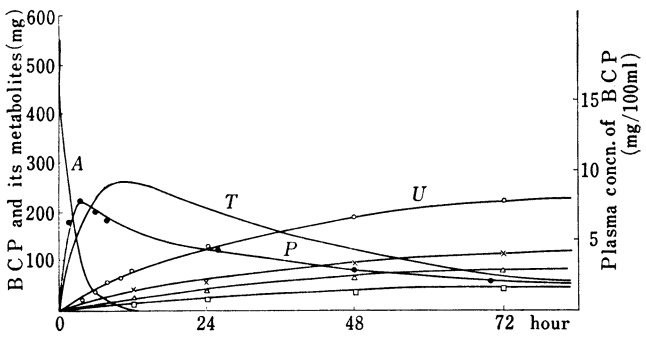

Fig. 7. Simulation Curves of Plasma Concentration (P), Tissue Amount (T), Alimentary Canal Amount (A) of BCP and Urinary Excretion (U) of BCP and Its Metabolites after Oral Administration of 600 mg of BCP to a Man (Subject 1, 62 kg, Model 1)

urinary excretion: total (○), total BCP (×), BCP equivalent of II (△) and IV (□) $k_1=0.50\ hr^{-1}$, $k_2=0.425$ liter · mg^{-1} · hr^{-1}, $k_{-2}=0$, $45hr^{-1}$, $k_3=17.80\ hr^{-1}$, $k_{-3}=0.125\ hr^{-1}$, $k_4=2.40\ hr^{-1}$, $k_5=3.25\ hr^{-1}$

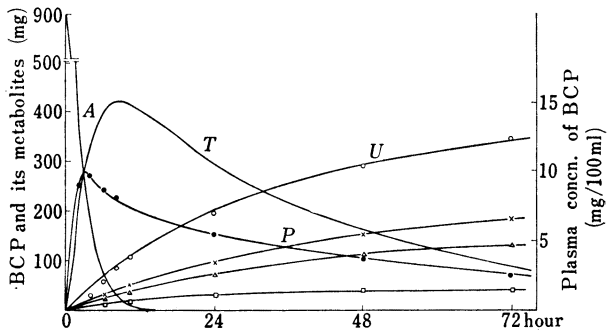

Fig. 8. Simulation Curves of Plasma Concentration (P), Tissue Amount (T), Alimentary Canal Amount (A) of BCP and Urinary Excretion (U) of BCP and Its Metabolites after Oral Administration of 900 mg of BCP to a Man (Subject 1, 62 kg, Model 1)

urinary excretion: total (○), total BCP (×), BCP equivalent of II (△), and IV (□) $k_1=0.50\ hr^{-1}$, $k_2=0.425$ liter · mg^{-1} · hr^{-1}, $k_{-2}=0.45\ hr^{-1}$, $k_3=17.80\ hr^{-1}$, $k_{-3}=0.125\ hr^{-1}$, $k_4=2.525\ hr^{-1}$, $k_5=3.25\ hr^{-1}$

Table IV. Relation between Dose and the Area under the Curve of the Amount of BCP in Plasma

Route			*p.o.*		*i.v.*[21]
Subject	1	3	4	5	1′—5′
Dose (mg)					
900	537.7(2.7)	557.5(2.8)	591.0(2.8)	476.5(2.4)	—
600	352.3(1.8)	—	—	—	—
400	—	—	—	—	191.5±56.2
300	196.5(1.0)	193.3(1.0)	212.5(1.0)	197.8(1.0)	—

amount of BCP in plasma: plasma concentration × volume of plasma; An area is expressed by the weight of the chart (mg). The value in the parenthesis is the ratio of the area to that in 300 mg of dose.

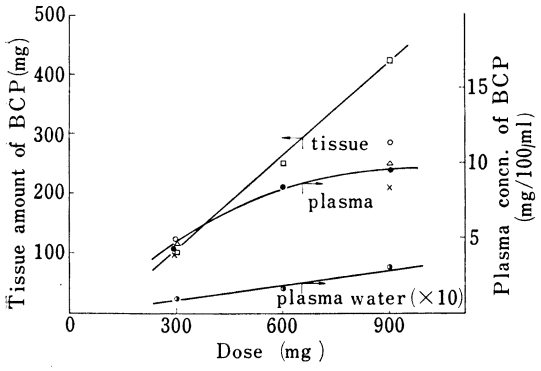

Fig. 9. Relations between Dose and the Maximum Values of Plasma Concentration, Plasma Water Concentration and Tissue Amount of BCP after Oral Administration of the Drug to a Man (Subject 1, 62 kg, Model 1)

plasma concentration: subject 1 (●), 3(×), 4(○) and 5(△). Plasma water concenrtation and tissue amount of BCP are for the subject 1.

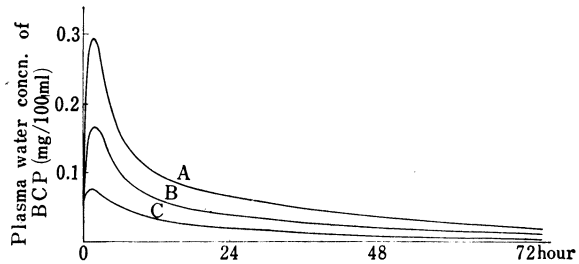

Fig. 10. Simulation Curves of plasma Water Concentrations after Oral Administration of BCP to a Man (Subject 1, 62 kg, Model 1)

dose: 900 mg (A), 600 mg (B) and 300 mg (C)

7. Simulation of BCP in Tissue Compartment

The calculated distribution amounts of BCP in tissue are also shown in Fig. 6, 7 and 8. The maximum tissue amounts were observed within 8—10 hours after the administration. There is apparently a linear relation between dose and the maximum tissue amount in contrast to the saturation of the plasma concentration (Fig. 9).

This characteristic behavior of BCP is elucidated by the reasons that 1) BCP shows high degree of binding to the plasma albumin ($k_1 = 1.08 \times 10^5 M^{-1}$) and the rate in the binding or transfer to the tissue compartment is high. 2) Hence, by increasing the dose, plasma concentration increases for a limiting value (13 mg/100 ml). On the other hand, plasma water concentration of BCP increases non linearly (concavely) by increasing the plasma concentration.[19] If plasma water concentration is plotted versus dose, these two relations are compensated and apparently a linear relation is held. 3) In this experiment, the dose was in a limited range (300—900 mg).

8. Individual Rate Constants for the Urinary Excretion of Total BCP, II and IV

The rate constant for the urinary excretion of total BCP and its metabolites II and IV was divided into individual rate constants assuming parallel first order processes. The values obtained are shown in Table V.

TABLE V. Rate Constants for the Urinary Excretion of BCP and Its Metabolites II and IV

Subject	Dose (mg)	k_4 (Total) (hr^{-1})	k_{41} (BCP)[a] (hr^{-1})	k_{42} (II) (hr^{-1})	k_{43} (IV) (hr^{-1})
1	900	2.53	1.35	0.89	0.29
	600	2.40	1.18	0.86	0.36
	300	3.00	1.43	1.16	0.41
Average ± S.D.		2.64 ± 0.32	1.32 ± 0.13	0.97 ± 0.17	0.35 ± 0.06
2	400	2.20	1.13	0.77	0.30
	300	2.20	1.26	0.74	0.20
Average ± deviation		2.20	1.20 ± 0.06	0.76 ± 0.02	0.25 ± 0.05

a) total BCP

19) B.K. Martin, *Nature*, **207**, 274 (1965).

9. Contribution Factors to the Long-Acting Behavior of BCP

As described before, urinary excretion was observed for almost 200 hours after BCP was administered orally to man. This might be ascribed to the slow release of BCP from tissue or from protein bound fraction. To know the degree of the contribution of these factors, time courses of the plasma concentration, urinary excretion and tissue amount were calculated with the analogue computer, maintaining first the tissue amount constant by cutting the supply and release of BCP at the tissue compartment ($k_3=k_{-3}=0$), second the amount of BCP bound to plasma protein constant by cutting the supply and release of BCP at the plasma protein compartment ($k_2=k_{-2}=0$) after an apparent steady state was attained. The calculated plasma concentration, urinary excretion and tissue amount were compared with the untreated simulation curves. As shown in Fig. 11, the decrease of plasma concentration of BCP at constant tissue amount of BCP was faster than the decrease of tissue amount at constant BCP bound to plasma protein. This indicates the release of BCP from the tissue compartment is a slower process, which might be the main cause of the long-acting behavior of BCP.

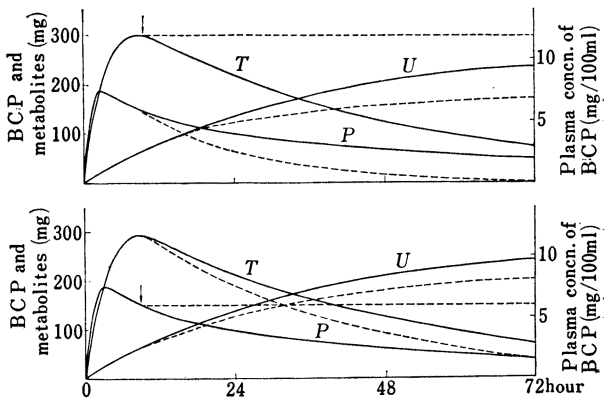

Fig. 11. Effect of Plasma Concentration (P) or Tissue Amount (T) of BCP on the Urinary Excretion (U) after Oral Administration of 600 mg of BCP to a Man of 55 kg

Dotted lines show the changes of the curves after the BCP bound to plasma protein or the tissue amount of BCP are kept constant. An arrow shows the time when the constancy is held.

10. Simulation in the Successive Oral Administration

As described before, the plasma concentrations in the chronic administration of BCP were reported in the clinical test.[8] According to the administration schedule of that experiments,[20] plasma concentration and the tissue amount were calculated with the rate constants

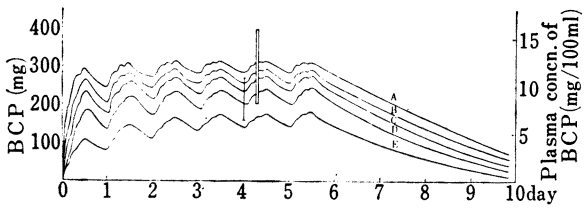

Fig.12. Simulation Curves of Plasma Concentrations of BCP after Successive Oral Administration of BCP to a Man of 55 kg (Model 1)

A: 600mg×3/day, B: 400mg×3/day, C: 300mg×3/day,
D: 200mg×3/day, E: 100mg×3/day
——: determined before breakfast (900—1200 mg/day)[8]
□: determined at 3.00 p.m. (600—1800 mg/day)[8]
$k_1=0.5$ hr^{-1}, $k_2=0.42$ liter · mg^{-1} · hr^{-1}, $k_{-2}=0.45$ hr^{-1}, $k_3=17.8$ hr^{-1}, $k_{-3}=0.15$ hr^{-1}, $k_4=2.4$ hr^{-1}, $k_5=2.9$ hr^{-1}

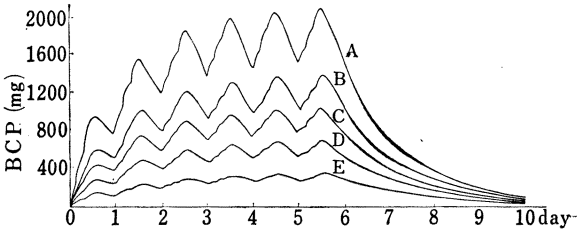

Fig. 13. Simulation Curves of Tissue Amount of BCP after Successive Oral Administration of BCP to a Man of 55 kg (Model 1)

A: 600 mg×3/day, B: 400 mg×3/day,
C: 300 mg×3/day, D: 200 mg×3/day,
E: 100 mg×3/day

20) Dose: 300, 600, 900, 1200 and 1800 mg/day; administration schedule: 3 times/day (8.00 a.m., noon and 4.00 p.m.); assay schedule: before breakfast and 3.00 p.m.

determined in the single dose. When the average body weight was assumed to be 55 kg, the constant plasma concentrations attained in 3—4 days were well within the range of the experimental variation (Fig. 12). The calculated constant tissue amounts were attained in 5—6 days and the values increased proportionally to the dose (300—1800 mg/day) as shown in Fig. 13.

Discussion

In Fig. 2, the urinary excretion rate of BCP decreased apparently in two steps which also suggested the applicability of a multi-compartmental model for the analysis.

In the clinical tests, BCP Na (400 mg as BCP) were administered intravenously to five patients who were subjected surgical operations and the areas under the curves of BCP in plasma were measured by weight method. As will be described elsewhere,[21] the average area was 191.5±56.2 mg, which was rather smaller than the expected value from the data in the oral administration, though direct comparison could not be made as the data were obtained in different experimental conditions, *i.e.*, oral administration to healthy subjects and *i.v.* administration to the patients after surgical operations (Table IV).

On the binding of BCP to human plasma protein, an assumption was made that the binding relation of BCP to bovine serum albumin was applicable in man (*in vivo*). However, detailed binding experiments using human plasma should be done in future to check the validity of application of the relation to the case of man.

The binding of BCP to plasma albumin and release from the bound fraction would be very fast[18] and it might be preferable to use the equilibrium constant K_1[22] instead of the rate constants k_2, k_{-2}. However, the rate of protein binding or dissociation of a drug *in vivo* had not been made clear enough and it was uncertain whether a true equilibrium could be attained instantly *in vivo*. Therefore, rate constants were used in this analysis. In the simulation curve with the rate constants (k_2=0.425 liter·mg^{-1}·hr^{-1}, k_{-2}=0.45 hr^{-1}), delay of the peak of plasma concentration from the peak of plasma water concentration was measured.

On the subject 1, though the delay were *ca.* 2 hours at 300 mg of dose, 1 hr at 600 mg and less than 1 hr at 900 mg, which were not significantly large considering the number and the the intervals of the experimental measurements.[23]

Also, the reported plasma concentrations after the successive oral administration were simulated well with the rate constants obtained in the single dose. Thus, though any experimental proof on the validity of the absolute values of k_2 and k_{-2} was not presented in this study and there was a possibility of the rate constants taking much larger values, those values described before were used for the practical analysis of the behavior of BCP *in vivo*. At the equilibrium, the apparent association constant calculated from k_2/k_{-2} is 2.49×10^5 M^{-1} in this case.

As for k_3 and k_{-3}, the same order of values were observed both in *p.o.* and *i.v.* administration,[21] which supported the estimation of k_3 and k_{-3} in the oral administration.

On the distribution volume, only apparent volume could be calculated, because when $dm_t/dt=0$, (m_t: tissue amount of BCP), the absorption from the alimentary canal was not finished completely. Assuming the concentration in plasma water was equal to the concentration in tissue, apparent distribution volume[24] at $dm_t/dt=0$, corresponded to 405 liter (subject 1), which was larger than a man. One of the reasons of this discrepancy might be due to the interaction between BCP and tissue protein.

21) Part V: T. Yashiki, T. Matsuzawa, M. Yamada, T. Kondo, H. Mima, M. Yamamoto, T. Yamada, M. Nakajima and K. Doi, *Chem. Pharm. Bull.* (Tokyo), **19**, 881(1971).

22) $K_1=1.08\times10^5$ M^{-1} for bovine serum albumin *in vitro*.[11]

23) As described in the "Experimental," blood samples were drawn at 0, 2, 4, 6, 8, 24, 48, and 72 hours after the administration and urines were collected at 0, 2, 4, 6, 8, 10 hours, then every 24 hours until the excretion was completed.

24) Calculated from the equation $V_d=(1+k_3/k_{-3})\ V_{pl}$ (V_d: distribution volume, V_{pl}: volume of plasma).

To examine the constancy of the renal clearance, relation between total urinary excretion rate and the plasma concentration of BCP was plotted (Fig. 14). At the dose of 300 mg, apparently linear relation was observed at any time. At 600 mg, until 12 hours and at 900 mg, until 24 hours after the administration, departures from the linearity were observed, but thereafter, apparently linear relation was held at low plasma concentration. If plasma water concentration was chosen as abscissa, however, constant renal clearance was observed always in the dosage range 300—900 mg.

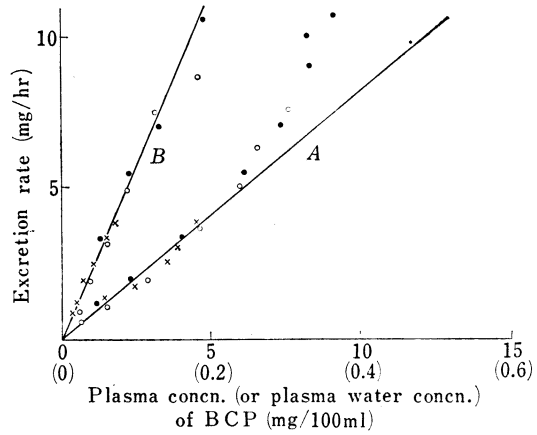

Fig. 14. Relation between Excretion Rate and Plasma Concentration (*A*) or Plasma Water Concentration (B) of BCP

dose: 900 mg (●), 600 mg (○), 300 mg (×)

In the model 1, to keep the concentrations of BCP in plasma and plasma water at the same order of values, an alternative set of rate constants was obtained, which gave resaonable fit to the plasma concentration and urinary excretion curves in the single doses of 300, 600 and 900 mg of BCP. The values for the rate constants are as follows: $k_0=0\ hr^{-1}$, $k_1=0.5\ hr^{-1}$, $k_2=0.01375$ liter·mg^{-1}·hr^{-1}, $k_{-2}=0.30\ hr^{-1}$, $k_3=1.05 hr^{-1}$, $k_{-3}=0.125 hr^{-1}$, $k_4=0.155\ hr^{-1}$, $k_5=0.20\ hr^{-1}$, $k_6=0\ hr^{-1}$. In the successive administration, however, the plasma concentrations increased almost proportionally to the dose, which did not fit the experimental results and this set of rate constants could not be accepted.

Besides the model 1, the applicability of some other models were examined.

Model 2: This model assumes that the biotransformation rate to the unknown[5] is proportional to the tissue amount of BCP. The calculated curves fitted the data in the single doses of 300, 600 and 900 mg of BCP and the plasma concentrations in the successive administration within the experimental variation ($k_0=0\ hr^{-1}$, $k_1=0.5\ hr^{-1}$, $k_2=0.29$ liter·mg^{-1}·hr^{-1}, $k_{-2}=0.29\ hr^{-1}$, $k_3=18.0\ hr^{-1}$, $k_{-3}=0.15\ hr^{-1}$, $k_4=2.4\ hr^{-1}$, $k_5=0\ hr^{-1}$, $k_6=0.03\ hr^{-1}$). However, the decrease of the tissue amount after cessation of the successive administration was too fast, because by the moel 1, the calculated tissue amount fitted the experimental data fairly well in rabbits.[21]

Model 3: This model assumes that only 50% of dose can be absorbed and the remain is changed to the unknown proportionally to the amount of BCP in the alimentary canal. The plasma concentrations and the urinary excretions in the single doses and the plasma concentrations in the successive administration could also be accounted by this model ($k_0=0.5\ hr^{-1}$, $k_1=0.5\ hr^{-1}$, $k_2=0.45$ liter·mg^{-1}·hr^{-1}, $k_{-2}=0.58\ hr^{-1}$, $k_3=4.8\ hr^{-1}$, $k_{-3}=0.16\ hr^{-1}$, $k_4=2.3\ hr^{-1}$, $k_5=0\ hr^{-1}$, $k_6=0\ hr^{-1}$). However, there were some reasons that could not accept this model, *i.e.* a) When 2-^{14}C-BCP was administered to rats, 72% of dose was excreted in urine and 6% in feces within 96 hours independently on the administration route or dosage form.[5] b) The decrease of the tissue amount after cessation of the successive administration was too fast. c) BCP was stable in the simulated gastric or intestinal juice.

Further, concerning with the absorption of BCP, the decrease in the availability was not observed by increasing the dose as the areas under the plasma concentration curves increased almost proportionally to the dose administered and the total excretion percents were almost unchanged with the dose. The average area under the curves of BCP in plasma at 300 mg (*p.o.*) was similar to that at 400 mg of dose (*i.v.*). Also, the decrease in the absorption rate constant was not observed in the dosage range 300—900 mg. These facts indicated the readiness in absorption of the drug from the alimentary canal.

From these results, only minor contributions of the model 2 and 3 are expected to explain the behavior of BCP, though linear relations between dose and the constant tissue amount attained in the successive administration were observed also in the model 2 and 3.

There are possibilities some other minor processes are taken part in the behavior of BCP *in vivo* such as active secretion, reabsorption from renal tubules and entero-hepatic circulation *etc.*

Strictly speaking, the real mechanisms might be much more complicated and in the Model 1, some uncertainty still remained on the absolute values for the accepted rate constants in the sense that the experiment for each step could not be done on human being and the obtainable data were less than the number of parameters to be determined.

However, the characteristic behaviors of BCP in man can be elucidated well with the set of rate constants accepted in the model 1.

If a pharmacological effect is more closely related to the concentration in tissue than that in plasma, the clinical efficacy will depend more on the concentration of a drug in tissue. In the case of BCP, increased effectiveness were reported clinically when larger doses such as 1200—1800 mg per day were administered orally[25-27] in spite of the saturation phenomenon of the plasma concentration, which suggests a possibility of being an intimate relation between the pharmacological effect and the tissue amount of BCP.

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25) T.Kawahara and H. Ashida, "Clinical Literature on Paramidin ®," Takeda Chem. Ind. Ltd., 1968, p. 12.
26) T. Mizukawa and S. Kawaguchi, "Clinical Literature on Paramidin®," Takeda Chem. Ind. Ltd., 1968, p. 106.
27) T. Ishibe and Y. Fujimoto, "Clinical Literature on Paramidin ® (Kinki, Chugoku,Shikoku and Hokuriku districts)," Takeda Chem. Ind. Ltd., 1968, p. 47.