

Studies on the Metabolic Fate and the Pharmacokinetics of 5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) in Man. I. Identification of the Metabolites of BCP in Human Urine¹⁾

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Urinary metabolites of a non-steroidal anti-inflammatory drug, 5-*n*-butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) were investigated in man.

Two hydroxylated compounds, 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine (II) and 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine (IV) were identified as the metabolites besides unchanged BCP, though two unknown minor peaks were observed by gas-liquid chromatography.

The glucuronides or sulfates of II and IV were not detected clearly.

5-*n*-Butyl-1-cyclohexyl-2,4,6-trioxoperhydropyrimidine (BCP) is a non-steroidal anti-inflammatory drug,³⁾ having low toxicity and no serious side effects. In order to know the metabolic fate of this compound, the urinary metabolites were investigated in man.

On the metabolism of barbiturates *in vivo*, the following pathways have been generally proposed.

1. Oxidation of substituents in position 5 of the barbiturate ring.
2. Dealkylation of substituents in position 5 of the barbiturate ring.
3. Dealkylation of substituents attached to the nitrogen atom.
4. Methylation to one of the nitrogen atoms in the barbiturate ring.
5. Hydrolytic cleavage of the barbiturate ring.
6. Desulfuration of thiobarbiturates.

BCP is different from so called barbiturate-drug in the points that it has a hydrogen atom in position 5 of the barbiturate ring, acts as a weak acid⁴⁾ and has no hypnotic action.

On a similar drug, phenylbutazone, which has also a hydrogen atom and a butyl group at the same carbon atom, the metabolic fate was investigated in man by Burns, Brodie, *et al.* to find two hydroxylated compounds, *i.e.*, ω -1 alcohol of the butyl group and *p*-hydroxy compound of the phenyl group.^{5,6)}

It is also known for general barbiturates that species-differences in the metabolism are rather quantitative than qualitative.⁷⁻⁹⁾ However, as the ultimate purpose is to know the metabolic fate in man, the investigation was carried out on human being.

- 1) Part of this work was presented at the Meeting of Kinki Branch, Pharmaceutical Society of Japan, Kyoto, March 1969.
- 2) Location: a) Higashiyodogawa-ku, Osaka; b) Mitahora, Gifu; c) Nagara, Gifu.
- 3) S. Senda, H. Izumi, and H. Fujimura, *Arzneimittel-Forsch.*, **17**, 1519 (1967).
- 4) H. Mima, Y. Asahi, K. Terada, T. Matsuzaki, E. Mizuta, and H. Izumi, *Takeda Kenkyusho Nempo*, **24**, 1 (1965).
- 5) J.J. Burns, R.K. Rose, S. Goodwin, J. Reichenenthal, E.C. Horning, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **113**, 481 (1955).
- 6) T.F. Yü, J.J. Burns, B.C. Paton, A.B. Gutman, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **123**, 63 (1958).
- 7) L.C. Mark, *Clin. Pharmacol. Therap.*, **4**, 504 (1963).
- 8) M.T. Bush and E. Sanders, *Ann. Rev. Pharmacol.*, **7**, 57 (1967).
- 9) M. Akagi, "Yakubutsu Taisha no Seikagaku," Nanzando, Tokyo, 1965, p. 109.

Experimental

Identification of the Urinary Metabolites in Man—1) Sample Urines: Healthy males ingested 300—900 mg of BCP crystals and the urines were collected for 24 hours.

2) Thin-Layer Chromatography (TLC): The CHCl_3 extract from the acid, neutral and alkaline urines were subjected to TLC along with the aqueous and ethanolic solutions of the lyophilized urines. The conditions were as follows. Plate: Kieselgel HF_{254} (250 μ). Solvent system: A) Benzene-Ether-AcOH-MeOH (60:30:9:1). B) Cyclohexanol-*iso*-PrOH-12.5% NH_4OH (5:13:2). C) $(\text{CH}_3)_2\text{CO}-\text{CHCl}_3-\text{EtOH}-\text{AcOH}$ (10:10:5:1). Detection: UV lamp (253.6 $m\mu$), HgNO_3 reagent and I_2 vapor. The location of a spot was expressed by the R_s value to a standard (Phenobarbital). $R_s=R_f$ of a sample/ R_f of a standard. To search for cyclohexylamine, the following solvent systems reported by Kojima and Ichibangase¹⁰⁾ were employed. a) $\text{AcOEt}-\text{AcOH}-\text{H}_2\text{O}$ (4:1:2). b) *n*-BuOH-Benzyl alcohol- H_2O -80% HCOOH (45:45:9:1). c) *n*-BuOH-AcOH- H_2O (4:2:1). d) AcOEt -80% $\text{HCOOH}-\text{H}_2\text{O}$ (4:1:2). Detection: 1% ethanolic solution of quinine hydrate.

3) Gas-Liquid Chromatography (GLC): Apparatus: Hitachi KGL-2B and for fractionation, Aerograph (Varian Associates) were employed. Method I: Column (50 cm length, 3 mm i.d.) was packed with 1% neopentyl glycol succinate (NGS) coated Celite 545 (60—80 mesh). Temperatures: Inlet 210°, Column 210° and Detector 230°. Carrier gas: He (50 ml/min). Detection: Flame ionization detector (FID). Method II: The next conditions were different from the Method I. NGS 2% on Gas Chrom P (60—80 mesh). Column: 50—80 cm in length. Temperatures: Inlet 270°, Column 210° and Detector 250°. Trimethylsilylation: To CHCl_3 extract was added hexamethyldisilazane 0.5 ml, trimethylchlorosilane 2 μ l and pyridine 5 μ l and the solution was kept at room temperature for 30 min.

4) Paper Partition Chromatography: Paper: Toyo Roshi No. 51. Solvent: BuOH-AcOH- H_2O (4:1:5). Detection: *p*-Dimethylaminobenzaldehyde, 2,4-dinitrochlorobenzene. Development: 16—17 hours. The authentic standards showed the spots at the following R_f values. Cyclohexylurea 0.92, *cis*-, *trans*-4-hydroxycyclohexylurea 0.65, cyclohexylamine 0.63 and urea 0.37.

Examination of Glucuronide and Sulfate—For glucuronide, β -glucuronidase (Sigma)¹¹⁾ was dispersed in pH 6.85 phosphate buffer (200 mg/ml). The following samples were incubated at 40° for 14—16 hours. a) Urine 3 ml, pH 6.85 phosphate buffer 5 ml, β -glucuronidase soln. 1 ml. b) Urine 3 ml, the buffer 5 ml, H_2O 1 ml. c) Blank urine 3 ml, the buffer 5 ml, β -glucuronidase soln. 1 ml. d) Blank urine 3 ml, the buffer 5 ml, H_2O 1 ml. For sulfate, similarly as in glucuronide, four samples with urine (or blank urine), pH 5.05 acetate buffer, and sulfatase¹²⁾ (or H_2O) were incubated at 40° for 22 hours. BCP and the metabolite II¹³⁾ were determined by UV absorption method¹⁴⁾ and the metabolite IV¹³⁾ was determined by GLC.¹⁵⁾

Identification of BCP, II and IV in the Urine of Rats Using 2-¹⁴C-BCP—1) Animal Experiment: To non-fasted Sprague-Dawley rats (SPF) weighing 180—200 g, 2-¹⁴C-BCP (Specific radioactivity: 0.98 mCi/mmol) was administered in the following dosage forms, *i.e.*, orally a) 0.01 N NaOH solution (3 mg 2-¹⁴C-BCP with 20 mg of BCP Na, 1.500×10^4 cpm), b) suspension with gum arabic (3 mg 2-¹⁴C-BCP with 17.1 mg BCP 1.437×10^4 cpm) and intraperitoneally, c) 50% ethanolic aqueous solution (1.5 mg 2-¹⁴C-BCP with 9.3 mg of BCPNa, 737.5×10^4 cpm). Urines were collected at 3, 6, 9, 12, 24, 48 and 96 hours and the feces were at 24, 48, 72 and 96 hours after the administration. The expired air was trapped in 50 ml of 2 N NaOH in an all-glass metabolic cage. The solution was acidified and kept in a sealed container for 30 min to trap the evolved ¹⁴CO₂ into Hyamine 10-X solution.¹⁶⁾

2) Measurement of Radioactivity: The radioactivity was measured by Tricarb 3000 liquid scintillation spectrometer.¹⁶⁾ Preliminary test on the quenching effect revealed that no correction was necessary both on the urine and $\text{CHCl}_3-\text{EtOH}$ (1:1) extract from the feces when less than 0.1 ml of the sample was diluted with 10 ml of a scintillator (PPO-POPOP in dioxane cellosolve). To measure the radioactivity in ex vivo redair, another scintillator (PPO-POPOP in toluene) was used.

3) Isotope Dilution Method: To heptane or CHCl_3 extract from the urine, were added large excess of the authentic standards (83.5—200 mg) and recrystallization were continued with various solvent systems until the constant specific radioactivities were obtained.

Result and Discussion

I. Search for the Urinary Metabolites

1. Thin-Layer Chromatography—In order to identify the urinary metabolites, authen-

10) S. Kojima and H. Ichibangase, *Chem. Pharm. Bull.* (Tokyo), **14**, 971 (1966).

11) Bacteria type and the specific activity was 80 000 units/g.

12) Origin was *Helix Pomatia* and the specific activity was 7 350 units/ml (Sigma).

13) The structures are shown in Table I.

14) T. Yashiki, T. Kondo, Y. Uda, and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 478 (1971).

15) T. Yashiki, Y. Uda, T. Kondo, and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **19**, 487 (1971).

16) Packard Instrument Inc.

tic standards of the expected metabolites were synthesized by Senda and Izumi.¹⁷⁻²⁰⁾ Table I lists the structures and the abbreviated name or the corresponding numbers of the expected metabolites.

Prior to the identification of the metabolites, TLC of the authentic standards were examined. Fig. 1 shows the chromatogram when a solvent system A²¹⁾ was used, where many standards show two spots.

Hayaishi, *et al.* reported that 5-methyl barbituric acid was oxidized readily by air to yield 5-hydroxy-5-methyl barbituric acid in an acid or neutral medium.²²⁾

TABLE I. BCP and Some of the Expected Metabolites in Man

R _I	R _{II}	R _{III}	Abbreviated name or number
<i>n</i> -C ₄ H ₉	H		BCP
<i>n</i> -C ₄ H ₉	OH		I
CH ₃ CHOHCH ₂ CH ₂	H		II
CH ₃ CHOHCH ₂ CH ₂	OH		III
<i>n</i> -C ₄ H ₉	H		IV
<i>n</i> -C ₄ H ₉	OH		V
CH ₂ OHCH ₂ CH ₂ CH ₂	H		VI
CH ₂ OHCH ₂ CH ₂ CH ₂	OH		VII
HOOCCH ₂ CH ₂ CH ₂	H		VIII
CH ₃ CHOHCH ₂ CH ₂	H		IX
CH ₃ CHOHCH ₂ CH ₂	OH		X
CH ₃ COCH ₂ CH ₂	H		XI
CH ₃ COCH ₂ CH ₂	H		XII
<i>n</i> -C ₄ H ₉	H	H	XIII
<i>n</i> -C ₄ H ₉	OH	H	XIV
			XV
<i>n</i> -C ₄ H ₉ CH ₂ CONHCONH-			XVI

17) S. Senda, H. Izumi, T. Tanimoto, and H. Fujimura, *Yakugaku Zasshi*, **89**, 254 (1969).

18) S. Senda, H. Izumi, and H. Fujimura, *Yakugaku Zasshi*, **89**, 260 (1969).

19) S. Senda and H. Izumi, *Yakugaku Zasshi*, **89**, 266 (1969).

20) S. Senda, H. Izumi, and K. Hirota, *Yakugaku Zasshi*, **89**, 272 (1969).

21) The condition is shown in the "Experimental."

22) O. Hayaishi and A. Kornberg, *J. Biol. Chem.*, **197**, 717 (1952).

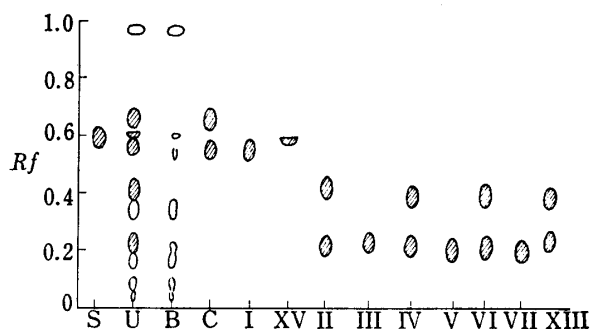


Fig. 1. TLC of BCP, Its Expected Metabolites and the Chloroform Extract from the Urine.

plate: Kiesel gel HF₂₅₄, solvent: benzene-ether-AcOH-MeOH(60:30:9:1), detection: UV lamp (253.6 mμ) (○) and with HgNO₃ reagent (●)

S: internal standard (phenobarbital), U: chloroform extract from the urine, B: chloroform extract from a blank urine, C: BCP

The number shows an expected metabolite whose structure is shown in Table I.

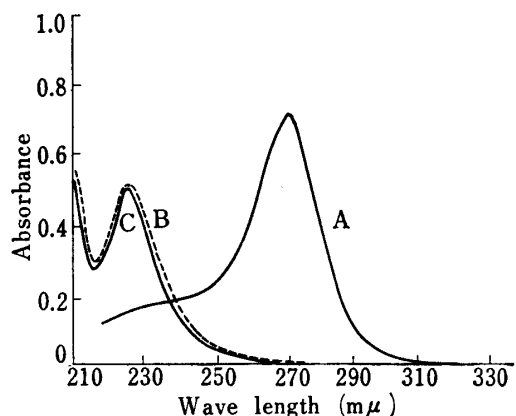


Fig. 2. Ultraviolet Absorption Spectra of BCP, I and the Extract from the Second Spot of BCP ($R_s=0.98$) on TLC in pH 6.5 Phosphate Buffer

A: BCP, ca. 10 μg/ml

B: I, ca. 28 μg/ml

C: extract from TLC, ca. 25 μg/ml

I: 5-*n*-butyl-5-hydroxy-1-cyclohexyl-2,4,6-trioxo-perhydropyrimidine

By comparing the TLC with those of the authentic standards, the spots of smaller R_s values²³⁾ were coincident with the spots of the corresponding 5-hydroxy compounds.

To examine the hydroxylation during TLC, two dimensional TLC was carried out on BCP with the same solvent system A. Three spots were found in the chromatogram, *i.e.*, one corresponding to BCP and two corresponding to I, which suggested I was made during TLC from BCP.

Further, to confirm the identification of I, the substance at $R_s=0.98$ was extracted with chloroform (CHCl₃) and was recrystallized with ethanol. The crystals, mp 131°, showed the maximum ultraviolet (UV) absorption at 225 mμ in pH 6.5 phosphate buffer, whereas BCP, mp 81°, showed the maximum absorbance at 271 mμ in the solution (Fig. 2).

The infrared (IR) absorption spectrum showed a band at 3395 cm⁻¹ (5-OH) and was coincident with the spectrum of I. The nuclear magnetic resonance (NMR) spectrum in deuterio-chloroform showed a signal at 5.95 τ (singlet, 5-OH), whereas BCP showed a signal at 6.5 τ (triplet, 5-H). Elementary analysis coincided with the theoretical values of I (*Anal.* Calcd. for C₁₄H₂₂O₄N₂: C, 59.55; H, 7.85; N, 9.92. Found: C, 59.80; H, 8.05; N, 9.87).

Chloroform Extract from the Acid Urines: The pK_{a1} value of BCP is 4.4 and it is dissolved 66% in CHCl₃ at 25°. As the similar properties were expected on the metabolites, an acidified urine was extracted with CHCl₃. Fig. 1 also showed the TLC for the extract with phenobarbital as the internal standard. Five spots, *i.e.*, $R_s=1.10$, 1.06, 0.98, 0.68 and 0.35 were observed by UV lamp and were positive to mercurous nitrate reagent. By comparing the R_s values with those of authentic standards, the following compounds were assumed. R_s , 1.10: BCP, 0.98: I, 0.68: II, IV and VI, 0.35: III, V and VII. The spot at R_s 1.06 was found to be an artifact produced when the CHCl₃ extract was heated (>40°) with some acid solution left during the evaporation of the solvent *in vacuo*. The spot at R_s 0.35 was found due to the corresponding 5-hydroxy derivatives of the compounds at R_s 0.68. Products by the ring cleavage, such as 1-cyclohexyl-3-hexanoylurea (XVI) or cyclohexylurea were not detected.

When an alkaline solvent system B was used for TLC, separation of the spots was poor. Two spots at R_s 1.08, 0.69 were observed by UV lamp and mercurous nitrate reagent from

23) $R_s=R_f$ of a sample/ R_f of a standard (phenobarbital).

the CHCl_3 extract. Comparing the R_s values with those of authentic standards, the following compounds were assumed. R_s , 1.08: BCP, I, II and IV. R_s , 0.69: III. The compound V showed a little larger R_s value, 0.81. The R_s values of VI, IX and XI were in the range of 0.90–0.96 and their existences were suspicious. Also, the extract did not give spots which were coincident with those of VII, VIII, XIII, XIV and XV.

Chloroform Extract from the Neutral and Alkaline Urines: The CHCl_3 extract from an alkaline urine (pH 12.0) and from a neutral urine (pH 6.5) which was made by adding sodium hydrogen carbonate to the acid urine once extracted with CHCl_3 were investigated by TLC with the solvent system A, B and C. No other clear spots were observed than blank. The decomposition of BCP to cyclohexylamine also could not be detected with the solvent systems by Kojima and Ichibangase.¹⁰⁾

From these observations in TLC, when BCP was administered to man, unchanged BCP, I, II, III, IV and V were found in the CHCl_3 extract from the urine. However, TLC could not tell whether the 5-hydroxy compounds were the metabolites or the artifacts produced during TLC.

2. Gas-Liquid Chromatography—GLC of the Chloroform Extract from the Acid Urines: Neopentyl glycol succinate (NGS) was selected as the liquid phase. Under the condition 1,²⁴⁾ the CHCl_3 extract showed four peaks and were attributed to the following compounds: t_R 2.3: BCP, 6.5 (trace): I, 8.2 (small): XV, 14.9 (broad): II and IV. The peak of I was trace and the small peak was observed even when the authentic sample of BCP

was added to a blank urine and treated similarly. The refore, I was not regarded as the metabolite. Also, the peak of XV was found to be an artifact that might be produced during the concentration process of the CHCl_3 extract. The easy conversion of II to XV by heating it in an acid medium was reported by Senda and Izumi.¹⁹⁾ Also in GLC, if the column temperature exceeded 210°, a part of II was converted to XV in this experiment.

GLC of the Chloroform Extract after Trimethylsilylation: Acetylation or trifluoroacetylation of the CHCl_3 extract did not separate the peaks of II and IV well. Trimethylsilylation, however, separated them enough and BCP, II and IV were identified from the extract by comparing the t_R 's with those of authentic standards (Fig. 3). Two small unknown peaks (U.K.1, U.K.2) were observed also. Table II shows the t_R 's of the authentic standards after trimethylsilylation.

The peaks at t_R 4.8 min was attributed to II which was considered the main metabolite of BCP. To confirm the assignment, the substance was fractionated with a preparative gas chromatograph. As will be described elsewhere,¹⁵⁾ though the IR spec-

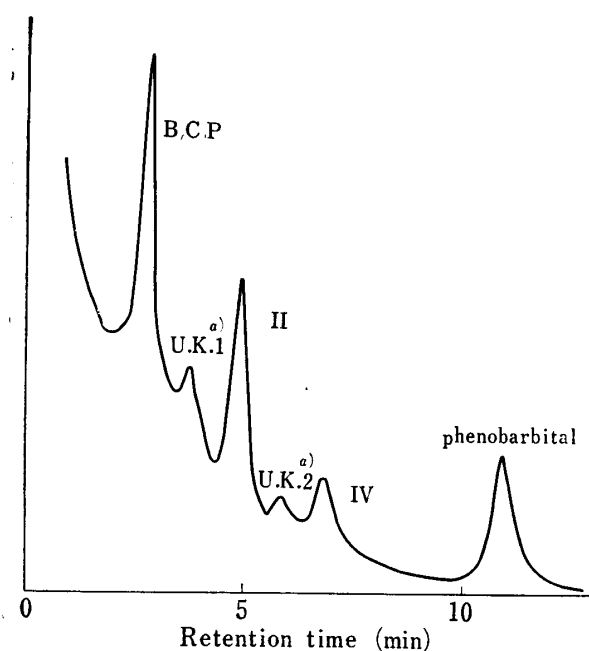


Fig. 3. Gas Chromatogram of the Trimethylsilyl Derivatives of the Chloroform Extract from a Urine

liquid phase: NGS 2% on Gas Chrom P (60–80 mesh), column: glass column, 50 cm (length), 3 mm (i.d.) temperatures: inlet 270°, column 210°, detector 250°, detection: F.I.D.

carrier: He gas, internal standard: phenobarbital, instrument: Hitachi KGL-2B.

a) the peaks of the unknown 1, and 2

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine

IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

24) The condition is shown in the "Experimental."

TABLE II. Retention Times of BCP and Some of the Expected Metabolites after Trimethylsilylation in the Gas-Liquid Chromatography

Authentic sample ^{a)}	<i>t_R</i> (min)	Authentic sample ^{a)}	<i>t_R</i> (min)
I	2.0	II	4.8
XIV	2.3	unknown 2	6.0
III	2.6	X	6.2
BCP ^{b)}	2.8	IV	6.9
XIII ^{b)}	3.7	VI	7.9
unknown 1	3.9	XV ^{b)}	10.9
VII	4.0	IX	13.8
V	4.3		

column: NGS 2% on Gas Chrom P (60—80 mesh). glass column (50 cm length, 3 mm i.d.)
 temperatures; inlet, 270°, column, 210°, detector, 250°.
 detection: F.I.D. carrier: He gas (50 ml/min)
 instrument: HitachiKGL-2B.

a) The structures are shown in Table I.

b) The *t_R*'s were not affected by trimethylsilylation.

trum of the fractionated substance coincided with that of XV, the conversion of II to XV would have happened during the fractionation.

For the unknown substances, the following behaviors were observed. a) These peaks appeared after the CHCl₃ extract was treated trimethylsilylation, b) Even with a moderate condition for trimethylsilylation, *e.g.*, with hexamethyldisilazane only, the peaks appeared. c) The addition of BCP, II and IV to a blank urine and similar treatment did not give the unknown peaks. d) Both substances were resistant to acid and alkali, *e.g.*, boiling a urine in 1*N* hydrochloric acid (HCl) for 30 min or shaking it in 1*N* sodium hydroxide (NaOH) for 30 min did not affect the peaks. e) The compound XIII, trimethylsilyl derivatives of V and VII gave the peaks close to the peak of the unknown 1. However, XIII showed the peak without trimethylsilylation and V or VII was unstable in an alkaline solution. f) The compound X showed a peak close to the peak of the unknown 2, but the *t_R* was not coincident.

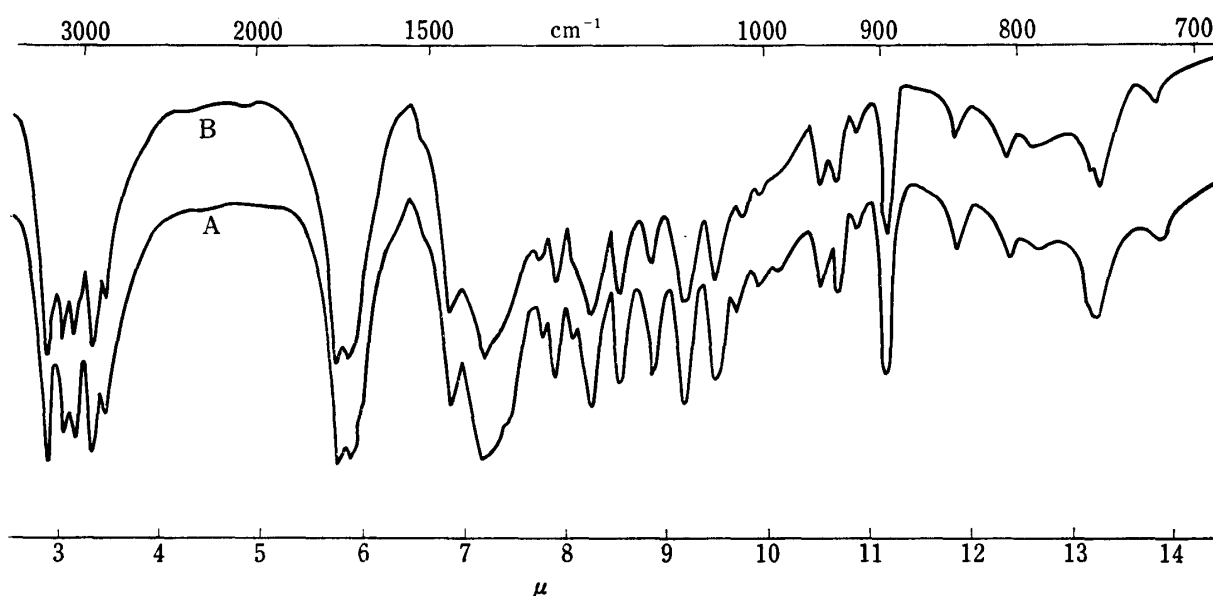


Fig. 4. IR Absorption Spectra of III and of the Crystal obtained from the Fraction of II with IV in TLC (KBr disk)

A: authentic standard of III, B: Crystal obtained from the fraction of II with IV in TLC.

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine

III: 1-cyclohexyl-5-hydroxy-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine

IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

3. Separation and the Identification of BCP and Its Metabolites—BCP: Aurine collected in 48 hours after the administration of BCP (600 mg) to a healthy male, was acidified with equal volume of 4N HCl and was extracted with heptane. The extract was recrystallized with hexane and subsequently with aqueous ethanol. The crystals, mp 82°, coincided with the authentic standard of BCP in UV ($\lambda_{\text{max}}^{\text{0.1M borax}}$: 271 m μ , $\lambda_{\text{max}}^{\text{1.2NHCl}}$: 224 m μ), IR (B form of BCP⁴⁾) and elementary analysis (Anal. Calcd. for C₁₄H₂₂O₃N₂: C, 63.13; H, 8.33; N, 10.52. Found C, 62.85; H, 8.58; N, 10.53).

The Compound II as III: The CHCl₃ extract was subjected to TLC with the solvent system A and the fraction of II was re-extracted with CHCl₃ after 0.1N HCl was added to reduce the activity of the silica gel. Recrystallization of the extract with ethylacetate gave the crystals, mp 180–185°, which were identical with III by UV ($\lambda_{\text{max}}^{\text{0.1M borax}}$: 252 m μ , $\lambda_{\text{max}}^{\text{0.1NHCl}}$: 228 m μ), IR (Fig. 4), mass spectra, TLC and GLC. The oxidation was also observed on the authentic standard of II during the same treatment.

The Compound II as XV: An acid urine once extracted with heptane was re-extracted with CHCl₃. The extract was heated in 2N HCl at 100° for 30 min. The crystals, mp >270°, obtained by recrystallization with aqueous methanol were identified as XV by UV ($\lambda_{\text{max}}^{\text{0.1N NaOH}}$: 274 m μ , $\lambda_{\text{max}}^{\text{1.2NHCl}}$: 263 m μ), IR, TLC and GLC.

4. The Metabolite IV as 4-Hydroxycyclohexylurea—The CHCl₃ extract was subjected to TLC with the solvent system A and the fraction of IV with II was re-extracted with CHCl₃.

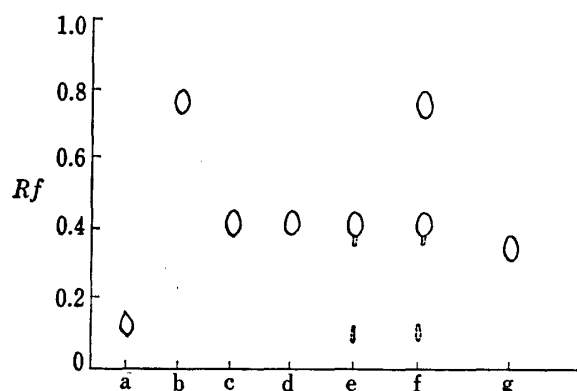


Fig. 5. TLC of 4-Hydroxycyclohexylurea, Hydrolyzed Products of IV and the Fraction of IV with II in TLC on the Chloroform Extract from a Urine and Related Compounds

plate: kieselgel HF₂₅₄, solvent: (CH₃)₂CO-CHCl₃-EtOH-AcOH (10:10:5:1), detection: *p*-dimethylaminobenzaldehyde

a: cyclohexylamine, b: cyclohexylurea, c: *cis*-4-hydroxycyclohexylurea, d: *trans*-4-hydroxycyclohexylurea, e: hydrolyzed product of IV; f: hydrolyzed product of the fraction of IV with II in TLC on the chloroform extract, g: urea

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine

V: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

The extract was hydrolyzed in 1N NaOH at 100° for 100 min. The solution was neutralized, concentrated and desalted with ethanol. TLC of the concentrate showed the spots corresponding to cyclohexylurea, 4-hydroxycyclohexylurea, urea and cyclohexylamine, though the hydrolyzed product of BCP or II did not show the spot of 4-hydroxycyclohexylurea (Fig. 5). The fraction of 4-hydroxycyclohexylurea was re-extracted with a mixed solvent of acetone-ethanol and refined three times by paper partition chromatography. Comparison of the IR spectrum with those of similarly treated authentic standards of 4-hydroxycyclohexylurea showed apparently better coincidence with *cis*-form than *trans*-form as shown in Fig. 6. However, owing to the scanty of the sample, the form could not be determined. The mass spectrum showed the molecular ion peak at *m/e*: 158 and agreed with those of the authentic standards.

II. Investigation on the Conjugates

On II and IV, the glucuronide or sulfate conjugation were expected. Also, some conjugation of BCP was assumed as the determined value increased when a urine was heated in an acid medium before extraction.²⁵⁾

1. Thin-Layer Chromatography—The lyophilized substance of a urine was dissolved in water or ethanol and subjected to TLC with the solvent system A. Besides faint spots

25) H. Mima, T. Matsuzawa, K. Terada, and T. Kondo, *Takeda Kenkyusho Nempo*, **24**, 9 (1965).

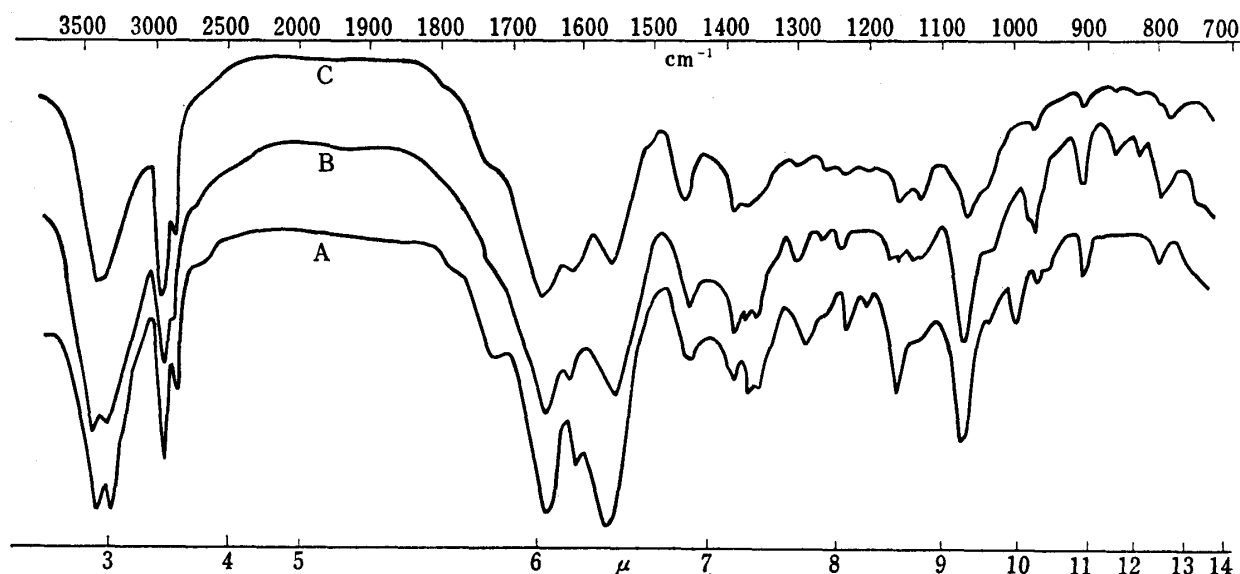


Fig. 6. IR Absorption Spectra of *cis*- and *trans*-4-Hydroxycyclohexylurea and the Hydrolyzed Product of the Fraction of IV with II in TLC (KBr Disk)

A^a): *trans*-4-hydroxycyclohexylurea, B^a): *cis*-4-hydroxycyclohexylurea, C: hydrolyzed product of the fraction of IV with II in TLC a) The same treatments were made as in C.
 II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydroypyrimidine
 IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydroypyrimidine

of BCP, I, II and IV, two spots were found at origin and *R_s* 0.17 which were positive to mercurous nitrate (white), *p*-dimethylaminobenzaldehyde (yellow) and negative to aniline hydrogen phthalate, ninhydrin and phosphomolybdic acid. Though urea showed the spot at *R_s* 0.17 (white with mercurous nitrate) and blank urines also showed the two spots, the solution of the lyophilized urine was more sensitive to mercurous nitrate. When the solution was acidified, only the spots of BCP and I were detected easily owing probably to the increase of the concentrations. The spots at *R_s* 0.17 and the origin did not change apparently at that time, but the CHCl₃ extract from the origin at 2N HCl also showed the spots of BCP and I. Treatment of a urine with β -glucuronidase or sulfatase did not affect the chromatogram.

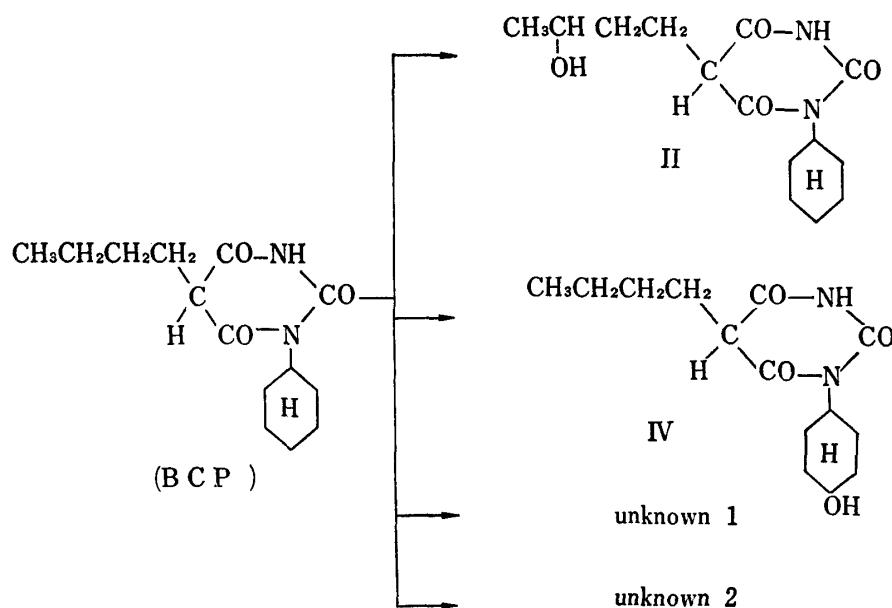


Fig. 7. Metabolism of BCP in Man

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydroypyrimidine
 IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydroypyrimidine

From the results, only BCP was recognized to have interactions with some substances in the urine, which released free BCP easily by the addition of HCl.

2. Examination on BCP—To examine on the interactions of BCP with the substances in a urine, uric acid, hippuric acid, creatinine, urea, glycine, cysteine, inorganic salts of magnesium, calcium and aluminum were added to an aqueous solution of BCP. No differences were found in the determination of free BCP with and without the adjuvants.

By treatment with β -glucuronidase and sulfatase, no conjugation were detected.

3. Examination on II and IV—As will be described elsewhere,¹⁴⁾ in the acidity range of 0.5–3*N* HCl, the recovery of the extraction of II from a urine did not change. Also, the examination with β -glucuronidase and sulfatase proved no glucuronide or sulfate on II and IV clearly.

III. Metabolic Fate of BCP in Man

Two hydroxylated compounds, II and IV were found as the urinary metabolites of BCP in man with two unknown minor peaks observed in GLC (Fig. 7).

Such an oxidation in position 3 or ω -1 of the butyl group was observed in the metabolism of barbiturates which had 5-alkyl substituents, *e.g.*, pentobarbital,²⁶⁾ amobarbital,²⁷⁾ secobarbital,²⁸⁾ thiopental²⁹⁾ and also in phenylbutazone⁵⁾ in man.

Concerning with the oxidation of cyclohexyl group, cyclohexanol, cyclohexanediol were observed as the metabolites when cyclohexane or cyclohexanol was administered respectively to rabbits.³⁰⁾ Murata investigated the metabolism of 1-ethynylcyclohexyl carbamate in man and found 4-hydroxy compound as the main metabolite and 50% of it was excreted as the glucuronide in the urine.³¹⁾

In the present investigation, IV coincided with 4-hydroxy compound in the physico-chemical properties and no further examinations were made on 2,3,5,6-hydroxy compounds in the cyclohexane ring.

Maynert identified ethyl (3-hydroxy-1-methylbutyl) barbituric acid as a metabolite of pentobarbital in dogs and obtained diastereoisomer (levo-rotatory alcohol: 33–36%, dextro-rotatory alcohol: 15%) from the urine collected in 24 hours after the administration.²⁶⁾

On the metabolite II, though the existence of the optically active diastereoisomer was also expected, the amount obtained was so small that no informations were obtained.

Further, BCP and the human metabolites, II and IV were searched in the urine of rats after the administration of 2-¹⁴C-BCP orally as a) 0.01*N* NaOH solution, b) suspension with gum arabic and intraperitoneally as c) 50% ethanolic aqueous solution. The urinary excretion of the radioactivity in 24, 96 hours and the fecal excretion in 96 hours were respectively as follows: a) $59.5 \pm 5.7\%$, $73.2 \pm 7.5\%$, $7.9 \pm 2.3\%$ ($n=4$), b) $61.5 \pm 0.5\%$, $69.4 \pm 1.5\%$, $5.9 \pm 1.3\%$ ($n=2$), c) $59.0 \pm 1.5\%$, $69.2 \pm 1.7\%$, $5.0 \pm 0.97\%$ ($n=2$). The radioactivity in the expired air was negligible.

In the urine collected in 24 hours after the administration, 12% of dose was found as BCP (12.6, 11.3% with heptane and 10% aqueous ethanol as the solvent respectively), 3% as II (3.3, 2.5% with benzene and acetone–water (1:1) as the solvent respectively), but IV was not identified clearly ($<1.7\%$ with 10% aqueous ethanol as the solvent) by isotope dilution method.

The anti-inflammatory activities of II and IV were already reported by Senda and Izumi.^{17,19)} From their data, the activities of the metabolites were somewhat lower than

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that of BCP, but the toxicities were decreased to one half (II) and a quarter (IV) of that of BCP.

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