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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. II.[1] Determination of BCP and Its Metabolite, 1-Cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine by Ultraviolet Absorption Method[2]

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The determination-procedures of BCP and its urinary metabolites, 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine (II) and 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine (IV) were investigated by ultraviolet absorption method.

Total and free BCP in a urine were determined separately by changing the conditions for extraction.

The metabolite II in a urine was determined selectively by converting it to 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione (XV), though IV was not determined by this method as the separation from the unknown metabolites was not successful.

In the previous paper,[1] the urinary metabolites of BCP was investigated in man and 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) were identified besides unchanged BCP.

In this paper, the determination-procedures for total and free BCP and the metabolite II in the urines were investigated by ultraviolet (UV) absorption method.

Experimental

Sample Urines——Three healthy males ingested 300—900 mg of BCP crystals and the urines were collected for 24 hours.

Gas-Liquid Chromatography (GLC) of the Heptane Extract from Urines——After addition of the equal volume of 1N HCl to a urine, it was heated at 100° for 30 min and was extracted with heptane. For GLC, Hitachi KGL-2B with a flame ionization detector and a glass column (50 cm in length, 3 mm *i.d.*) was used. The column was packed with 1.5% neopentyl glycol succinate (NGS) coated Gas Chrom P (60—80 mesh). The carrier gas was helium and the column was operated isothermally at 210°, the inlet at 270° and the detector at 240°.

Partition of II, IV and XV[4] between Heptane or $CHCl_3$ and Buffers of Various pH's——To 20 ml of the buffer solutions of various pH's, II and IV were dissolved and they were shaken with equal volume of heptane or $CHCl_3$ for 2 hours at 25°. In the case of XV, it was dissolved in the organic solvents. The contents of II, IV and XV in the buffers were determined by UV absorption method.

Contribution of XV to the Increment of the Absorbance at 271 mμ——To 2 ml of a urine, were added equal volume of HCl of various concentrations and they were heated at 100°. Periodically, the urines were extracted with 25 ml of heptane and 20 ml of which were shaken with 10 ml of 0.1M borax solution to measure the absorbances at 271 mμ ($A_{271m\mu}$) on the aqueous phase. The same procedures were carried out on the

1) 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).
2) This work was presented at the Meeting of Kinki Branch, Pharmaceutical Society of Japan, Kyoto, March 1969.
3) Location: *Higashiyodogawa-ku, Osaka.*
4) 3-Cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione.

unheated urines. The concentration of XV were determined by $A_{263m\mu}$ after the 0.1M borax solution were acidified with HCl. Subtraction of the $A_{271m\mu}$ due to XV from the total $A_{271m\mu}$ gave the absorbance due to BCP.

Extraction of II and IV with $CHCl_3$ from Buffers and Blank Urines——To 10 ml of buffers of various pH's were fortified the authentic samples of II and IV for extraction with 30 ml of $CHCl_3$ by shaking for 25 min. After centrifugation, 20 ml of the extract was shaken with 20 ml of 0.1N NaOH and the $A_{271m\mu}$ was measured. In the case of a blank urine, the $CHCl_3$ extract was washed with 3 ml of the aqueous solution of 50% $ZnCl_2$.

Determination of the Conditions to Form XV from II——Authentic samples of II (10.6 μg/ml) in 10 ml of HCl of various concentrations were heated at 100° for different periods. After cooling, $A_{263m\mu}$ was measured. On urines, the standard determination-procedures were applied.

Result and Discussion

I. Determination of Unchanged BCP in Urines

The determination-procedures of BCP from urines by UV absorption method were already reported.[5,6] However, the determined values increased when the urines had been heated in acid media before extraction, which suggested the existence of conjugates or interactions of BCP with some substances in the urines. Thus, in order to find the determination-procedures for the total and free BCP in human urines, an investigation was made on the behavior of the interactions.

The Compositions extracted with Heptane from a Urine——The heptane extract from the unheated and heated urines in acid media were analyzed by GLC. Besides unchanged BCP, 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione (XV) was found in the extract from the heated urine, while only BCP was found from the unheated urines (Fig. 1).

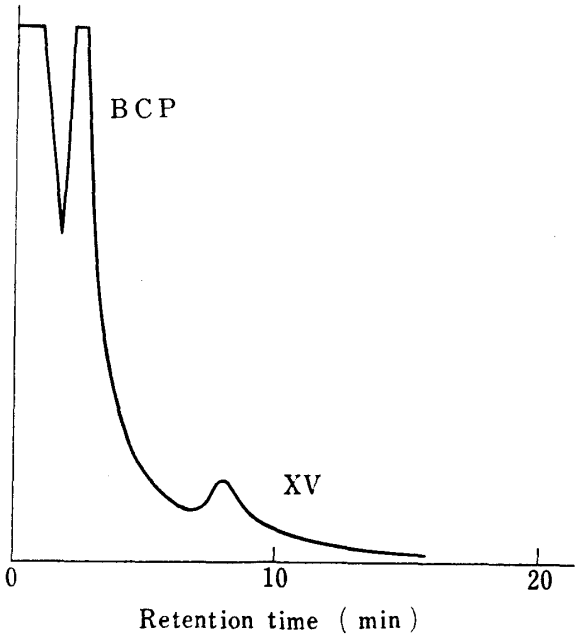

Fig. 1. Gas Chromatogram of the Heptane Extract from a Urine after Heating it in an Acid Medium

NGS 1.5% on Gas Chrom P (60—80 mesh): temperatures: inlet 270°, column 210°, detector 240° detection: FID, Carrier: He gas
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione

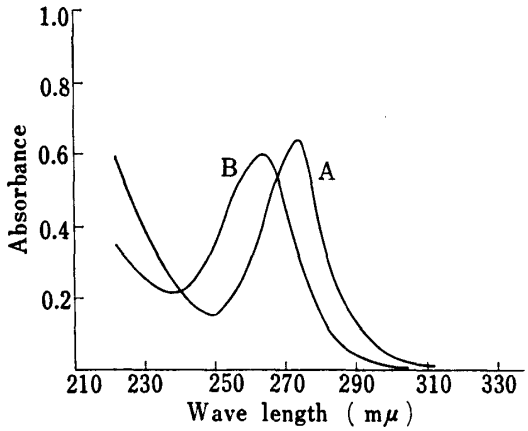

Fig. 2. Ultraviolet Absorption Spectra of XV as a Derivative of the Metabolite II

A: *ca.* 16 μg/ml XV in 0.1N NaOH
B: *ca.* 15 μg/ml XV in 1.2N HCl
II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione

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

As XV was not the metabolite of BCP and the conversion from II during GLC[1] was excluded in the conditions, the conversion must have occurred during the heating process of the urines.[1,7] The gas chromatogram was almost unchanged by trimethylsilylation[1] on the heptane extract and the extraction of II and IV with heptane were actually negligible.

The compound XV shows the maximum UV absorbance at 273 mμ in 0.1M borax solution ($\varepsilon=1.03\times10^4$) which would interfere the determination of BCP performed by measuring the absorbance at 271 mμ (A_{271} mμ) in the solution. The maximum absorbance shifts to 263 mμ ($\varepsilon=1.06\times10^4$) in an acid medium as shown in Fig. 2.

The partition coefficients between buffers of various pH's and heptane or chloroform ($CHCl_3$) showed that *ca.* 75% of XV was transferred to heptane from the equal volume of 2N hydrochloric acid (HCl) and by shaking again the heptane layer with equal volume of 0.1M borax solution, *ca.* 88% of XV in the heptane was transferred to the aqueous phase (Table I). These results suggested that in the increment of A_{271} mμ, the absorbance due to XV was included besides that of BCP when a urine was heated in an acid medium before extraction.

Table I. Distribution of XV between Heptane or Chloroform and Buffers of Various pH's (25°)

Buffers	Heptane	Chloroform
2N HCl	74.5%[a]	100%[a]
pH 1.05	67.6	100
pH 2.30	68.0	100
pH 5.05	67.8	100
pH 7.00	68.6	100
0.1M Borax	11.6	98.8
0.1N NaOH	0	12.5

a) Percent stands for the distribution amount of XV in the organic solvent to the total amount used.
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione

To know the degree of the contribution of XV to the increment, A_{271} mμ along with the concentration of XV were determined periodically during the heating process of a urine. As shown in Fig. 3, A_{271} mμ increased with the concentration of HCl added even if the urine was not heated. When a urine was heated, the absorbance increased with heating period and showed a constant value by heating for more than 15 min. The constant value in 2N HCl was slightly higher than that in 1N HCl, and a small value was observed in 0.1N HCl.

On the other hand, the formation of XV (A_{263} mμ in 2N HCl) was negligible in an unheated urine at the acidities below 2N HCl. However, in a heated urine, the concentration of XV (A_{263} mμ) increased with heating period and showed a constant value also by heating a urine for more than 15 min. The constant value in 2N HCl was slightly higher than that in 1N HCl. From the increments of A_{271} mμ and A_{263} mμ observed after heating of a urine, 60% of the increment of A_{271} mμ in 1N HCl and all of the increment in 2N HCl were due to the formation of XV from II and the rest were due to the release of free BCP.

Determination of Total BCP——The fact that the concentration of BCP no more increased when a urine was heated in 2N HCl suggested the interaction of BCP was weak toward acid. Therefore, an examination was carried out to determine the total BCP with an unheated urine. Fig. 4 shows the relation between A_{271} mμ due to BCP versus the concentration of HCl added. The concentration of BCP increased until 2N HCl, then showed a constant value in spite of the increase of the acidity. On the other hand, the concentration

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

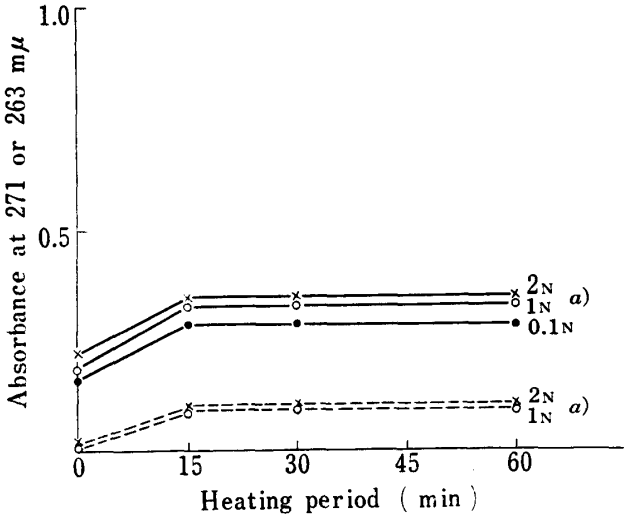

Fig. 3. Relation between the Absorbance at 271 mμ or 263 mμ and the Heating Period (at 100°) in Various Concentrations of HCl

a) normal of HCl in the solution
—— : for the absorbance at 271 mμ
------ : for the absorbance at 263 mμ

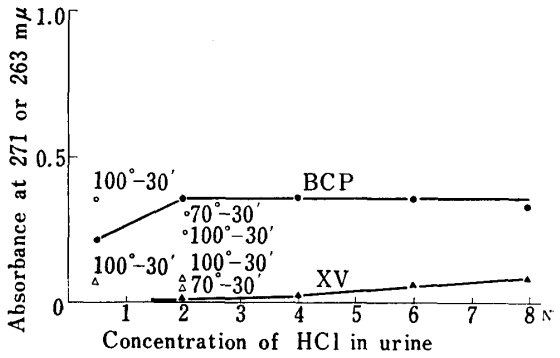

Fig. 4. Relation between Absorbance at 271 mμ or 263 mμ and the Concentration of HCl in a Urine

●, ○: absorbances at 271 mμ due to BCP in unheated (●) and heated urine under the conditions described above (○)
▲, △: absorbances at 263 mμ due to XV in unheated (▲) and heated urine under the conditions described above(△)
XV : 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano(2,3-*d*)pyrimidine-2,4-dione

of XV (A_{263} mμ) increased at the acidities more than 3N HCl. For comparison, A_{271} mμ due to BCP determined on the heated urine in 0.5N and 2N HCl were also plotted in Fig. 4, which were almost coincident with the constant absorbance of BCP on the unheated urine.

From these results, the total BCP could be determined with an unheated urine at 3N HCl without the significant influence of XV.

Determination of Free BCP——Authentic samples of BCP were added to buffers or a blank urine and after the pH's were adjusted with buffers of various pH's, BCP were extracted with heptane to know the relation between pH and the recovery of extraction.

As shown in Fig. 5, BCP were extracted quantitatively at the pH's below 3.4 in both cases. The recovery did not change when 1N acetate or 0.5M citrate buffer was used.

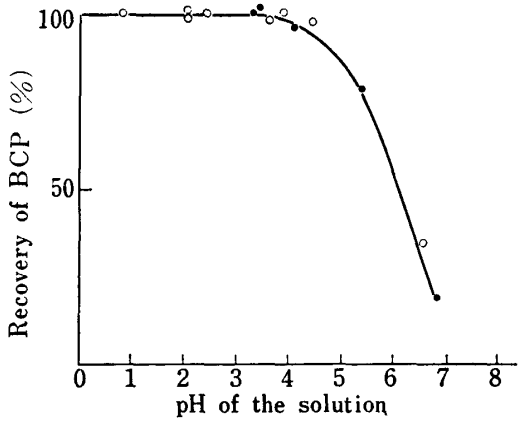

Fig. 5. Relation between pH and the Recovery in the Extraction of BCP with Heptane (29.3 μg/ml)

—●—: from buffers
—○—: from blank urines

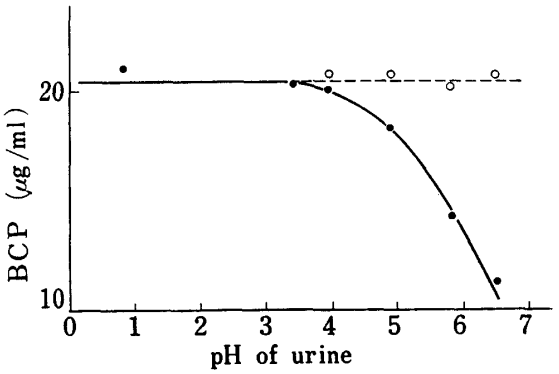

Fig. 6. Relation between pH and the Determined Concentration of BCP from a Urine

—●—: observed value
—○—: Correction was made for the recovery of extraction from the relation in Fig. 5.
The pH's of the sample-urines were adjusted by adding equal volume of HCl and buffers of various pH's; pH 0.8: 0.5N HCl, pH 3.4—5.8: acetate buffer (1N AcOH-1N AcONa), pH 6.5: water.

BCP were also extracted at pH's 4.0, 4.9, 5.8 and 6.5 from the urine and the recoveries of extraction were corrected by the relation shown in Fig. 5. The corrected concentrations of BCP in the urine at pH's 4.0—6.5 were coincident with that determined at pH 3.4 as shown in Fig. 6. This means that the fraction of BCP with some kind of interactions does not release free BCP anymore at pH 3.4.

As total BCP is extracted with heptane from a urine at 3N HCl and free BCP at pH 3.4, the difference in the determination-values corresponds to the content of BCP which has some kind of interactions.

Determination-Procedures of BCP from a Urine——In a 50 ml centrifuge tube, 1—5 ml of a urine and equal volume of 6N HCl are added, which is extracted with 25—30 ml of heptane by shaking it for 25 min. Twenty milliliter of the heptane layer is transferred to another centrifuge tube to which is added 10 ml of 0.1M borax solution and they are shaken for 20 min. The A_{271} mμ is measured for total BCP on the borax solution after centrifugation.

The same procedures are available except a buffer of pH 3.4 is employed instead of 6N HCl for the determination of free BCP.

With these standard procedures, total and free BCP in a human urine collected after the administration of BCP crystals to males were determined. As shown in Table II, the contents of free BCP were 25—54% of the total, which indicated that a large portion of BCP existed with some kind of interactions in the urine.

Table II. Total and Free BCP excreted in the Urine after Administration of 300 and 900 mg of BCP to Healthy Males

Exp. No.	Hours	Dose 300 mg Total BCP (mg)	Dose 300 mg Free BCP (mg)	Free/Total	Dose 900 mg Total BCP (mg)	Dose 900 mg Free BCP (mg)	Free/Total
1	2	0.85	0.34	0.40	0.22	0.07	0.32
	4	4.79	1.67	0.34	6.37	2.71	0.43
	6	3.16	1.17	0.37	9.75	5.25	0.54
	9(8)[a]	6.28	2.83	0.45	8.64	4.27	0.50
2	2	0.72	0.34	0.47	0.98	0.32	0.33
	4	4.62	2.26	0.49	8.35	2.06	0.25
	6	5.81	2.73	0.47	9.38	2.50	0.27
	8	3.71	1.82	0.49	9.93	3.31	0.33

a) At the dose of 300 or 900 mg, the urine was collected for 9 or 8 hours respectively.

II. Determination of a Metabolite II in Urines

Before the experiments on the partition coefficients, pK_a values of II and IV were determined by UV absorption method. The metabolite II showed pK_{a1}=4.6, pK_{a2}=13.4 and IV showed pK_{a1}=4.4, pK_{a2}=13.5 at 25° and they were almost consistent with those for BCP.[8]

The partition coefficients of II and IV, as shown in Table III, indicate that they are distributed only 2—3% to heptane from an acidified urine, though BCP is distributed quantitatively.[1,8] To $CHCl_3$, the metabolites are transferred quantitatively and by shaking the $CHCl_3$ layer with 0.1N sodium hydroxide (NaOH), they are transferred selectively to the aqueous phase again. From the results, however, the separation of II form IV, *vice versa*, by extraction was not successful.

Extraction of II and IV with Chloroform——One of the most important problems in the determination of metabolites from a $CHCl_3$ extract by UV absorption method is how to remove

8) H. Mima, Y. Asahi, K. Terada, T. Matsuzaki, E. Mizuta, and H. Izumi, *Takeda Kenkyusho Nempo*, **24**, 1 (1965).

Table III. Partition Coefficients of the Human Metabolites of BCP *i.e.*, II and IV at 25°

pH	Heptane/Buffer			Chloroform/Buffer	
		II	IV	II	IV
	26.3 μg/ml	9.0 μg/ml	10.0 μg/ml	30.0 μg/ml	30.0 μg/ml
1.05	0.029	0.032	0.035	189	127
5.28	0.017	0.026	0.028	0.835	1.15
7.24	0.011	0.019	0.022	0.041	0.072
9.41	0.011	0.017	0.022	0.041	0.053
0.1N NaOH	0.008	0.010	0.013	0.028	0.029

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

the significant blank value of the extract. Washing the extract with an aqueous solution of 50% zinc chloride was quite effective in elimination of the blank without significant loss of the metabolites.

To know the recoveries of the extraction, authentic samples of II and IV were added to buffers or blank urines of various pH's and were extracted with $CHCl_3$. The average recoveries were 91.8%, 92.0% for II and IV respectively from the blank urines at the pH's below 1.3, though 96.4%, 98.1% were obtained from the buffers at the same pH's.

Determination of II as XV——The both metabolites, II and IV show the same maximum absorbances at 271 mμ in 0.1M borax or 0.1N NaOH (ε_{II}=18860, ε_{IV}=18890) and at 223 mμ in 2N HCl (ε_{II}=6550, ε_{IV}=6860) which are almost the same as in BCP.[8]

To separate II from IV, a selective reaction was applied that II was converted to XV by heating it in an acid medium.[7] As described before, XV(has the maximum absorbance at 273 mμ in 0.1N NaOH and at 263 mμ in 2N HCl, but the A_{263} mμ due to BCP, II and IV are actually negligible in the acid solution.

Apparently, the sum of the contents of II and IV is determined by measuring A_{271} mμ after they are re-extracted with 0.1N NaOH from the $CHCl_3$ extract and II can be determined selectively by measuring the A_{263} mμ after the conversion of II into XV. Subtraction of the content of II from the sum, the content of IV must be determined.

However, the contents of IV thus determined were always larger than those determined by GLC. This might be due to the interferences of the unknown substances[1] and unless IV was separated from the unknown, the determination of IV by UV absorption method was not available.

The investigation on the conditions for conversion of II into XV revealed that heating a sample of II in 2N HCl at 100° for 30 min was enough for the reaction.

As the apparent solubility of XV in 2N HCl was 38.3 μg/ml at 23°, the concentration in a sample solution should be adjusted below this concentration.

The calibration line of II as XV is shown in Fig. 7. The average recoveries in the determination were 93.3% from 2N HCl, 85.0±3.1 (S.D.)% from blank urines and 84.6±2.9% from the urines which were fortified with II respectively. The last samples were prepared to check the effects of the compositions in the urine which were not contained in a blank urine on the determination.

Effect of the Unknown Substances on the Determination of II——Two unknown substances observed by GLC were not separated successfully[1] and the effects of them on the determination of II were not examined directly. However, as they were not transferred to heptane, though XV was, the sample solution for measuring the UV absorbance was extracted five times with heptane after the concentration of XV was measured by A_{263} mμ. The extraction of XV decreased the A_{263} mμ to the value obtained from a blank urine. Also, in the gas chromato-

gram, the peak of XV disappeared, whereas the peaks of the unknown were almost unchanged by the extraction (Fig. 8). These facts suggested the effects of the unknown on the A_{263} mμ were actually negligible.

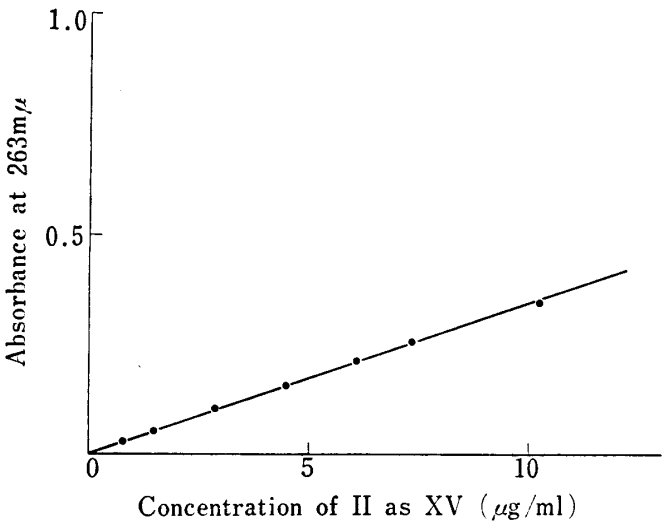

Fig. 7. Calibration Line of the Metabolite II as XV

A blank urine was added with the authentic samples of II for the determination. The average recovery was 85.0±3.1 (S.D.) %.

II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4-dione

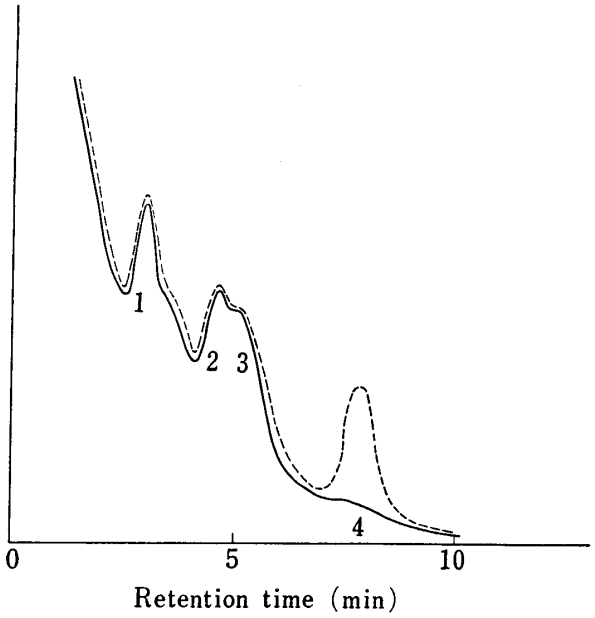

Fig. 8. Gas Chromatograms showing the Removal of XV with Heptane

-----; before extraction
——; after extraction with heptane
1; unknown 1, 2; unknown 2, 3; IV, 4; XV
The chloroform extract was treated trimethylsilylation for gas-liquid chromatography.
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano (2,3-*d*) pyrimidine-2,4,-dione

Determination-Procedures of II in a Urine by UV Absorption Method——To 50 ml centrifuge tube, are added 1—5 ml of a urine, equal volume of 6N HCl and 30 ml of heptane and they are shaken for 25 min to extract total BCP. The metabolites are extracted with 30 ml $CHCl_3$ from 1—9 ml of the residual urine by shaking it for 25 min. The $CHCl_3$ layer is washed with 3—4 ml of an aqueous solution of 50% zinc chloride by shaking it for 5—10 min. After dehydration of the extract with sodium sulfate, 20 ml of it is shaken with 15 ml of 0.1N NaOH for 20 min, then 2 ml of 12N HCl is added to 10 ml of the 0.1N NaOH and the solution is heated at 100° for 30 min. After cooling, A_{263} mμ is measured to determine the concentration of II as XV. The recovery of the determination is usually 85%, though corrections are necessary according to the blank values.

III. Examples of the Determination

To prove the applicability of the method, authentic samples of BCP, II and IV were added to a blank urine simultaneously and were determined according to the standard procedures.

As shown in Table IV, the average recoveries and the standard deviations were 101.6 ±2.98% (n=15), 86.06±5.40% (n=12) for BCP and II respectively. When the concentration was more than 13.8 μg/ml, the recovery of II was 86.42±1.53% (n=8).

The determination was also carried out on a urine collected in 6—8 hours after the administration of 900 mg of BCP to a healthy male. The average determination values (n=10) and the standard deviations were 55.86±0.58 μg/ml for BCP and 83.10±1.58 μg/ml for II.

Table IV. Recoveries of the Determination of BCP and Its Metabolites, II and IV from a Blank Urine

Exp. No.	Concentration of the authentic standards BCP (μg/ml)	II (μg/ml)	IV (μg/ml)
1	38.6	27.6	43.12
2	19.3	13.8	21.56
3	9.65	6.9	10.78

Exp. No.	Recoveries of the determination BCP (%)	II (%)	IV (%)
1	103.59±2.26[a)]	87.93±0.99[b)]	92.44±1.06[b)]
2	101.22±1.01	84.91±0.87	97.05±1.28
3	98.66±3.11	85.35±9.91	92.31±1.87
Total	101.16±2.98	86.06±5.40 (86.42±1.53)[d)]	93.93±2.64[c)]

a), *b*): the average and standard deviation, $n=5$ for *a*), $n=4$ for *b*)
c): Determination can be made by UV absorption method when authentic sample is added to a blank urine.
d): the average of the recoveries in Exp. No. 1 and 2
II: 1-cyclohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine

IV. Determination of BCP and the Metabolite II in Plasma

BCP——To adjust the pH's, equal volume of HCl (6—0.5N) or double volume of 1N acetate buffer (pH 3.4—5.8) were added to plasma which were obtained after BCP were administered to men (or rats) and BCP in the plasma were extracted with heptane at various pH's. The recoveries of the extraction were almost coincident within the pH-range (pH 3.4–6N HCl) as shown in Fig. 9.

The fraction of BCP with some kind of interaction was not observed in the plasma in contrast with the case of the urine. Thus, for the determination of BCP in plasma, the determination-procedure by Mima, *et al.*[5] can be applied, *i.e.*, in a 50 ml centrifuge tube, 1—3 ml of a plasma and equal volume of 1N HCl are added, which is extracted with 25—30 ml of heptane by shaking it for 25 min. Twenty milliliter of the heptane layer is transferred to another centrifuge tube to which is added 10 ml of 0.1M borax solution and they are shaken for 20 min. The A_{271} mμ is measured on the borax solution after centrifugation. The blank value is determined with the plasma obtained just before the administration of the drug.

The Metabolite II——When $CHCl_3$ was used for the extraction, the recoveries of II from a blank plasma were almost unchanged (98.6±0.5 (S.D.) %) within the pH-range (pH 4–6N HCl). Thus, equal volume of 1N HCl was added to a plasma for the extraction of II. The determination–procedure is as follows.

To 50 ml centrifuge tube, are added 1—3 ml of a plasma, equal volume of 1N HCl and 25—30 ml of heptane and they are shaken for 25 min to extract BCP. The metabolites are extracted with 30 ml of $CHCl_3$ from 1—5 ml of the residual plasma solution by shaking it for 25 min. Twenty milliliter of the $CHCl_3$ layer is shaken with 15 ml of 0.1N NaOH for 20 min, then 2 ml of 12N HCl is added to 10 ml of the 0.1N NaOH and the solution is heated at 100° for 30 min. After cooling, A_{263} mμ is measured to determine the concentration of II as XV.

When authentic samples of BCP, II and IV were added to a blank plasma simultaneously, the calibration lines are shown in Fig. 10.

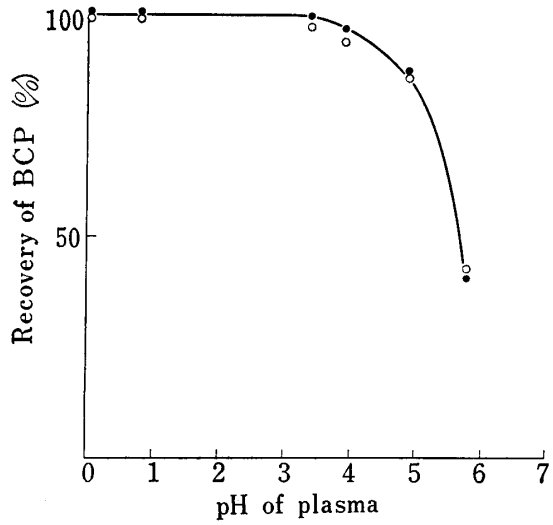

Fig. 9. Relation between pH and the Extracted Amount of BCP from Human and Rat's Plasma

○: human plasma, ●: rat's plasma
The pH's of the samples were adjusted by adding equal volume of HCl or double volume of the acetate buffer (1N AcOH–1N AcONa). The ordinate shows the relative extracted amount of BCP to the amount when 6N HCl was added.

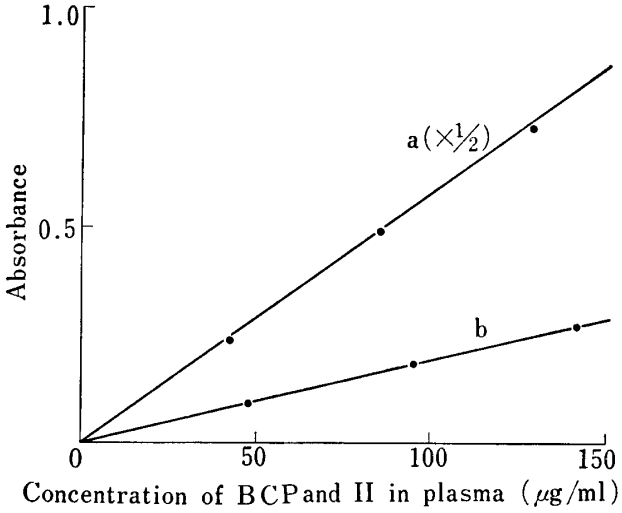

Fig. 10. Calibration Lines of BCP and II as XV from Plasma

a: BCP from A_{271} mμ; b:II as XV from A_{263} mμ
The concentrations of BCP, II and IV in the plasma were shown below respectively; 1) 128.01, 141.81 and 130.00 μg/ml; 2) 85.40, 94.55 and 86.67 μg/ml; 3) 42.67, 47.27 and 43.33 μg/ml. Two milliliter of the samples were used. The recoveries of the determination were 97.66 ± 1.26 (S.D.)% for a and 84.79±1.54 (S.D.)% for b.
II: 1-cylcohexyl-5-(3-hydroxybutyl)-2,4,6-trioxoperhydropyrimidine
IV: 5-*n*-butyl-1-(4-hydroxycyclohexyl)-2,4,6-trioxoperhydropyrimidine
XV: 3-cyclohexyl-7-methyl-1,2,3,4,6,7-hexahydro-5-*H*-pyrano(2,3-*d*) pyrimidine-2,4-dione

In the plasma, which was obtained 2—3 hours after the administration of BCP[9] to rats, 13.5 μg/ml of II was determined, though the concentration of BCP was 151.5 μg/ml. This fact showed that the concentration of II in the plasma was very low, owing probably to the rapid excretion.

On the other hand, for the determination of II by this method, more than 50 μg of II in the plasma is preferable and when the concentration is low, the volume must be increased.

9) Suspension with gum arabic (10 mg/rat).