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Determination of Phenylbutazone and Its Metabolites in Plasma by Gas-Liquid Chromatographic Procedure[1)]

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A gas-liquid chromatographic (GLC) method is described for the determination of phenylbutazone and its metabolites, oxyphenbutazone and γ-hydroxyphenylbutazone, in human or rabbit plasma following administration of phenylbutazone. A modified Herrmann's extraction method has been used and coupled with the GLC procedure without derivative formation ation for phenylbutazone and using trimethylsilylation for the metabolites. This method is accurate and sufficiently sensitive for use in routine clinical assay and the estimation of pharmacokinetic parameters of phenylbutazone and its metabolites. The utility of the procedure was corroborated by its application to the determination of phenylbutazone and its metabolites in the plasma samples obtained from rabbits and patients chronically treated with phenylbutazone.

Phenylbutazone has been widely used in the treatment of reumatoid arthritis and other inflammatory disorders. The drug is almost completely metabolized in man and animals.[3)] Two metabolites, 1-phenyl-2-(*p*-hydroxyphenyl)-3,5-dioxo-4-*n*-butylpyrazolidine (oxyphenbutazone) and 1,2-diphenyl-3,5-dioxo-4-(3-hydroxybutyl)-pyrazolidine (γ-hydroxyphenyl butazone), were isolated from the urine of human subjects receiving phenylbutazone.[4)] Oxyphenbutazone has an anti-inflammatory activity approximately equal to that of the parent drug, while γ-hydroxyphenylbutazone formed by the introduction of an alcohol group in the butyl side-chain possesses an enhanced uricosuric activity.[5,6)]

Several ultraviolet (UV) spectrophotometric methods have been developed to determine phenylbutazone in biological fluids.[3,7–13)] Burns, *et al.*[3)] extracted phenylbutazone from acidified biological material into *n*-heptane or *n*-heptane containing 3% (v/v) isoamyl alcohol, and then the phenylbutazone transferred into 2.5N sodium hydroxide solution was measured spectrophotometrically at 265 nm. This method was improved by repeated washing of

1) This work was presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April 1972.
2) Location: *a) Hongo, Bunkyo-ku, Tokyo; b) Yayoi-cho, Chiba.*
3) J.J. Burns, R.K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **109**, 346 (1953).
4) J.J. Burns, R.K. Rose, S. Goodwin, J. Reichenthal, E.C. Horning, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **113**, 481 (1955).
5) T.F. Yü, J.J. Burns, B.C. Paton, A.B. Gutman, and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **123**, 63 (1958).
6) G. Wilhelmi, *Arzneim-Forsch.*, **10**, 129 (1960).
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8) W. Fuchs, *Münch. Med. Wschr.*, **107**, 1267 (1965).
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11) G. Stajer, E. Vinkler, and A.E. Szabo, *Pharmazie*, **25**, 126 (1970).
12) G.R. van Petten, H. Feng, R.J. Withey, and H.F. Lettau, *J. Clin. Pharmacol. New Drugs*, **11**, 177 (1971).
13) E. Jähnchen and G. Levy, *Clin. Chem.*, **18**, 984 (1972).

the organic phase with phosphate buffer (pH 8.0) to remove most of its metabolites which interfered with the assay.[7,12] However, these methods still suffer from a lack of specificity in the presence of other neutral or acidic drugs extracted into the organic phase. Oxyphenbutazone produced from phenylbutazone was detected for prolonged time in human blood after administration of phenylbutazone.[4] On the other hand, the blood concentration of γ-hydroxyphenylbutazone was presumed to be negligible as compared with that of oxyphenbutazone,[7] since γ-hydroxyphenylbutazone disappeared considerably faster than oxyphenbutazone.[4] This is supported by the fact that the plasma concentrations of oxyphenbutazone decreased slowly with a biological half-life averaging 3 days, which is similar to that reported for phenylbutazone, and in contrast, those of γ-hydroxyphenylbutazone decreased relatively rapidly with a biological half-life averaging 10 hr in the same human subject.[5] Herrmann[7] showed that the serum concentration of oxyphenbutazone could attain to that of phenylbutazone on prolonged therapy and the average serum concentration of oxyphenbutazone was approximately half that of phenylbutazone in five patients. To determine the serum concentration of oxyphenbutazone in the presence of phenylbutazone, Herrmann used the improved UV spectrophotometric method. In the improved procedure, the absorbance of 2.5 N sodium hydroxide solution obtained after repeated washing of the organic phase with phosphate buffer (pH 8.0) was subtracted from the absorbance of 2.5 N sodium hydroxide solution obtained without the washing of the organic phase. The difference was assumed to be practically equivalent to the serum or plasma concentration of oxyphenbutazone, although the difference is virtually the sum of absorbances of oxyphenbutazone, γ-hydroxyphenylbutazone and other interfering substances.

Wallace[9] described the specific spectrophotometric determination of phenylbutazone by oxidation to azobenzene with permanganate. This method was adapted for small volumes of blood samples (0.1—0.5 ml) by Jähnchen and Levy.[13] They determined the time-course of phenylbutazone concentrations for the same plasma samples after an intravenous injection of 50 mg/kg to rats by both this oxidation method and the method of Burns, *et al.*[3] The data obtained by both of these methods were similar initially, but the latter method yielded appreciably higher concentration values after 10 hr. This finding indicates the lack of specificity in the latter UV spectrophotometric method. However, any γ-hydroxyphenylbutazone present can not be distinguished from the parent drug even by this oxidation method, although oxyphenbutazone does not interfere with this assay. Jähnchen and Levy[13] claimed in this regard that γ-hydroxyphenylbutazone was unlikely to interfere with the assay under ordinary conditions, since an absorbance of γ-hydroxyphenylbutazone was about 30% that of an equal concentration of phenylbutazone and the plasma concentration of γ-hydroxyphenylbutazone was presumed to be negligible after administration of phenylbutazone.[14]

Recently, the gas-liquid chromatographic (GLC) analyses of phenylbutazone alone[16,17] or phenylbutazone and oxyphenbutazone[18] in biological fluids have been reported. There is, however, no published GLC method which determined both phenylbutazone and its two known metabolites, oxyphenbutazone and γ-hydroxyphenylbutazone. It is desirable to have a more sensitive and specific method for pharmacokinetic studies and monitoring of the plasma concentration of phenylbutazone in clinical situations.

14) During the preparation of this manuscript, it was found that Bakke, *et al.*[15] have carried out the study on the metabolism of phenylbutazone in the rat using ^{14}C-phenylbutazone and that unchanged phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone were estimated to be 55, 25, and 12% of the extractable activity by the TLC of extracts with 1,2-dichloroethane from pooled plasma at 6 hr after administration of 50 mg/kg of ^{14}C-phenylbutazone. This datum indicates the fact that the plasma concentration of γ-hydroxyphenylbutazone is not negligibly small.

15) O.M. Bakke, G.H. Draffan, and D.S. Davies, *Xenobiotica*, **4**, 237 (1974).

16) R. Perego, E. Martinelli, and P.C. Vanoni, *J. Chromatog.*, **54**, 280 (1971).

17) I.J. McGilveray, K.K. Midha, R. Brien, and L. Wilson, *J. Chromatog.*, **89**, 17 (1974).

18) R.B. Bruce, W.R. Maynard, and L.K. Dunning, *J. Pharm. Sci.*, **63**, 446 (1974).

The purposes of the present study are to describe a GLC method for determining phenylbutazone and its two known metabolites in plasma following administration of phenylbutazone, and to clarify the extent to which oxyphenbutazone and γ-hydroxyphenylbutazone produced from phenylbutazone accumulate in patients during chronic administration and in the rabbit after single administration in order to further our understanding of their roles in the total observable drug effect.

Experimental

Apparatus——A Hitachi Model F-6 gas chromatograph equipped with a flame ionization detector and a Hitachi Model QPD-33 recorder were employed in this study. The chromatographic column consisted of stainless steel tubing (2.5 m × 3 mm i.d.) prepacked with Gas-Chrom Q (60—80 mesh) coated with 3% polysulfone (Gaschro Kogyo Co., Tokyo). Prior to use, the packed column was conditioned under nitrogen flow for 20 hr at 300°. The column was maintained isothermally at 260° with both the detector and injection port at 300°. The efficiency of the column was conditioned each day at the end of determination by injecting about 30 μl of Silrest.[19] Nitrogen gas was used as a carrier at a flow rate of 32 ml/min (inlet pressure of 1.6 kg/cm^2), and hydrogen and air flow rates were adjusted to give a maximal response. The electrometer sensitivity ranged between 2.5×10^{-9} and 2.5×10^{-10} A for full scale deflection, and the recorder speed was set at 4 cm/min.

Materials——Phenylbutazone and oxyphenbutazone were recrystallized from methanol and ether-petroleum ether, respectively, and stored in vials under nitrogen to prevent atomospheric oxidation.[20a] Sodium phenylbutazone was prepared by addition of equimolar NaOH dissolved in methanol to the phenylbutazone. The solvent was evaporated to dryness under reduced pressure, and the product was kept in a desiccator. Sodium phenylbutazone thus obtained was estimated to have two molecules of water of crystallization by comparing its absorbance at 265 nm with that of phenylbutazone. The side-chain hydroxylated phenylbutazone, γ-hydroxyphenylbutazone, was prepared from ketophenbutazone[20b] by the method of Denss, *et al.*[21] Recrystallization of the product from ethanol gave the lactone form of γ-hydroxyphenylbutazone (δ-caprolactone α-carbonic acid-N,N'-diphenylhydrazide), mp 181—184°. *Anal.* Calcd. for $C_{19}H_{20}O_3N_2 \cdot C_2H_5OH$: C, 68.09; H, 7.07; N, 7.56. Found: C, 68.04; H, 7.06; N, 7.59.[22] The lactone is very hygroscopic and easily hydrolyzed to γ-hydroxyphenylbutazone in alkaline solution.[21] It exists as the enolate form of γ-hydroxyphenylbutazone in alkaline solution. γ-Hydroxyphenylbutazone is converted into the lactone on standing in a closed container for several months. The lactone was used in this study for convenience, since it showed the same retention time as that of γ-hydroxyphenylbutazone after trimethylsilylation under the GLC conditions described in the "Apparatus" section. N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and *n*-heptane (special grade for spectrophotometry) were obtained from Tokyo Kasei Kogyo Co., Tokyo. All other solvents and reagents used were reagent grade and required no further purification.

Internal Standard Solution——Fluoranthene (4 μg/ml) in *n*-heptane was used as an internal standard for the assay of phenylbutazone and its metabolites. Fluoranthene is stable in organic solvents, does not react with BSTFA and gives a symmetrical peak which has retention time of 4.3 min under the GLC conditions described above.

Extraction of Phenylbutazone and Its Metabolites from Plasma——Phenylbutazone and its metabolites from human or rabbit plasma were extracted according to the minor modification of the method of Herrmann.[7] One milliliter of plasma was added to 3 ml of 0.1N NaOH in a 50 ml glass-stoppered centrifuge tube, and then acidified by adding 0.5 ml of 5N HCl. The mixture was allowed to stand at room temperature for 10 min to permit the liberation of drug bound to plasma proteins. After addition of 20 ml of 1,2-dichloroethane (EDC), the mixture was shaken with a mechanical shaker for 30 min and centrifuged at 3000 rpm for 5 min. As much of the upper aqueous phase as possible was then aspirated, and 18 ml of the EDC phase was transferred into a clean glass-stoppered centrifuge tube containing 12 ml of 0.067M phosphate buffer solution at pH 8.0. The mixture was shaken for 10 min and centrifuged as before. The buffer washing was repeated further twice with 10 ml of the phosphate buffer solution. Ten milliliters from each of the

19) Column conditioner for GLC obtained from Tokyo Kasei Kogyo Co., Tokyo.

20) *a*) Phenylbutazone and oxyphenbutazone were kindly supplied from Fujisawa Pharmaceutical Industries, Osaka; *b*) Ketophenbutazone was kindly supplied from Kyowa Hakko Kogyo Co., Tokyo.

21) R. Denss, F. Häfliger, and S. Goodwin, *Helv. Chim. Acta*, **40**, 402 (1957).

22) The lactone is recrystallized as a structure including one molecule of crystallization solvent from ethanol, acetone or benzene. This fact was confirmed by infrared (IR), nuclear magnetic resonance (NMR), and the elementary analysis. Thus, it can be presumed that the lactones in α- and β-forms reported by Denss, *et al.*[21] corresponded to those containing one molecule of ethanol and acetone as crystallization solvent, respectively.

three buffer washing were combined in another centrifuge tube. The residual EDC phase and the combined buffer solution were used for analyses of phenylbutazone and the metabolites, respectively.

Analysis of Phenylbutazone——After removing the remaining buffer phase, 17 ml of the EDC phase was transferred into a glass-stoppered centrifuge tube containing 11 ml of 0.1N NaOH. The mixture was shaken for 10 min and centrifuged. Ten milliliters of the NaOH extract was acidified by adding 2 ml of 5N HCl, and then 11 ml of *n*-heptane was added. Again the mixture was shaken for 10 min and centrifuged. Five to 10 ml of the *n*-heptane extract was transferred into a 25 ml round-bottom flask containing 2 ml of the internal standard solution. The mixture was evaporated to dryness on a rotary-vacuum evaporator with the flask immersed in a water bath lower than 20°. The flask was returned to atomospheric pressure from vacuum by giving dry nitrogen. The residue was transferred into a clean test tube of 4.5 cm in length and 6 mm in inside diameter by washing twice the flask with about 0.3 ml of EDC, and the EDC extract was evaporated to dryness. The test tube was closed with a silicone-rubber stopper after blowing dry nitrogen. Then, 30 μl of chloroform was added to the dry residue. Approximately 1—3 μl of the solution was then injected onto the gas chromatographic column, and the peak area ratio of phenylbutazone to the internal standard was calculated.

Analyses of Metabolites——The combined buffer solution (30 ml) was acidified with 2 ml of 5N HCl, shaken with 11 ml of EDC for 15 min and centrifuged. Ten milliliters of the EDC phase was transferred into a 25 ml round-bottom flask containing 2 ml of the internal standard solution. The mixture was evaporated to dryness in the same manner as described under "Analysis of Phenylbutazone." The dry residue in the test tube was dissolved into 10 μl of chloroform dried with addition of an anhydrous sodium sulphate, and 30 μl of BSTFA was added. After the tube was allowed to stand at room temperature for 1 hr, approximately 1—3 μl of the solution was injected directly onto the gas chromatographic column. The peaks of trimethylsilylated γ-hydroxyphenylbutazone and oxyphenbutazone have retention times of approximately 5.8 and 7.7 min, respectively. The peak area ratio of oxyphenbutazone to the internal standard was calculated.

On the other hand, the peak of γ-hydroxyphenylbutazone at retention time of 5.8 min was affected by phenylbutazone transferred into the buffer solution. To investigate the interference of phenylbutazone with the assay of γ-hydroxyphenylbutazone, a series of plasma samples at various concentrations (5, 10, 25, 50, 100, 125, 150, and 200 μg/ml) of phenylbutazone was determined after the extraction and the trimethylsilylation with BSTFA as described above. It was found that phenylbutazone at lower concentrations than 30 μg/ml did not interfere with the assay of γ-hydroxyphenylbutazone. However, the area ratio of phenylbutazone (AR_{PB}), which added to the area ratio of γ-hydroxyphenylbutazone to the internal standard, was given at higher concentrations of phenylbutazone (C_{PB}, μg/ml) than 30 μg/ml by:

$$AR_{PB} = 0.001C_{PB} - 0.029 \quad (1)$$

The corrected area ratio of γ-hydroxyphenylbutazone to the internal standard ($AR_{\gamma\text{-OHPB}}$), therefore, may be calculated by:

$$AR_{\gamma\text{-OHPB}} = \text{apparent } AR_{\gamma\text{-OHPB}} - AR_{PB} \quad (2)$$

Where, the apparent $AR_{\gamma\text{-OHPB}}$ is a ratio of the peak area at a retention time of 5.8 min to that of the internal standard.

Calibration Curves——A series of samples containing various quantities of phenylbutazone, oxyphenbutazone and the lactone form of γ-hydroxyphenylbutazone was prepared by dissolving each compound in 0.1N NaOH at approximate concentrations and by adding 1 ml of each solution to 1 ml of human or rabbit plasma (μg phenylbutazone/μg oxyphenbutazone/μg γ-hydroxyphenylbutazone: 0/0/0, 200/5/5, 150/10/10, 125/20/20, 100/35/35, 75/50/50, 25/35/35, 12/20/20, 6/10/10, and 3/5/5). The plasma samples were then assayed according to the procedure as described above. The corrected peak area ratio of γ-hydroxyphenylbutazone and the peak area ratio of phenylbutazone and oxyphenbutazone to the internal standard were plotted against the concentration of each compound. To establish the reproducibility of the GLC system the calibration curves were prepared each day prior to analysis.

Sample Collection——Blood samples were drawn into a heparinized syringe from an antecubital vein in patients and from a marginal ear vein in rabbits. Plasma samples were obtained by centrifugation at 3000 rpm for 15 min and either analyzed immediately or stored at 4°.

Results and Discussion

Assay of Phenylbutazone and Its Metabolites in Plasma

Oxyphenbutazone and γ-hydroxyphenylbutazone could not be chromatographed directly without trimethylsilylation by GLC. Various silylating reagents (N,O-bis-trimethylsilylacetamide, hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine, N-

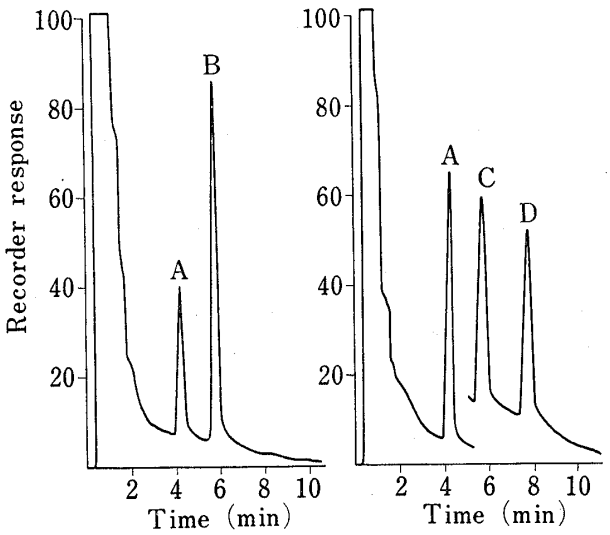

Fig. 1. Chromatograms of Plasma Extract Taken from a Patient during Chronic Treatment with Phenylbutazone

peak A: internal standard (fluoranthene)
B: phenylbutazone
C: trimethylsilylated γ-hydroxyphenylbutazone
D: trimethylsilylated oxyphenbutazone
conditions: 3% Polysulfone on Gas-Chrom Q, 2.5m×3 mm i.d., column temperature 260°, N_2 32 ml/min

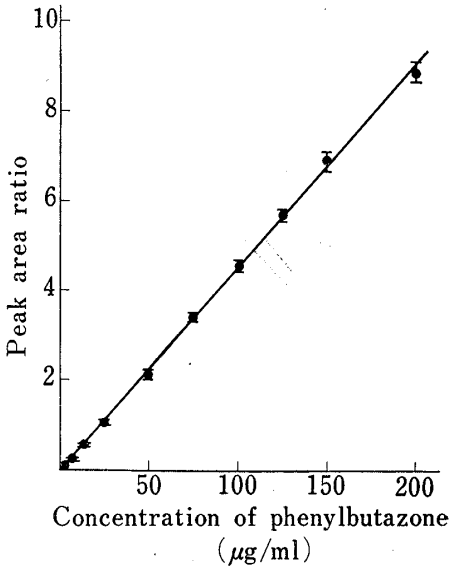

Fig. 2. Standard Calibration Curve for Phenylbutazone Added to Human Plasma

Each point represents the mean of five determinations. Vertical bars indicate standard deviations. The peak area ratio of phenylbutazone to the internal standard (fluoranthene) is plotted as ordinate.

trimethylsilylimidazole, and BSTFA) were used, and BSTFA was chosen as the best silylating reagent since it produced the smallest solvent front under the GLC conditions. Fluoranthene was used as an internal standard for the analyses of phenylbutazone and its metabolites, and it is free from trimethylsilylation by BSTFA. Typical chromatograms of phenylbutazone and its metabolites extracted from human plasma are shown in Fig. 1. The peaks of the internal standard and phenylbutazone are good symmetrical peaks and have retention times of approximately 4.3 and 5.8 min, respectively. The peaks due to the trimethylsilylation of γ-hydroxyphenylbutazone and oxyphenbutazone are nearly symmetrical with slight tailing and have retention times of approximately 5.8 and 7.7 min, respectively. The peak area ratios of oxyphenbutazone and γ-hydroxyphenylbutazone to the internal standard were found to reach the respective maximum values within 30 min at room temperature after addition of BSTFA and remained constant for 7 hr. The retention time of peak of phenylbutazone was the same as that of the trimethylsilyl derivative of γ-hydroxyphenylbutazone. The peak of phenylbutazone, however, was not interfered by γ-hydroxyphenylbutazone, since γ-hydroxyphenylbutazone could not be chromatographed directly without prior trimethylsilylation.

Phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone were added to human or rabbit plasma at various concentrations and were determined using the procedure involving two GLC injections described in the Experimental. A blank human or rabbit plasma showed no peak even at the electrometer attenuation used for the highest sensitive analysis. Thus, injection of the sample onto the column could be repeated every 8 min for phenylbutazone and every 10 min for the metabolites. The corrected peak area ratio of γ-hydroxyphenylbutazone and the peak area ratios of phenylbutazone and oxyphenbutazone to the internal standard were calculated and plotted against the concentration of each compound. Plots of peak area ratio *versus* concentration gave straight line graphs over the range of 6 to 200 μg/ml for phenylbutazone and also over the range of 5 to 50 μg/ml for oxyphenbutazone and γ-hydroxyphenylbutazone (Fig. 2 and 3). The calibration curve of phenylbutazone passes nearly through the origin, but those of metanolites have an intercept of approximately 1 μg/ml

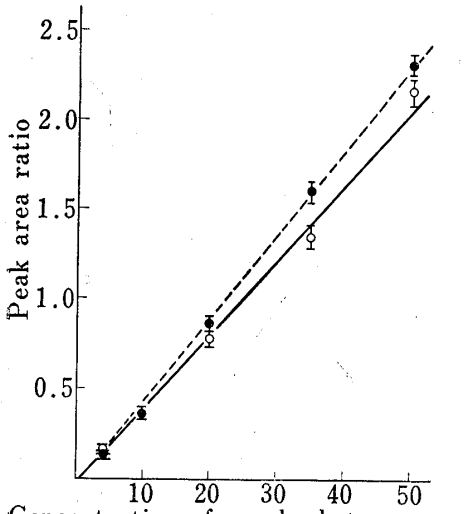

Fig. 3. Standard Calibration Curves for Oxyphenbutazone and γ-Hydroxyphenylbutazone Added to Human Plasma

—○—: oxyphenbutazone, ---●---: γ-hydroxyphenylbutazone.
Each point represents the mean of five determinations. Vertical bars indicate standard deviations. The peak area ratio of trimethylsilylated oxyphenbutazone or γ-hydroxyphenylbutazone to the internal standard (fluoranthene) is plotted as ordinate.

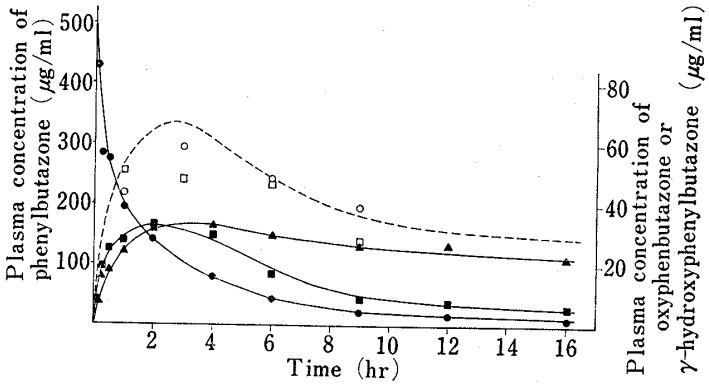

Fig. 4. Plasma Concentrations of Phenylbutazone and Its Metabolites after Single Intravenous Administration of Sodium Phenylbutazone (50 mg/kg as Phenylbutazone) in a Rabbit

—●—: phenylbutazone, —■—: oxyphenbutazone, —▲—: γ-hydroxyphenylbutazone
The dotted line shows the sum of plasma concentrations of oxyphenbutazone and γ-hydroxyphenylbutazone, and the plots (○ and □) show the serum concentrations of oxyphenbutazone determined by the Herrmann's improved UV spectrophotometric method taken from Reference 7.

TABLE I. Reproducibility of the Analyses of Phenylbutazone, Oxyphenbutazone, and γ-Hydroxyphenylbutazone in Rabbit Plasma following Intravenous Administration of Sodium Phenylbutazone

Sampling time (hr)	Plasma concentration (μg/ml)[a]					
	Phenylbutazone mean±S.D.	C.V. %	Oxyphenbutazone mean±S.D.	C.V. %	γ-Hydroxyphenylbutazone mean±S.D.	C.V. %
0.5	193.1±2.8	1.5	12.5±0.6	4.5	15.0±0.7	4.8
3.0	50.2±0.8	1.6	16.7±0.6	3.4	19.8±1.1	5.5
7.0	20.2±0.7	3.5	6.1±0.5	7.3	13.1±0.7	5.5

a) Mean±standard deviation (S.D.) and coefficient of variation (C.V.) for six determinations measured on three different days

on the axis of abscissa. In order to minimize loss by adsorption onto the solid phase of column, a concentrated solution of phenylbutazone, its metabolites trimethylsilylated and the internal standard was injected repeatedly prior to determination.

Reproducibility Studies

To illustrate the reproducibility of the GLC method described in this study, a male rabbit weighing approximately 3 kg was intravenously administered a single dose of sodium phenylbutazone (50 mg/kg as phenylbutazone). Approximately 15 ml of whole blood was drawn by heart puncture at three different time periods after administration. Two 1 ml aliquots from the plasma sample at each sampling time were analyzed on three different days. Initial duplicate determinations were immediately made after sampling. The remaining samples were kept on a refrigerator after being flushed with nitrogen and determined in two successive

days. The results are shown in Table I. The mean coefficients of variation for phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone were calculated to be 2.2, 5.1, and 5.3%, respectively.

Plasma Concentrations in Rabbit

The plasma concentrations of phenylbutazone and its metabolites were determined in an adult male rabbit following intravenous administration of sodium phenylbutazone (50 mg/kg as phenylbutazone). Blood samples were taken prior to administration and thereafter at 5, 15, 30, and 45 min, and 1, 2, 4, 6, 9, 12, and 16 hr after administration. The plasma concentrations of phenylbutazone and its metabolites against time are shown in Fig. 4. The plasma concentrations of phenylbutazone declined with a biological half-life of 2.7 hr and the metabolites were detected within 5 min after administration. The maximal plasma concentrations of oxyphenbutazone and γ-hydroxyphenylbutazone were shown to be 31.8 μg/ml at 2 hr and 33.7 μg/ml at 4 hr, respectively. The plasma concentration of the former fell to 5.9 μg/ml at 16 hr, but that of the latter showed a high value of 22.6 μg/ml even at 16 hr. The plasma concentration of γ-hydroxyphenylbutazone became higher than that of oxyphenbutazone with time, although the former was comparable to the latter during the initial 4 hr after administration. The serum concentrations of oxyphenbutazone in the same dose as reported by Herrmann[7] are considerably higher than the plasma concentrations of oxyphenbutazone in our study (Fig. 4). However, the sum of oxyphenbutazone and γ-hydroxyphenylbutazone concentrations at various times after administration are shown to be nearly equal to the oxyphenbutazone concentrations reported by Herrmann.[7] This fact indicates that the determination of oxyphenbutazone by the Herrmann's UV spectrophotometric method leads to a considerable error in the rabbit.

Plasma Concentrations in Patients

Plasma concentrations of phenylbutazone and its metabolites were determined in patients therapeutic doses (200—400 mg/day) of Butazolidin® for periods longer than two weeks. The results are shown in Table II. The plasma concentrations of phenylbutazone ranged from 48.5 to 105.7 μg/ml, while those of oxyphenbutazone and γ-hydroxyphenylbutazone ranged from 3.1 to 17.6 μg/ml and 4.7 to 19.5 μg/ml, respectively. The plasma concentrations of phenylbutazone in the patients were found to be almost the same therapeutic ranges as

Table II. Plasma Concentrations of Phenylbutazone, Oxyphenbutazone and γ-Hydroxyphenylbutazone in Patients Receiving Chronic Oral Doses of Phenylbutazone[a]

Patient	Daily dose (mg)	Plasma concentration (μg/ml)		
		Phenylbutazone	Oxyphenbutazone	γ-Hydroxyphenylbutazone
JU	200	53.2	7.3	12.3
TW	300	67.8	17.6	15.2
TI	300	74.6	11.4	7.0
MF	300	82.6	15.6	4.7
YJ	300	92.2	14.4	5.2
HK	300	105.7	10.9	10.9
MT	300	99.9	16.7	12.3
KW	300	76.3	3.1	7.8
KT	300	48.5	5.8	7.3
MK[b]	300	50.9	6.5	7.0
MK[c]	400	56.2	6.3	19.5

a) The patients had been received Butazolidin® for periods longer than two weeks.
b) determined on January 31, 1972
c) determined on March 20, 1972 after the daily dose of the above patient (MK) had been increased to 400 mg

those suggested by other workers.[23] The plasma concentrations of oxyphenbutazone and γ-hydroxyphenylbutazone were much lower than those of phenylbutazone. Herrmann[7] showed that the serum concentration of oxyphenbutazone in patients could attain therapeutically active levels during chronic administration of phenylbutazone. This finding suggests that part of the anti-inflammatory activity of phenylbutazone may be attributed to the presence of oxyphenbutazone. However, this question seems to be not of great practical importance, since the plasma concentrations of oxyphenbutazone determined by the specific GLC method in our study were shown to be significantly lower than those in the Herrmann's report[7] and since the parent drug and oxyphenbutazone had the same intensity of action.[24]

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