(5) V. Cody, W. L. Duax, K. Yasuda, Y. Osawa, and D. A. Noeton, *ibid.*, **28**, 5683 (1972).
(6) S. R. Byrn, C. W. Graber, and S. L. Midland, *J. Org. Chem.*, **41**, 2283 (1976).
(7) G. R. Parker, N. K. Hilgendorf, and J. G. Lindberg, *J. Pharm. Sci.*, **65**, 585 (1976).
(8) G. R. Parker and E. C. Moore, *ibid.*, **66**, 1040 (1977).
(9) W. Dresler and R. Stein, *Justus Liebigs Ann. Chem.*, **150**, 242 (1869).
(10) R. E. Harmon, J. C. Dabrowiak, D. J. Brown, S. K. Gupta, M. Herbert, and D. Chitharanjan, *J. Med. Chem.*, **13**, 577 (1970).
(11) G. Clifton, S. R. Bryant, and C. G. Skinner, *ibid.*, **13**, 377 (1970).
(12) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr.*, **A27**, 368 (1971).
(13) I. K. Larsen and B. Jerslev, *Acta Chem. Scand.*, **20**, 983 (1966).
(14) H. Berman and S. H. Kim, *Acta Crystallogr.*, **23**, 180 (1967).
(15) N. Armagan, J. P. G. Richards, and A. A. Uraz, *ibid.*, **B32**, 1042 (1976).
(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed., Cornell University Press, Ithaca, N.Y., 1960, p. 281.
(17) N. L. Alpert, W. E. Keiser, and H. A. Szymanski, "The Theory and Practice of Infrared Spectroscopy," Plenum, New York, N.Y., 1970, p. 281.
(18) R. H. Adamson, *Proc. Soc. Exp. Biol. Med.*, **119**, 456 (1965).

ACKNOWLEDGMENTS

The authors thank Miss Barbara Stearns, Squibb Institute of Medical Research, for supplying the hydroxyurea.

GLC Determination of Phenylbutazone in Human Plasma

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Received March 1, 1977, from *Ciba-Geigy Biopharmaceutical Research Center, 92506 Rueil-Malmaison Cedex, France.* Accepted for publication May 11, 1977.

Abstract □ A GLC method for phenylbutazone at concentrations down to 10 ng/ml in human plasma is described. After addition of an internal standard, phenylbutazone is extracted at pH 5 into benzene. The dry extract is dissolved in benzene, and phenylbutazone is determined by GLC using a ^{63}Ni-electron-capture detector.

Keyphrases □ Phenylbutazone—GLC analysis in human plasma □ GLC—analysis, phenylbutazone in human plasma □ Antirheumatic agents—phenylbutazone, GLC analysis in human plasma

Many methods have been proposed for the quantitative assay of phenylbutazone[1] (I) in biological fluids. The first UV method (1, 2) (extraction of phenylbutazone with heptane in acidic medium) was modified (3, 4) and automated (5), for the serial determination of phenylbutazone in human plasma. The UV determination of phenylbutazone after oxidation to azobenzene by alkaline permanganate was described (6) and applied to small-volume samples (7). The method of Burns (1, 2) was also applied to 1 drop of capillary blood (8). Stevens (9) determined phenylbutazone by UV absorption after fast extraction in hexane from an acidic medium.

Several investigators studied the determination of phenylbutazone by GLC. McGilveray *et al.* (10) described a procedure using 1 *N* HCl and heptane for extraction and diphenyl phthalate as the external standard. Midha *et al.* (11) published an elaborated method for determining phenylbutazone and oxyphenbutazone. These two compounds were derivatized by flash-heater methylation with trimethylanilinium hydroxide, but each yielded two peaks.

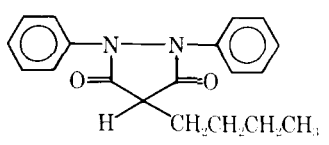
A GLC technique was also used to determine phenylbutazone and oxyphenbutazone in the plasma and urine of horses and dogs (12). The extraction was performed with 2 *N* H_2SO_4 and 3 × 20 ml of benzene. Phenylbutazone was assayed by GLC without derivatization, but oxyphenbutazone was separated as its heptafluorobutyryl derivative.

Tanimura *et al.* (13) determined phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone in human or rabbit plasma, using fluoranthene as the internal standard and *N,O*-bis(trimethylsilyl)trifluoroacetamide as the silylating reagent. A GLC method in rat serum or urine, using promethazine as the external standard, was also described (14). The retention times were between 15 and 18 min. This method was checked for its specificity. Finally, a high-pressure liquid chromatographic (HPLC) technique was recently reported (15).

The GLC method described here is simple and fast, and it specifically determines phenylbutazone in human plasma with a high sensitivity. A phenylbutazone analog, 4-butyl-1,2-bis(*p*-tolyl)-3,5-pyrazolidine (II), is used as the internal standard.

EXPERIMENTAL

Reagents—A pH 5 buffer[2] was prepared by diluting the contents of seven vials with 1000 ml of water. Benzene was analytical grade[3]. The



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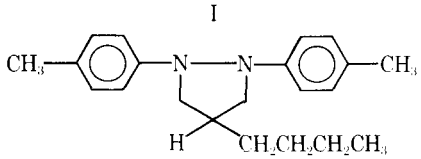

II

[1] Active ingredient of Butazolidin (Geigy).

[2] Titrisol, Merck.
[3] Mallinckrodt.

Table I—Precision and Accuracy of the Assay Applied to Spiked Human Plasma Samples (1 ml and 100 μl)

Amount Added, ng/ml	Amount Found[a]		CV, %	
	1 ml	100 μl	1 ml	100 μl
10	10	—	3.1	—
25	25	—	5.7	—
50	51[b]	—	2.6	—
100	—	100	—	6.2
150	150	—	1.1	—
500	509	503	2.2	2.6
1,000	988	—	2.5	—
1,500	—	1,515	—	1.5
5,000	4914	4,769	3.3	1.0
10,000	—	10,214	—	4.4
25,000	—	25,069	—	3.6
50,000	—	50,389	—	1.0

[a] Average of six assays. [b] Four assays only.

benzene internal standard solution contained 1 μg of II[4]/ml.

Materials—The glassware was dried at 100° and immersed for 0.5 hr in an ultrasonic bath, first in water and then in methanol. A gas chromatograph[5] equipped with a linear 15-mCi ^{63}Ni-electron-capture detector[6] was used.

The peak areas were given by an electronic integrator[7]. The column was operated at 250°, the injector was at 300°, and the detector was at 300° with an argon–methane (90:10) flow rate of 70 ml/min. Glass columns were washed with 1 *N* HCl, distilled water, acetone, and benzene and then silanized with a 1% (v/v) solution of hexamethyldisilazane in benzene.

After this treatment, the columns were washed again with benzene and dried at 100°. The column packing was 5% OV-17[8] on 80–100-mesh Chromosorb WHP[8]. The filled column was flushed with the carrier gas at 50° for 30 min and then gradually heated to 300° at 1°/min with a flow rate of 30 ml/min. The column temperature was held overnight at 300°. The temperature was then repeatedly increased at 8°/min from 150 to 300° over 24 hr. During the five last cycles, 20 μl of silylating reagent[9] was injected between 150 and 220°.

Extraction—A 500-μl aliquot of the internal standard solution was added to a stoppered glass tube, and the solution was taken to dryness under a nitrogen stream. Then 100 μl of the sample, 1 ml of pH 5 buffer, and 4 ml of benzene were introduced in the tube; the tube was shaken mechanically[10] for 20 min at 400 rpm and centrifuged at 5000 rpm for 10 min.

An aliquot volume of the benzene phase was transferred to another tube and taken to dryness under a nitrogen stream in a water bath at 37°.

GLC—Benzene, 500 μl, was added to the dry residue, and the tube was shaken on a mixer[11]. A 3-μl portion of the benzene solution was then injected into the gas chromatograph using the solvent-flush technique.

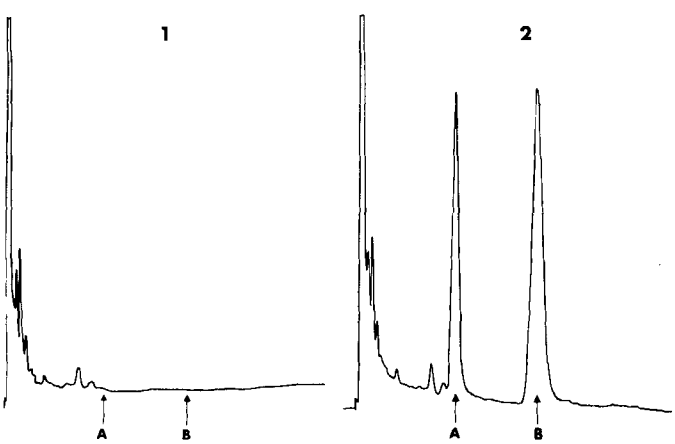

Figure 1—*Chromatograms of a human plasma blank (1) and 150 ng of phenylbutazone/ml (A) and 500 ng of internal standard/ml (B) in human plasma (2).*

[4] Ciba-Geigy, Basel, Switzerland.
[5] Hewlett-Packard 5710 A.
[6] Hewlett-Packard 18 713 A.
[7] Hewlett-Packard 3380 A.
[8] Applied Science Laboratories.
[9] Silyl 8, Pierce.
[10] Infors.

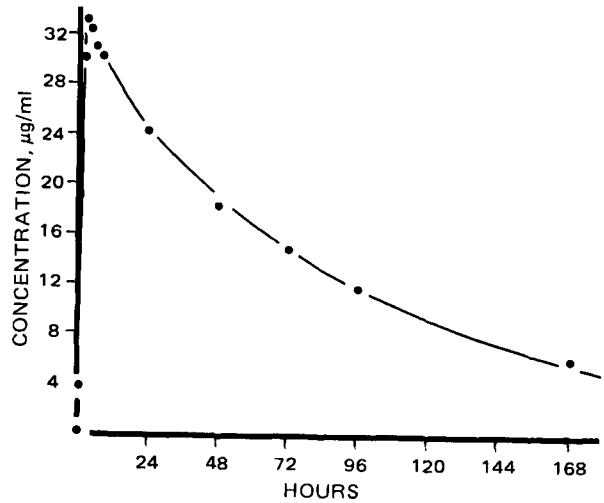

Figure 2—*Plasma phenylbutazone concentrations after oral administration of 300 mg (mean of six subjects).*

The phenylbutazone content was calculated from the peak area ratio by reference to a calibration curve. This curve was plotted on the basis of a benzene solution containing 1 μg of I/ml. Aliquot parts of this solution were taken to dryness, and plasma was added to yield plasma solutions containing 100–50,000 ng/ml.

The phenylbutazone and internal standard solutions were stable for 1 week at 4°. After 5–7 days, there was a progressive change in the peak area ratio of the two compounds, indicating some degradation.

Experiment in Humans—Six healthy volunteers were given 300 mg of phenylbutazone in a cachet. Blood samples were withdrawn 0.5, 1, 2, 3, 4, 6, 8, 24, 48, 72, 96, and 168 hr after administration, transferred to heparinized tubes, and centrifuged immediately. Plasma was removed and stored at −20° until analysis.

RESULTS AND DISCUSSION

Analytical Yield—Extraction was studied at several acidic pH values with a 1-μg/ml plasma ^{14}C-phenylbutazone solution. Acidic pH solutions were: 2 *N* HCl; 1 *N* HCl; and pH 1, 2, 3, 4, 5, and 6 buffers (seven vials[2] diluted with 1000 ml of water). At all of these pH values, phenylbutazone recovery was 88 ± 6% (average of five assays). When the plasma was simply diluted with distilled water, the pH was 7.2 and the average extraction yield of five assays was 50 ± 4%.

Phenylbutazone is a carbon acid with a pKa of 4.5–4.7 (16), and it may appear surprising that it is extracted with a high recovery at pH 5. Stella (17) showed that phenylbutazone has a hindered dissolution at pH values greater than its pKa because of noninstantaneous ionization kinetics.

Sensitivity and Accuracy—Table I gives the results obtained when the described procedure was applied to spiked plasma samples of different volumes.

The coefficients of variation were calculated on the basis of six replicate analyses of each sample. With a 100-μl sample, concentrations down to 100 ng/ml can be determined accurately. If a 1-ml sample is used, the assay limit is lowered to 10 ng/ml, and even smaller concentrations can be detected.

Phenylbutazone and the internal standard do not contain functional groups commonly associated with high electron-capture ability. Nevertheless, both are well detected.

Plasma Interference—Figure 1 shows the chromatograms of a human plasma extract and of the same plasma spiked with 150 ng of phenylbutazone and 500 ng of the internal standard. No interference by the normal plasma components was recorded.

Specificity—Three unconjugated metabolites of phenylbutazone have been described (18): oxyphenbutazone (III), γ-hydroxyphenylbutazone (IV), and *p*,γ-dihydroxyphenylbutazone (V). These metabolites account for 23, 2, and 0.5%, respectively, of the total radioactivity in the plasma after administration of ^{14}C-labeled drug.

The proposed technique was applied to plasma solutions containing

[11] Vortex.

50 μg of III/ml and 5 μg of IV and V/ml. These concentrations greatly exceed those expected in plasma. None of the three metabolites was recorded.

Application—The technique was applied to study phenylbutazone elimination after oral administration to humans. The mean plasma phenylbutazone concentration–time curve is depicted in Fig. 2. The mean elimination half-life was 68.5 hr.

The technique has been applied in studying the pharmacokinetics of phenylbutazone in humans after the administration of single and multiple doses[12].

REFERENCES

(1) J. J. Burns, *J. Pharmacol. Exp. Ther.*, **109**, 346 (1953).

(2) *Ibid.*, **113**, 481 (1955).

(3) B. Herrmann, *Med. Exp.*, **1**, 170 (1960).

(4) G. R. Van Petten, H. Feng, R. J. Withey, and H. F. Lettau, *J. Clin. Pharmacol.*, **11**, 177 (1971).

(5) B. G. Lukas, C. B. Borman, and S. B. Zak, *J. Pharm. Sci.*, **65**, 86 (1976).

(6) G. E. Wallace, *ibid.*, **57**, 2053 (1968).

(7) E. Jähnchen and G. Levy, *Clin. Chem.*, **18**, 984 (1972).

(8) M. W. Fuchs, *Rhumatologie*, **16**, 489 (1964).

(9) H. M. Stevens, *Clin. Chem.*, **16**, 437 (1970).

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

(11) K. K. Midha, I. J. McGilveray, and C. Charette, *J. Pharm. Sci.*, **63**, 1234 (1974).

(12) R. B. Bruce, W. R. Maynard, and L. K. Dunning, *ibid.*, **63**, 446 (1974).

(13) Y. Tanimura, Y. Saitoh, F. Nakagawa, and T. Suzuki, *Chem. Pharm. Bull.*, **23**, 651 (1974).

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

(15) N. J. Pound and R. W. Sears, *J. Pharm. Sci.*, **64**, 284 (1975).

(16) J. J. Burns, T. F. Yu, P. Dayton, L. Berger, A. B. Gutman, and B. B. Brodie, *Nature*, **182**, 1162 (1958).

(17) V. J. Stella, *J. Pharm. Sci.*, **64**, 706 (1975).

(18) W. Dieterle, J. W. Faigle, F. Früh, H. Mory, W. Theobald, K. O. Alt, and W. J. Richter, *Arzneim.-Forsch.*, **26**, 572 (1976).

[12] A. Sioufi, F. Caudal, and D. Grimault, to be published.

ACKNOWLEDGMENTS

The authors thank Dr. J. W. Faigle and Dr. W. Dieterle (Pharmacological Chemistry Department, Ciba-Geigy, Basel, Switzerland) for information on phenylbutazone metabolism. They are also grateful to Dr. W. Theobald (Medical Department, Ciba-Geigy, Basel, Switzerland) and to Dr. J. P. Schoeller (Medical Department, Ciba-Geigy, Rueil, France) for performing the clinical experiments.

Effect of Enzyme-Inducing and Enzyme-Inhibiting Agents on Drug Absorption I: 3-*O*-Methylglucose Transport in Rats

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Received February 22, 1977, from the *Department of Pharmaceutics, University of Houston, Houston, TX 77004.* Accepted for publication May 26, 1977.

Abstract □ The influence of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride on 3-*O*-methylglucose transfer across the everted rat small intestine was examined. The active and passive components of the sugar transport mechanism were evaluated using phlorizin, a potent inhibitor of active transport of sugars. Pretreatment of the animals with phenobarbital did not alter 3-*O*-methylglucose transfer characteristics. Pretreatment with intraperitoneally administered benzo[*a*]pyrene increased mucosal to serosal transfer of the sugar at low (0.1 m*M*) sugar concentrations. Enhancement of the active transfer of 3-*O*-methylglucose by pretreatment with proadifen hydrochloride was noted at low sugar concentrations. The passive transfer of the sugar was reduced after pretreatment with proadifen hydrochloride.

Keyphrases □ 3-*O*-Methylglucose—transfer across everted rat small intestine, effect of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride □ Transport, drug—3-*O*-methylglucose, transfer across everted rat small intestine, effect of preadministration of phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride □ Enzyme inducers—phenobarbital, benzo[*a*]pyrene, and proadifen hydrochloride, effect on transfer of 3-*O*-methylglucose across everted rat small intestine □ Phenobarbital—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine □ Benzo[*a*]pyrene—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine □ Proadifen hydrochloride—effect of preadministration on transfer of 3-*O*-methylglucose across everted rat small intestine

Intestinal glucose absorption is an active energy-expending process (1) believed to be controlled enzymatically and susceptible to alteration by chemical agents known to affect cell metabolism (2–4). The structural specificity of this particular mechanism has been well documented (5, 6). The 3-*O*-methyl derivative possesses transport characteristics similar to glucose; however, it is not metabolized by animal tissue (7). Thus, this sugar is useful for studying the sugar transport process across intestinal tissue.

Recent investigations showed the ability of various hepatic enzyme-inducing agents to enhance drug metabolism in the intestinal mucosa (8). Phenobarbital produced a striking hypertrophy of the small intestinal endoplasmic reticulum with an increase in this tissue's *N*-demethylase activity (9). However, no difference in mucosal to serosal transfer of ^{14}C-3-*O*-methylglucose or ^{14}C-palmitic acid was observed compared to controls. Previously, evidence that phenobarbital pretreatment heightened the mucosal transfer rate of ^{59}Fe-iron sulfate in duodenal rat intestinal sacs was reported (10). That effect may be due, in part, to an increased synthesis of a carrier molecule. In addition, subcutaneous phenobarbital injections enhanced active bile salt ileal transport (11).

It was of interest, therefore, to examine the possible effect of pretreatment with known hepatic enzyme-inducing agents on the active transport of 3-*O*-methylglucose