and similar biological activities, as estimated by the measurement of thyroid weight in rats on chronic administration of these substituted 2-thiouracils (*Reference 27* and Table I).

A possible mechanism of antithyroid activity of thiouracils is by their oxidation to the disulfide by iodine in the thyroid gland (27). Since oxidation is the loss of electrons, it would be expected that substituents which increase the electron density of sulfur would also increase the antithyroid activity. However, this increase in electron density is also what would be expected if complex formation were the mechanism. If we assume that either complexation or oxidation is the antithyroid mechanism of thiouracils, then the pK'a values of the thiouracil acids, which are measures of the sulfur electron density, and the related log K_1 values for the complexes (1) could be used to predict the structure of thiouracil derivatives which have greatly enhanced antithyroid activity, provided that the solubilities and ability to cross biological membranes are of equivalent magnitude in such a comparative series.

REFERENCES

(1) E. R. Garrett and D. J. Weber, *J. Pharm. Sci.*, **59,** 1383 (1970).

(2) *Ibid.*, **60,** 845(1971).

(3) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed., vol. 1, Interscience, New York, N. Y., 1952, (*a*) p. 362, (*b*) p. 228, (*c*) chap. XII, (*d*) chap. XI, (*e*) p. 52.

(4) F. J. C. Rossotti and H. Rossotti, "The Determination of Stability Constants," 1st ed., McGraw-Hill, New York, N. Y., 1961, p. 176.

(5) E. L. Colichman, *J. Amer. Chem. Soc.*, **72,** 4036(1950).

(6) R. W. Schmid and C. N. Reilley, *ibid.*, **80,** 2087(1958).

(7) D. N. Hume, D. D. DeFord, and G. C. B. Case, *ibid.*, **73,** 5323(1951).

(8) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," 1st ed., Interscience, New York, N. Y., 1962, p. 750.

(9) R. Weiss and H. Venner, *Z. Physiol. Chem.*, **317,** 82(1959).

(10) D. R. Crow, "Polarography of Metal Complexes," Academic, New York, N. Y., 1969, chap. 4 and 5.

(11) J. Jeyrovsky and J. Kuta, "Principles of Polarography," Academic, New York, N. Y., 1966, (*a*) chap. VIII, (*b*) p. 105.

(12) W. M. Latimer, "Oxidation Potentials," 2nd ed., Prentice-Hall, Englewood Cliffs, N. J., 1952, pp. 12, 30, 184.

(13) E. D. Potter, "Electrochemistry," 1st ed., Cleaver-Hume Press, Ltd., London, England, 1956, p. 112.

(14) N. N. Lange, "Handbook of Chemistry," 9th ed., Handbook Press, Sandusky, Ohio, 1956, p. 950.

(15) E. I. Onstott and H. N. Laitinen, *J. Amer. Chem. Soc.*, **72,** 4724(1950).

(16) "Instruction Manual, Sargent Recording Polarograph, Model XV," section 1.1, 1963.

(17) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., Wiley, New York, N. Y., 1962, p. 283.

(18) W. C. Schneider and I. F. Halverstady, *J. Amer. Chem. Soc.*, **70,** 2626(1948).

(19) C. Berecki-Biedermann, *Arkiv Kemi*, **9,** 175(1956).

(20) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed., vol. 2, Interscience, New York, N. Y., 1962, p. 494.

(21) R. G. Pearson, *Science*, **151,** 172(1966).

(22) S. E. Livingstone, *Quart. Rev.*, **19,** 386(1965).

(23) M. C. Day and J. Selbin, "Theoretical Inorganic Chemistry," 1st ed., Reinhold, New York, N. Y., 1962, chap. 9.

(24) H. Irving and R. J. Williams, *J. Chem. Soc.*, **1953,** 3192.

(25) R. J. Irving and W. C. Fernelius, *J. Phys. Chem.*, **60,** 1427 (1956).

(26) E. B. Astwood, *J. Pharmacol. Exp. Ther.*, **78,** 79(1943).

(27) E. B. Astwood, A. Bissell, and A. M. Hughes, *Endocrinology*, **37,** 456(1945).

(28) F. Horak and M. Samel, *Collect. Czech. Chem. Commun.*, **30,** 1229(1965).

(29) R. E. Stuckey, *J. Pharm. Pharmacol.*, **1,** 382(1949).

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GLC Determination of Sulthiame in Plasma

KEITH J. SIMONS and RENÉ H. LEVY▲

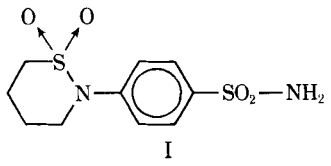
Abstract □ A GLC method is presented for the determination of sulthiame in plasma. A chloro derivative of sulthiame was synthesized for use as an internal standard. Both compounds can be chromatographed directly without derivatization, the method being quantitative over the range 3–20 mcg. The utility of the procedure was demonstrated by its application to the determination of sulthiame in the plasma of drug-treated rabbits and patients.

Keyphrases □ Sulthiame—GLC analysis in plasma □ GLC—analysis, sulthiame in plasma

Sulthiame (I)[1], a sulfonamide derivative, was first synthesized by Helferich and Kleb (1) and was later found to have potent anticonvulsant properties (2). It is primarily used in Europe[2] and Australia[2] for treatment of psychomotor (temporal lobe) and, to a lesser degree, for major motor (grand mal) seizures. It is cur-

[1] The USAN chemical name is *p*-(tetrahydro-2*H*-1,2-thiazin-2-yl)-benzenesulfonamide, *S,S*-dioxide.

[2] Ospolot, Farbenfabriken Bayer A. G.; Elisal, Specia Rhône-Poulenc.



I

rently under investigation for use in the United States[3]. Good results also have been reported in myclonic and petit mal seizures.

Von Duhm *et al.* (3, 4) studied the fate of the drug in rats using ^{35}S-labeled sulthiame. In the same year, Diamond and Levy (5) also used ^{35}S-labeled sulthiame to detect the excretion of unchanged drug and a metabolite in the urine of rats and human subjects. More recently, Olesen (6) extracted the drug from the plasma and urine of patients on long-term sulthiame therapy and, after TLC separation, determined the concentration by UV spectrophotometry. A few months ago, Kupferberg and Hunninghake (7) reported the determination of sulthiame by GLC after derivatization with trimethylanilinium hydroxide, but their method has not been published to date. In our laboratory it was found that under appropriate conditions, sulthiame can be chromatographed directly. This paper describes a rapid and direct determination of sulthiame in plasma by GLC.

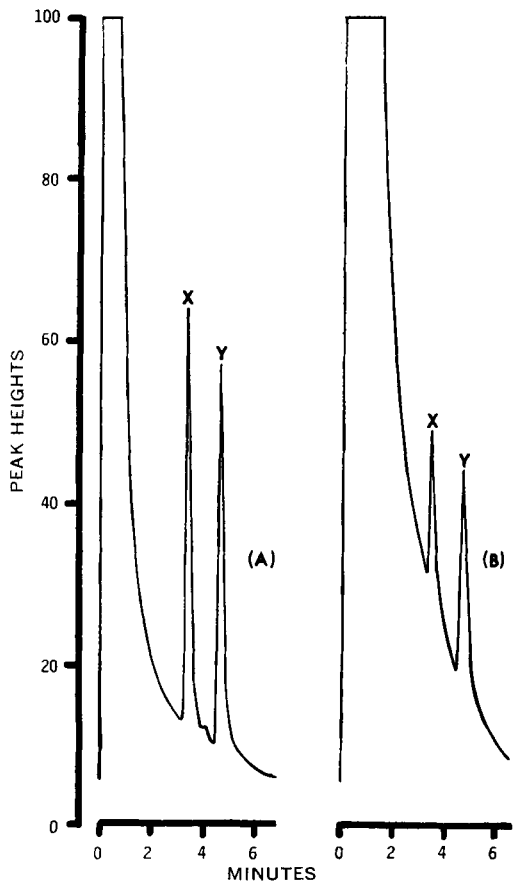


Figure 1—*Gas chromatogram of sulthiame (X) and internal standard (Y) (A = 20 mcg. sulthiame and 20 mcg. internal standard; B = 3 mcg. sulthiame and 2.5 mcg. internal standard) extracted from human plasma.*

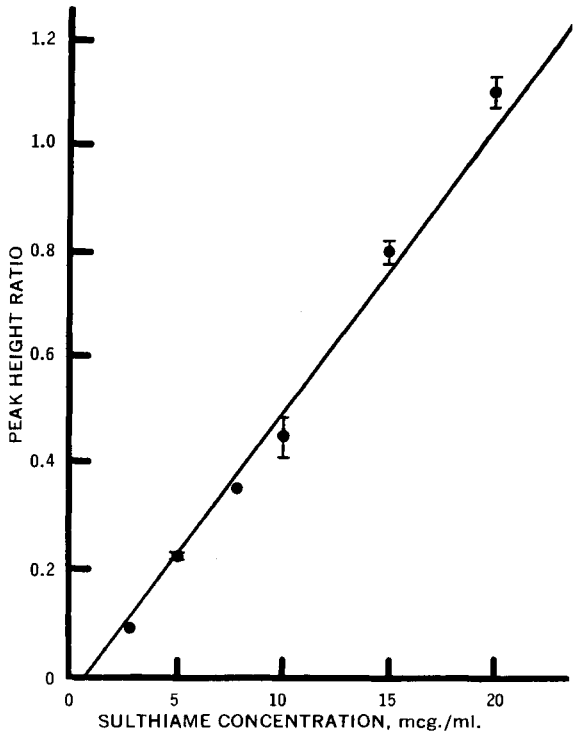


Figure 2—*Calibration curve for sulthiame extracted from human plasma.*

EXPERIMENTAL

Reagents—The following were used: stock solutions of sulthiame[4] containing 1, 3, 5, 8, 10, 15, and 20 mcg./50 μl. in water, with methanol as a wetting agent; stock solutions of internal standard containing 2.5, 10, and 20 mcg./20 μl. in methanol; and buffer solutions of 0.25 *M* K_2HPO_4 (pH 7.2) and 0.2 *M* K_3PO_4 (pH 12.8). All solvents were reagent grade.

Biological—*Animals*—New Zealand strain albino rabbits (3–5 kg. body weight) were used in the study. The animals were given food and water *ad libitum*.

Dosing—Sulthiame was dissolved in polyethylene glycol 400[5] to yield a solution (50 mg./ml.) which was sterilized by heat. The dose (20 mg./kg.) was administered intravenously (peripheral ear vein) over a 2-min. period using a 26-gauge, 1.3-cm. (0.5-in.) needle. Warming the solution to slightly above room temperature prior to injection aided administration.

Blood Collection—Prior to administration of the drug, an arterial shunt[6] was set up in the midline artery of the ear not used for dosing. Blood samples (3–4 ml.) were drawn at −5, 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min., collected in heparinized tubes, and centrifuged at 2000 r.p.m. for 10 min. The plasma was harvested and frozen.

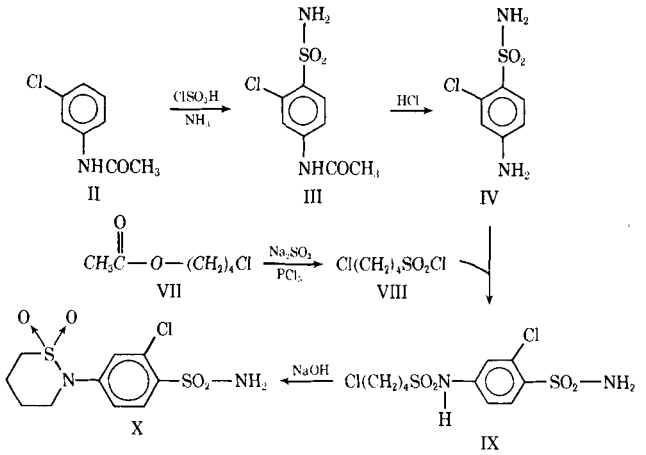
Preparation and Extraction of Plasma Standards and Specimens from Dosed Animals—*Rabbit Plasma Standards*—To 1-ml. control plasma samples in Teflon-lined screw-capped, 15-ml., Pyrex test tubes were added 50 μl. of each aqueous sulthiame solution, 20 μl. of the appropriate internal standard solution, and 1 ml. phosphate buffer (pH 7.2). The samples were extracted with 10 ml. ethylene dichloride which was accomplished by manual shaking for 2 min., followed by centrifugation at 2000 r.p.m. for 5 min. The aqueous phase, including precipitated plasma proteins, was aspirated and the organic phase was transferred to a clean tube. After addition of 5 ml. buffer (pH 12.8), the samples were shaken and

[3] Trolone, Riker Labs. Inc., Northridge, CA 91324

[4] Sulthiame-SN#D0960, compliments of Riker Labs. Inc., Northridge, CA 91324

[5] Fisher Scientific Co., Fair Lawn, N. J.

[6] Disposable Butterfly Infusion Set 21, Abbott Labs. Inc., North Chicago, Ill.



Scheme I

centrifuged as before. The maximum amount possible of the buffer phase was transferred to a 12-ml. glass-stoppered centrifuge tube, acidified with 0.2 ml. 10 *N* HCl, and extracted as before with 7 ml. ethylene dichloride. The aqueous phase was aspirated, and the organic phase was evaporated to dryness at 90° with the aid of a dry nitrogen stream. Samples were dissolved in 20 μl. chloroform and held under refrigeration, being removed only for GLC injection.

Plasma Samples from Dosed Rabbits—Appropriate aliquots (0.2–1 ml.) of plasma were pipeted into 15-ml. tubes. Following addition of the internal standard, the samples were extracted as the plasma standards.

GLC—GLC was carried out on a gas chromatograph[7] equipped with a flame-ionization detector. Glass columns [coiled 1.83 m. × 0.63 cm. (6 ft. × 0.25 in.), 2 mm. i.d.], packed with 3% OV-17, coated 60–80-mesh, acid-washed Chromosorb W-DMCS[8], were used throughout the study. Helium as a carrier gas was maintained at 30 ml./min., with air and hydrogen at 300 and 30 ml./min., respectively.

The column was maintained isothermally at 295° with both the injector and detector at 310°. The column was conditioned at the end of each day with 30 μl. Silyl 8[9]. Under these conditions, sulthiame and the internal standard have retention times of ~4 and ~5 min., respectively (Fig. 1).

Calculations—Peak height ratios were calculated by dividing the height of the peak due to sulthiame by the height of the peak due to the internal standard. Calibration curves were constructed daily from the results of "spiked" control plasma samples by plotting the concentration of sulthiame (micrograms per milliliter plasma) against the peak height ratios. A typical calibration curve is shown in Fig. 2.

Internal Standard—The internal standard was synthesized according to the following procedures (Scheme I). The 4-amino-2-chlorobenzenesulfonamide (IV) was prepared according to the procedure outlined by Adams *et al.* (8). A slight modification (9) was applied whereby the 3-chloroacetanilide (II) was heated with chlorosulfonic acid ($ClSO_3H$) for 7 hr. instead of only 30 min., thus increasing the yield of IV by 50%. The 4-chlorobutyl acetate (VII) was purchased[10] but could also be synthesized as described by Cloke and Pilgrim (10). The original method of Helferich and Kleb (1) was followed to prepare the 4-chlorobutanesulfonyl chloride (VIII), which was allowed to react with IV to form N^4-(4-chloro-1-butylsulfonyl)-2-chlorosulfanilamide (IX) (3). This reaction yielded a large amount of polymeric material from which IX had to be extracted with chloroform prior to cyclization to *N*-(2′-chloro-4′-sulfamylphenyl)-1,4-butanesultam (X) using sodium hydroxide (3).

[7] Aerograph 200, Varian Aerograph, Walnut Creek, CA 94598; equipped with Speedomax W Recorder, Leeds and Northrup, Philadelphia, Pa.

[8] Varian Aerograph, Walnut Creek, CA 94598

[9] Pierce Chemical Co., Rockford, IL 61105

[10] Eastman Organic Chemicals, Rochester, N. Y.

RESULTS AND DISCUSSION

GLC—Figure 1 shows typical chromatograms obtained after addition of 20 mcg. (1A) and 3 mcg. (1B) of sulthiame to 1-ml. plasma samples. In both instances, sulthiame and the internal standard gave well-resolved symmetrical peaks. Under the conditions of the assay, they eluted at ~4 and ~5 min., respectively, with the absence of interfering peaks even at later times. Consequently, injections could be repeated as frequently as every 8 min., a significant advantage.

Attempts were made to chromatograph sulthiame and the internal standard directly on a column of 3% SE-30, coated 80–100-mesh, acid-washed Chromosorb W-DMCS under various conditions of column temperature and carrier gas flow rates. Unfortunately, in all instances the peaks were unsymmetrical and/or unresolved, which precluded quantification.

Sulthiame and the internal standard elute on the downslope of the solvent front; at lower concentrations, this becomes a limiting factor governing the ease and accuracy of measuring the peak height ratios (Fig. 1B). The importance of selecting the most appropriate solvent will be discussed further.

To test the linearity of the system (extraction and response of the flame-ionization detector), a calibration curve was constructed using spiked human plasma samples (Fig. 2). The plot of sulthiame concentration *versus* peak height ratio was linear in the range 3–20 mcg., although lower amounts could be detected. Several curves were constructed and, in most instances, a positive intercept of approximately 1 mcg. was observed. This is probably due to adsorption and/or decomposition (injector), but the phenomenon prevents accurate quantitation only at concentrations below 3 mcg./ml. From a practical standpoint, however, this phenomenon is probably insignificant because of the relatively high levels of sulthiame found in the plasma samples of patients treated with this drug. Nevertheless, a concentrated solution of drug and standard (20 mcg. of each in 20 μl. chloroform) was injected at the beginning of each run to minimize adsorption.

The recovery of sulthiame added to human plasma controls was calculated from the data used to plot the calibration curves. The results show that 94.60 ± 4.49% (mean ± standard deviation) of the sulthiame added was recovered (Table I).

The choice of solvent is critical, both for extraction and GLC. Using UV spectrophotometry, Olesen (6) demonstrated that only 60% of the sulthiame content could be extracted with chloroform over the pH range 1–7. From a selection of several solvents tested at pH 7.2 in this laboratory, Olesen's results with chloroform were confirmed but ethylene dichloride was found to extract about 85% of the sulthiame content. Olesen's work also showed that the pKa of sulthiame lies somewhere in the range 11–12, which was also confirmed in this laboratory. This explains the importance of buffering to pH > 12.5 when back-extracting sulthiame from ethylene dichloride.

The criteria for selection of the GLC solvent were the ability to dissolve sulthiame after evaporation of ethylene dichloride and the width and tailing of the solvent front. A study of various potential solvents (ethylene dichloride, methanol, ether, acetone, dimethylformamide, chloroform, and carbon disulfide) was carried out. Carbon disulfide would have been ideal because of the minimal response of the flame-ionization detector but, unfortunately, sulthiame proved to be insoluble in carbon disulfide. The results from comparative GLC injections demonstrated that under the conditions of the assay, chloroform produced the smallest solvent front, thus becoming the solvent of choice for sulthiame chromatography.

Very few methods have been published for the determination of sulthiame in plasma. Following the administration of ^{35}S-labeled sulthiame to rats, von Duhm *et al.* (3, 4) measured the loss of activity from most body organs, blood, serum, and erythrocytes and were able to account for 80–90% of the administered activity in urine and 10–20% in feces. Diamond and Levy (5) also used ^{35}S-labeled sulthiame to detect the excretion of unchanged drug in the urine of rats and human subjects. For both species, 60–75% of the radioactivity in urine was attributed to unchanged drug and 25–50% to an unidentified metabolite, while 10–15% was accounted for in the feces.

Olesen (6) obtained urine and plasma samples from patients on long-term therapy with sulthiame. These samples were buffered and extracted with chloroform, and the resulting residue was chro-

Table I—Recovery of Sulthiame Added to Human Plasma

Number of Samples	Sulthiame Added, mcg./ml.	Sulthiame Found, mcg./ml.[a]	Percent Recovery[a]
2	3	2.83	94.33
5	5	5.00 ± 0.12	100.00 ± 2.40
2	8	7.17	89.63
7	10	8.91 ± 0.30	89.10 ± 3.00
7	15	14.43 ± 0.32	96.20 ± 2.13
7	20	19.67 ± 0.42	98.35 ± 2.10

[a] Mean ± standard deviation.

matographed on TLC plates to separate the drug. After detection of the sulthiame spots, these were scraped off and extracted with methanol. The sulthiame content was then assayed spectrophotometrically at 243 and 280 nm. This method was reported to be quantitative over the range 1–40 mcg.

Kupferberg and Hunninghake (7) reported on the determination of sulthiame by GLC. On-column methylation of sulthiame with trimethylanilinium hydroxide (11), using 3-methoxycholesterol as an internal standard, yielded symmetrical peaks on a 3% OV-17 column maintained isothermally at 300°. This method was reported to be quantitative over the range 1–20 mcg. but has not been published.

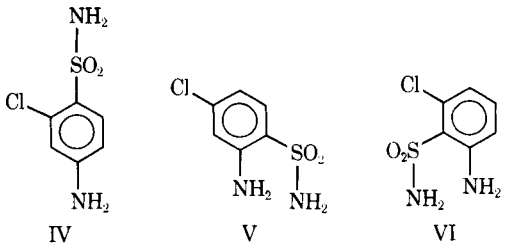
In the present work, it was found that sulthiame can be chromatographed directly (without derivatization) on a 3% OV-17 column maintained isothermally at 295°. Attempts to use 3-methoxycholesterol as an internal standard proved to be unsatisfactory because it cannot be added directly to the plasma sample, thus requiring that all volume measurements in the various transfer steps of the extraction procedure be quantitative. It also elutes earlier than sulthiame, so interference with the solvent front makes quantitation difficult at lower concentrations.

This situation instigated the search for a satisfactory internal standard. An ideal standard should be structurally related to sulthiame in order to have similar partition properties for solvent extraction as well as column retention. As a derivative of sulthiame was unobtainable, it was necessary to synthesize one. The compound synthesized was a chloro derivative of sulthiame, *N*-(2′-chloro-4′-sulfamylphenyl)-1,4-butanesultam.

Internal Standard—When II was heated with chlorosulfonic acid, substitution of the sulfonyl chloride moiety could occur at either the 2-, 4-, or 6-position because of the *ortho*- and *para*-directing ability of the chloro and acetamido substituents. This then would yield one of the three possible chlorosulfanilamide isomers: IV, 2-amino-4-chlorobenzenesulfonamide (V), or 2-amino-6-chlorobenzenesulfonamide (VI).

It was necessary to prove that substitution occurs at the 4-position (IV) to confirm the structure of X. The reported melting point of 4-acetamido-2-chlorobenzenesulfonamide (III) is 194–197° (9), while the corresponding compound that was synthesized has a melting point[11] of 201–203°. The reported melting-point values of IV, V, and VI are 176.5–177.5° (9), 143–144° (12), and 140–141° (12), respectively. Since the corresponding compound that was synthesized had a melting point of 178–180°, it would indicate that Structure IV is indeed correct. The work of Behnisch *et al.* (13), where VIII was reacted with various sulfanilamides (including IV), showed that IV can only yield IX and, subsequently, X. The reported melting point of X is 214–215° (13), while the compound synthesized in this laboratory had a melting point of 210–210.5°. Furthermore, mass spectroscopy[12] showed that the mass of the parent ion is 323.99873, which is consistent with the calculated value 324.000495 for the empirical formula $C_{10}H_{13}ClN_2O_4S_2$.

The advantages of this internal standard include both ease and accuracy because it can be added directly to the plasma samples. Therefore, the volume measurements in the transfer steps of the extraction procedure do not have to be quantitative since quantitation is based on the calculation of drug to standard peak height ratios rather than absolute peak heights of sulthiame. Furthermore, since the internal standard is a derivative of sulthiame, both compounds should produce a similar detector response. This can be an important factor since detector response (for a flame-ionization detector) is not always constant and varies with the unavoidable fluctuations in flow rates of hydrogen, air, and carrier gas. Moreover, any solvent front limitations on the assay of lower concentrations are now dependent on sulthiame itself since it elutes before the internal standard. In addition, both compounds elute within 5 min., so injections may be repeated as frequently as every 8 min., and an entire assay can be completed in 45–75 min.



Attempts to use on-column methylation (11) for sulthiame and its internal standard proved that both compounds form derivatives that yield symmetrical peaks and shorter retention times under the conditions of the assay. Measurements of peak height ratios after repeated injections were performed to test the reproducibility of methylation. The variability obtained with this approach was significantly larger than that obtained from corresponding injections of the underivatized compounds. This may be due to the fact that the reaction mechanism of flash-heater methylation and the experimental factors affecting it have not been completely elucidated and are difficult to control. Such experimental factors might be injection rate, carrier gas flow rate, and injection port temperature, all of which affect the reaction time in the injection port. Hence, completeness and reproducibility of methylation of sulthiame and its chloro derivative might require more carefully controlled conditions than those generally found under operating conditions of most routine procedures in GLC.

Determination of Sulthiame in Rabbit and Human Plasma—This

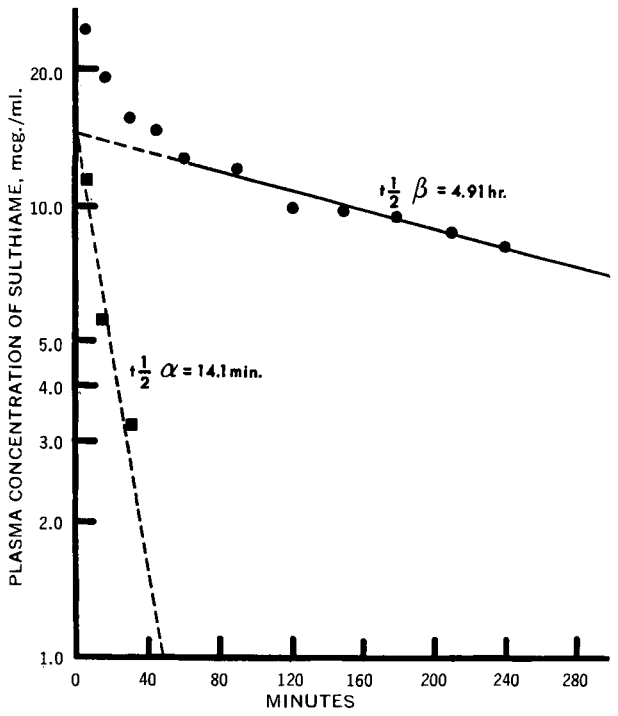


Figure 3—*Concentrations of sulthiame in plasma after a single intravenous administration of 20 mg./kg. to Rabbit C. Key:* ●—●, *plot of experimental values; and* ■---■, *plot of differences between the extrapolated β-phase and the plasma levels at early times (α-phase).*

[11] Hoover capillary melting-point apparatus, Thomas Co., Philadelphia, Pa.

[12] Picker AEI MS9 mass spectrometer, Picker Nuclear Division, White Plains, N. Y.

Table II—Basic Pharmacokinetic Parameters of Sulthiame in Rabbits

Rabbit	$t^1/_2\alpha$[a], min.	$t^1/_2\beta$[b], hr.	V_p[c], l./kg.	Clearance[d], l./hr./kg.
A	12.3	3.35	0.70	0.38
B	10.0	3.76	0.64	0.24
C	14.1	4.91	0.71	0.19

[a] Half-life of the α-phase (rapid). [b] Half-life of the β-phase (slow). [c] Volume of the central compartment two-compartment model. [d] Portion of the volume of the central compartment that is totally cleared of drug per unit time ($k_{el}V_p$).

method has been used to follow the kinetics of disappearance of sulthiame from plasma of rabbits given 20 mg./kg. of the drug intravenously. Twelve blood samples were taken over a 4-hr. period, and the sulthiame concentration was determined in the plasma. A semilogarithmic plot of plasma concentration (micrograms per milliliter sulthiame) *versus* time (minutes) (Fig. 3) showed a biexponential decay curve, *i.e.*, a rapid α-phase followed by a slower β-phase. After appropriate pharmacokinetic interpretation using a two-compartment model, the usual basic parameters were calculated (Table II) (14). The biological half-life ($t_{1/2}\beta$) of sulthiame in rabbits was determined to be about 4 hr. Kupferberg and Hunninghake (7) found the biological half-life to be 4.5 hr. in a dog.

This method was also used to determine the sulthiame concentration in several plasma samples obtained from a few patients on sulthiame therapy. The plasma levels ranged from 11 to 50 mcg./ml.; consequently, only a fraction of a milliliter (0.2–0.8 ml.) was required for the assay. There appeared to be no interfering foreign peaks from possible metabolites of sulthiame or other medication taken by the patients.

CONCLUSIONS

The method for the determination of sulthiame in plasma utilizes direct GLC of the underivatized drug and a chloro derivative synthesized in this laboratory as an internal standard. The extraction procedures and GLC conditions described provide quantitative determination of sulthiame over the range 3–20 mcg.

This method compares more than favorably with those presently available, all of which require lengthy TLC separation prior to quantitation. The presented method is relatively rapid as well as sensitive and accurate.

REFERENCES

(1) B. Helferich and K. Kleb, *Justus Liebigs Ann. Chem.*, **635,** 91(1960).

(2) W. Wirth, F. Hoffmeister, H. Friebel, and S. Sommer, *Deut. Med. Wochenschr.*, **85,** 2195(1960).

(3) B. von Duhm, W. Maul, H. Medenwald, K. Parzschke, and L. Wegner, *Z. Naturforsch.*, **18b,** 475(1963).

(4) B. von Duhm, W. Maul, H. Medenwald, K. Parzschke, and L. Wegner, *Med. Chem., Abh. Med.-Chem. Forschungsstaetten Farbewerke Hoechst.*, **7,** 315(1963); Farbenfabriken-Bayer A. G.

(5) S. Diamond and L. Levy, *Curr. Ther. Res.*, **5,** 325(1963).

(6) O. Olesen, *Acta Pharmacol. Toxicol.*, **26,** 22(1968).

(7) H. Kupferberg and D. Hunninghake, Abstr., APhA Academy of Pharmaceutical Sciences, San Francisco meeting, Mar. 1971.

(8) "Laboratory Experiments in Organic Chemistry," 6th ed., R. Adams, J. Johnson, and C. Wilcox, Jr., Eds., Collier MacMillan, London, England, 1970, pp. 310–313.

(9) M. Bullock and G. Rohrbacker, Jr., U. S. pat. 3,435,100; through *Chem. Abstr.*, **71,** 12830(1969).

(10) J. Cloke and F. Pilgrim, *J. Amer. Chem. Soc.*, **61,** 2667 (1939).

(11) E. Brochmann-Hanssen and T. O. Oke, *J. Pharm. Sci.*, **58,** 370(1969).

(12) J. A. Christensen, German pat. 1,135,483; through *Chem. Abstr.*, **58,** 11279(1963).

(13) R. Behnisch, R. Hoffmeister, H. Horstmass, E. Schraufstatter, and W. Wirth, *Med. Chem., Abh. Med.-Chem. Forschungsstaetten Farbewerke Hoechst.*, **7,** 296(1963); Farbenfabriken-Bayer A. G.

(14) S. Riegelman, J. Loo, and M. Rowland, *J. Pharm. Sci.*, **57,** 117(1968).

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