Zwitterion—This compound was prepared in the same manner as the 6-methoxy analog. A 68% yield of red-brown solid was obtained, mp 185° dec.

Anal.—Calc. for $C_{13}H_{10}F_3NS_2$: C, 51.82; H, 3.32; N, 4.65. Found: C, 52.16; H, 3.49; N, 4.35.

6-Bromo-1-methyl-2-bis(2-methylthio)vinylquinolinium Iodide—A mixture of 6-bromo-1-methylquinolinium-2-dithioacetic acid zwitterion (6.81 g, 0.0208 mole), iodomethane (10 ml), and dimethylformamide (40 ml) was allowed to stand at room temperature, with occasional shaking, for 17 hr. The dark-brown solid was filtered, washed with acetone, and dried at 23° (0.2 torr) for 6 days, giving 4.81 g (49% yield), mp 194–197° dec.; NMR (dimethyl sulfoxide-d_6): δ 2.50 (s, 3H, SCH_3), 2.63 (s, 3H, SCH_3), 4.35 (s, 3H, NCH_3), 6.65 (s, 1H, vinyl H), and 8.2–9.0 (m, 5H, ring H) ppm.

Anal.—Calc. for $C_{14}H_{15}BrINS_2$: C, 35.91; H, 3.23; N, 2.99; S, 13.70. Found: C, 35.55; H, 3.08; N, 2.94; S, 13.12.

1-Methyl-2-bis(2-methylthio)vinylquinolinium Iodide—The same procedure was used as the for 6-bromo analog. The resulting dark-yellow solid was washed with ether, recrystallized from water, and dried at 25° (1.5 torr), giving a 15.5% yield of yellow crystals, mp 198–201° dec. [lit. (9) mp 203° dec.]; NMR (dimethyl sulfoxide-d_6): δ 2.55 (s, 3H, SCH_3), 2.70 (s, 3H, SCH_3), 4.40 (s, 3H, NCH_3), 6.80 (s, 1H, vinyl H), and 8.3–9.0 (m, 6H, ring H) ppm.

REFERENCES

(1) W. O. Foye, Y. J. Lee, K. A. Shah, and J. M. Kauffman, *J. Pharm. Sci.*, **67**, 962 (1978).

(2) B. K. Sinha, R. M. Philen, R. Sato, and R. L. Cysyk, *J. Med. Chem.*, **20**, 1528 (1977).

(3) K. N. Campbell and I. J. Schaffner, *J. Am. Chem. Soc.*, **67**, 86 (1945).

(4) L. Horowitz, *ibid.*, **77**, 1687 (1955).

(5) J. Willems and J. Nys, *Bull. Soc. Chim. Belg.*, **66**, 502 (1957). P. Lochon and M. Jambu-Geoffroy, *Bull. Soc. Chim. Fr.*, **1965**, 393.

(6) L. G. S. Booker and L. A. Smith, *J. Am. Chem. Soc.*, **59**, 67 (1937).

(7) R. J. N. Sahay, A. K. Sinha, and J. C. Banerji, *J. Indian Chem. Soc.*, **43**, 255 (1966).

(8) E. Rosenhauer, *Chem. Ber.*, **57**, 1294 (1924).

(9) R. Gompper, B. Wetzel, and W. Elser, *Tetrahedron Lett.*, **1968**, 5519.

(10) K. Mizuyama, Y. Tominaga, J. Matsuda, and G. Kobayashi, *Yakugaku Zasshi*, **94**, 702 (1974).

(11) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972).

(12) K. N. Campbell, C. H. Helbing, and J. F. Kerwin, *J. Am. Chem. Soc.*, **68**, 1840 (1946).

(13) N. S. Kozlov and O. E. Kostromina, *J. Gen. Chem. USSR*, **25**, 907 (1955).

(14) W. Cocker and D. G. Turner, *J. Chem. Soc.*, **1941**, 143.

(15) K. L. Moudgill, *J. Am. Chem. Soc.*, **43**, 2257 (1921).

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Differential Pulse Polarographic Analysis of Thyroid Hormone: Determination of Iodine, Thyroxine, and Liothyronine

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Received June 30, 1978, from the *Food and Drug Administration, Department of Health, Education, and Welfare, Brooklyn, NY 11232.* Accepted for publication August 24, 1978.

Abstract ☐ A differential pulse polarographic method for the analysis of thyroid and thyroid tablets for total iodine, thyroxine, and liothyronine is described. The procedure for iodine, which is also applicable to individual tablet assay, consists of ashing the sample, converting iodide to iodate, and analyzing by differential pulse polarography. The procedure for thyroxine and liothyronine involves hydrolysis of the sample with barium hydroxide and isolation and separation of the iodoamino acids using ion exchangers, followed by differential pulse polarographic determination in a supporting electrolyte composed of 0.5 N Na_2CO_3 in 20% 2-propanol containing 1% tetrabutylammonium bromide. The differential pulse polarographic results for iodine agree with values obtained using the USP XIX procedure, and the quantities of thyroxine and liothyronine found agree with literature values.

Keyphrases ☐ Thyroid—differential pulse polarographic analysis of iodine, thyroxine, and liothyronine in hormone and tablets ☐ Polarography, differential pulse—analysis of iodine, thyroxine, and liothyronine in thyroid hormone and tablets ☐ Iodine—differential pulse polarographic analysis in thyroid hormone and tablets ☐ Thyroxine—differential pulse polarographic analysis in thyroid hormone and tablets ☐ Liothyronine—differential pulse polarographic analysis in thyroid hormone and tablets

The thyroid gland produces various iodoamino acids that are derivatives of thyronine and tyrosine including thyroxine, liothyronine, 3,3′,5′-triiodothyronine, 3,5-diiodothyronine, diiodotyrosine, and monoiodotyrosine (1, 2). The major biological activity is present only in thyroxine and liothyronine. 3,3′,5′-Triiodothyronine and 3,5-diiodothyronine have little or no biological activity and are present in small amounts; diiodotyrosine and monoiodotyrosine have no biological activity.

BACKGROUND

The USP XIX method for the estimation of thyroid potency involves the determination of total iodine. The compendial assay requires ashing the sample with potassium carbonate, which releases iodide ion. The chemical transformations consist of oxidation of iodide to iodate, reduction to iodine, and, finally, titration with sodium thiosulfate.

This titrimetric procedure is not applicable to individual tablet analysis because it lacks sufficient sensitivity. For this reason, a procedure that requires a Schöniger flask oxygen combustion treatment followed by the chemical transformations is used. However, the determination of total iodine is a nonspecific test for thyroid potency since iodine is available from biologically inactive compounds as well as the biologically active hormones. This assay is used because total iodine is relatively simple to determine accurately.

A better indication of thyroid potency would be the determination of the biologically active hormones, thyroxine and liothyronine. The development of a simple and reliable method for their determination has been a challenge. The approach has been to hydrolyze the sample by

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heating in an alkaline medium, followed by isolation of the hormones by extraction into 1-butanol prior to the determinative step. The separation of thyroxine from liothyronine recently was accomplished using ion exchangers (3). Several methods involve TLC (1) and GLC after derivatization (2).

This paper reports an investigation of differential pulse polarography in the analysis of thyroid. Iodine is determined by conventional dc polarography, and thyroxine and tyrosine also have been determined by this method (4-6). Its application to thyroid analysis is not practical, however, because of the lack of sensitivity, which is largely due to the capacitance current contribution to the Faradaic current. In differential pulse polarography, this difficulty is reduced so that this technique is well suited to the analysis of thyroid for total iodine, thyroxine, and liothyronine.

EXPERIMENTAL

Apparatus and Polarographic Conditions—A polarographic analyzer[1] was equipped with a drop timer in conjunction with three electrodes: dropping mercury, saturated calomel, and platinum wire auxiliary. The drop timer was set at 1 sec, and the dropping mercury electrode had a flow rate of 0.80 mg/sec. The height of the mercury column was 70 cm.

Other conditions were: current range, 1-5 μamp; pulse amplitude, 100 mv; and scan rate, 5 mv/sec. The potential range scans, in volts, were: iodate, from −0.9 to −1.50; and iodoamino acids, from −0.6 to −1.7. All polarograms were recorded on an x-y recorder[2].

A pH meter[3] was fitted with a combination glass-saturated calomel electrode.

Reagents and Chemicals—All chemicals were reagent grade, unless otherwise specified.

The potassium carbonate was purified to remove iodide. The following procedure was used. Dissolve about 100 g of potassium carbonate in 200 ml of water, add 25 g of 20-50-mesh ion-exchange resin[4], and agitate for 30 min. Filter, evaporate to dryness on a hot plate, and heat at 675° for 25 min. Then cool to room temperature.

The iodoamino acids[5] used were thyroxine (T_4, 3,5,3′,5′-tetraiodothyronine), liothyronine (T_3, 3,5,3′-triiodothyronine), 3,3′,5′-triiodothyronine ($T_{3'}$), 3,5-diiodothyronine (T_2), diiodotyrosine, and monoiodotyrosine. The ion-exchange resins used were 200-400-mesh AG 1 × 2[6], QAE-Sephadex A-25[7], and SP-Sephadex C-25[7].

Preparation of Solutions—*Standard Solutions*—The standard iodate stock solution containing 1000 μg of iodine/ml is prepared by dissolving 1.6860 g of potassium iodate in water and diluting to 1 liter.

The working standard iodate solution contains 1 μg of iodine/ml. Dilute with water, stepwise, aliquots of the standard iodate stock solution to achieve a concentration of 10 μg of iodine/ml. Pipet 10 ml of this solution into a 100-ml volumetric flask containing 8.0 g of potassium carbonate dissolved in about 20 ml of water. Then add 1 ml of bromine water and mix. Add about 10 mg of sodium sulfite to remove the excess bromine, mix, and dilute to volume.

Standard thyroxine and liothyronine solutions are prepared in the same manner. Add 10 mg of compound, accurately weighed, to separate 10-ml volumetric flasks. Add about 5 ml of water, then add dropwise 1 N NaOH to dissolve the amino acid salts, dilute to volume with water, and mix. Protect from light.

Supporting Electrolyte for Liothyronine and Thyroxine—Dissolve 5.3 g of sodium carbonate in about 30 ml of water. Then add 20 ml of 2-propanol, 1.0 g of tetrabutylammonium bromide, and 10 ml each of saturated aqueous solutions of sodium sulfite and disodium ethylenediaminetetraacetate. Dilute to 100 ml with water and mix. Adjust the pH to 11.6 with 5 N NaOH.

Elution Solutions—Solution A contains 0.02 M NaOH in water. For Solution B, dissolve 12.1 g of tris(hydroxymethyl)aminomethane (I) and 11.8 g of succinic acid in 1 liter of water. Adjust the pH to 8.7 with 0.1 N NaOH.

To prepare Solution C, dissolve 12.1 g of I and 11.8 g of succinic acid in 1 liter of 30% (v/v) 2-propanol in water. Adjust the apparent pH to 8.7 with a solution of 4 g of sodium hydroxide in 1 liter of 30% (v/v) 2-propanol in water.

For Solution D, dissolve 12.1 g of I and 11.8 g of succinic acid in 1 liter of 30% (v/v) 2-propanol in water. Adjust the apparent pH to 6.3 with a solution of 4 g of sodium hydroxide in 1 liter of 30% (v/v) 2-propanol in water.

Solution E is 2.5 M acetic acid in water, and Solution F is 12.0 M acetic acid in water.

Preparation of Columns—Plastic, 1-cm diameter[8] columns are packed as follows: Column 1, fill to a depth of 4 cm with QAE-Sephadex A-25 in 0.02 N NaOH; Column 2, fill to a depth of 4 cm with a mixed-bed exchanger, 40% SP-Sephadex C-25 and 60% QAE-Sephadex A-25 in Solution B; and Column 3, fill to a depth of 1 cm with 200–400-mesh AG 1 × 2 resin in 7 M acetic acid in water. Place a small pledget of glass wool on top of the resin.

Determination of Iodine—*Composite Assay Solution*—Weigh and finely powder not less than 20 tablets. Weigh a portion of powder containing about 60 mg of thyroid into a Vycor crucible, mix with 4 g of potassium carbonate, and overlay with 4 g of potassium carbonate. Place the crucible in a preheated muffle furnace at 675–700° for 25 min. Cool, dissolve the residue in 20 ml of hot water, and filter through a glass wool plug into a 100-ml volumetric flask.

Wash the crucible with two additional 20-ml portions of hot water and add these portions through the glass wool plug to the volumetric flask. Add 1 ml of bromine water, mix, add about 20 mg of sodium sulfite, and mix. Cool to room temperature and dilute to volume.

Individual Tablet Assay Solution—Weigh and finely powder each tablet. Weigh a portion of powder containing about 15 mg of thyroid into a Vycor crucible and mix with 1 g of potassium carbonate. Place into a preheated muffle furnace at 675–700° for 25 min. Cool, dissolve the residue in 10 ml of hot water, and filter through a glass wool plug into a 25-ml volumetric flask.

Wash the crucible with two 5-ml portions of hot water and add these portions through the glass wool plug to the volumetric flask. Add 1 ml of bromine water, mix, add about 20 mg of sodium sulfite, and mix. Cool to room temperature and dilute to volume.

Polarographic Procedure—Polarograph individually the composite assay solution, the individual tablet assay solution, and the working standard iodate solution as follows. Add about 10 ml of the solution to be assayed to the polarographic cell, deaerate for 5 min with nitrogen, and then polarograph using the described conditions. Measure the peak heights of the sample and the standard peaks that occur at approximately −1.18 v *versus* the saturated calomel electrode and calculate the percent iodine in the sample.

Determination of Thyroxine and Liothyronine—Weigh and finely powder not less than 20 tablets. Weigh a portion of powder containing about 0.2 g of thyroid into a 20-ml polyethylene screw-capped vial. Add 1.5 g of barium hydroxide octahydrate and 4 ml of water. Close the vial tightly, shake vigorously to mix the contents, and place in an oven at 95° for 6 hr. Transfer the contents of the vial with the aid of water to a 25-ml volumetric flask, cool to room temperature, dilute to volume, and mix. Allow any precipitate to settle. Label this solution the sample solution.

To another 25-ml flask, add 100 μl each of standard thyroxine and liothyronine solutions, 1.5 g of barium hydroxide, and about 20 ml of water. Mix and then dilute to volume. Label this solution the standard solution. Carry 15 ml of the standard solution through the chromatographic procedure in the same manner as the sample solution.

Feed a 15-ml aliquot of the sample solution to Column 1 and wash the column with 10 ml of Solution B or until the eluate pH is 8.7. Discard this eluate. Arrange the columns so that Column 1 elutes into Column 2, which then elutes into Column 3, which elutes into a waste beaker. Elute with 20 ml of Solution C. Remove Column 3, which now retains liothyronine. Place a new Column 3 under Columns 2 and 1 and elute with 20 ml of Solution D. Remove this second Column 3, which now retains thyroxine. Wash both columns with 5 ml of Solution E and discard the washings.

Elute liothyronine and thyroxine from the individual columns into glass evaporating dishes with 5 ml of Solution F. Evaporate the eluates to dryness at 60° with a current of nitrogen. Reserve the residue obtained from the liothyronine and thyroxine standard solutions for later use. Dissolve the sample residue in 5 ml of supporting electrolyte for liothyronine and thyroxine and transfer to the polarographic cell. Wash the evaporating dish with one 3-ml and one 2-ml portion of the supporting

[1] Princeton Applied Research model 174.
[2] Houston Omnigraphic model 2000.
[3] Beckman Zeromatic 55-3.
[4] Amberlite IRA-400.
[5] Courtesy of Joseph H. Graham, Food and Drug Administration, Washington, D.C.
[6] BioRad Laboratories, Richmond, Calif.
[7] Pharmacia Fine Chemicals, Piscataway, N.J.
[8] Chromaflex disposable columns, Kontes Glass Co., Vineland, N.J.

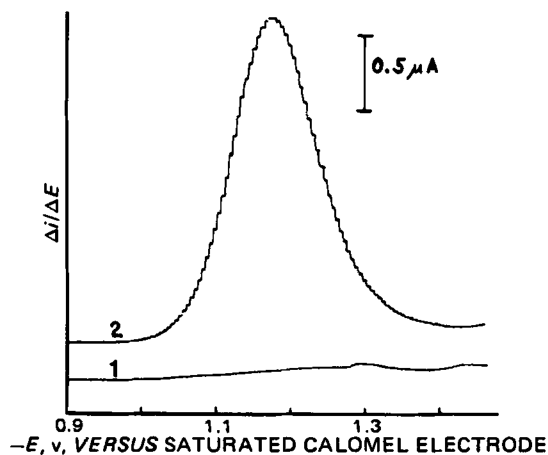

Figure 1—*Differential pulse polarogram of iodate. Key: 1, reagent blank; and 2, thyroid tablets.*

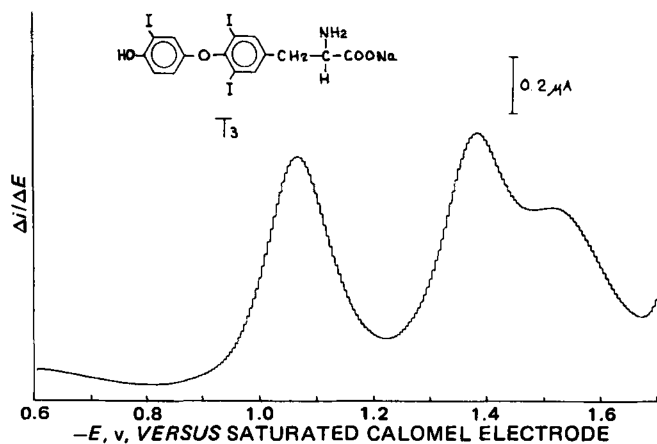

Figure 2—*Differential pulse polarogram of liothyronine standard.*

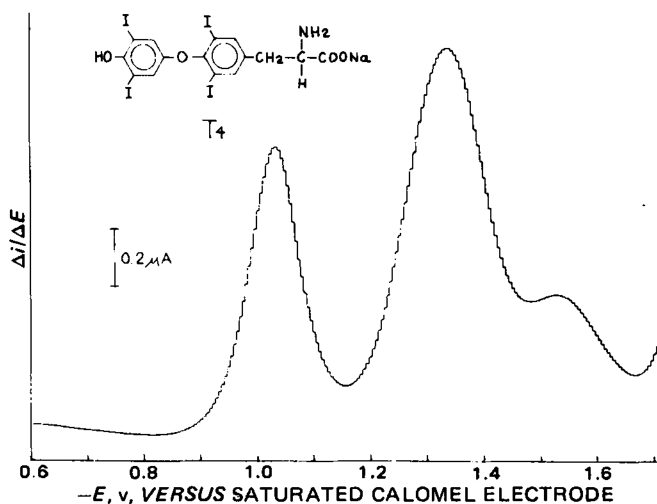

Figure 3—*Differential pulse polarogram of thyroxine standard.*

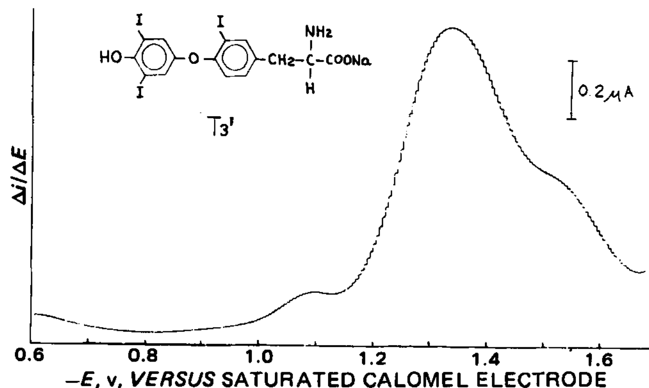

Figure 4—*Differential pulse polarogram of 3,3',5'-triiodothyronine standard. (This standard contained an impurity, probably liothyronine.)*

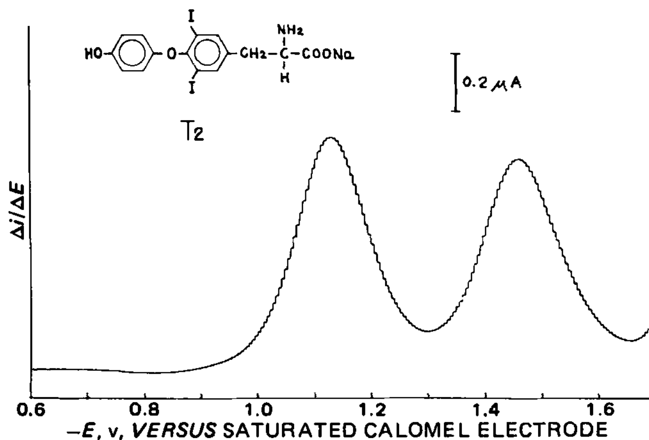

Figure 5—*Differential pulse polarogram of 3,5-diiodothyronine standard.*

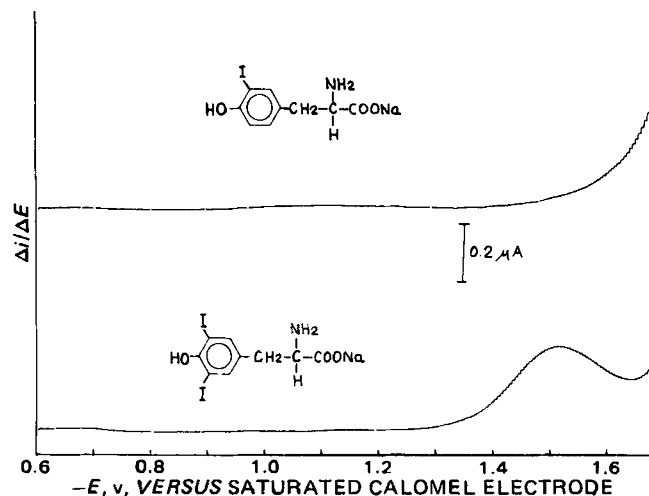

Figure 6—*Differential pulse polarograms of monoiodotyrosine and diiodotyrosine standards.*

electrolyte for liothyronine and thyroxine and transfer to the polarographic cell. Bubble nitrogen through the cell for 5 min and polarograph according to the described conditions.

Add the solution from the polarographic cell containing the sample to the evaporating dish containing the appropriate standard residue (either liothyronine or thyroxine) and swirl to dissolve. Quantitatively transfer the solution to the cell, wash the evaporating dish with solution in the cell, and then transfer back to the cell. Bubble in nitrogen for 3 min and polarograph using the same conditions as before. Calculate the percent of the hormone in the sample by the method of standard additions.

RESULTS AND DISCUSSION

Typical differential pulse polarograms of iodate and the iodoamino acid standards are shown in Figs. 1–6. Although only liothyronine and thyroxine are of interest, 3,3',5'-triiodothyronine, 3,5-diiodothyronine, diiodotyrosine, and monoiodotyrosine also were polarographed for comparison. Apparently, the sequential reduction of organically bound

Table I—Qualitative and Quantitative Differential Pulse Polarographic Behavior of Iodate and Iodoamino Acids Found in Thyroid

Compound	E_p, v, *versus* Saturated Calomel Electrode	Solution Concentration[a], μg/ml					Correlation Coefficient[b]
Iodate	−1.18	0.20 (0.22)	0.70 (0.75)	1.70 (1.66)	3.70 (4.02)	8.70 (9.74)	1.000
Liothyronine[c]	−1.06, −1.38, −1.53	2.34 (0.05)	8.19 (0.18)	19.89 (0.44)	31.59 (0.70)	43.29 (1.02)	0.999
Thyroxine[c]	−1.03, −1.34, −1.53	2.48 (0.06)	8.68 (0.21)	21.08 (0.55)	33.48 (0.95)	45.88 (1.26)	0.999
3,3′,5′-Triiodothyronine	−1.34, −1.53						
3,5-Diiodothyronine	−1.13, −1.46						
Diiodotyrosine	−1.52						
Monoiodotyrosine	N.R.[d]						

[a] Corresponding peak height in microamperes is given in parentheses. [b] Linear correlation coefficient showing relationship between concentration and peak height. [c] First peak used for quantitation. [d] No reduction before the decomposition of supporting electrolyte.

Table II—Analysis of Thyroid Powder for Iodine, Liothyronine, and Thyroxine Using the Differential Pulse Polarographic Method

	Liothyronine, %	Thyroxine, %	Iodine[a], %
	0.017	0.051	0.023
	0.016	0.046	0.200
	0.016	0.052	0.204
	0.015	0.049	0.206
Average	0.016	0.050	0.203
SD	0.001	0.003	0.003

[a] USP XIX method for iodine shows 0.202%.

Table III—Analysis of Thyroid Tablets of Various Strengths Using the Differential Pulse Polarographic Method and the USP XIX Procedure

Strength, mg	Differential Pulse Polarography			USP XIX,
	Liothyronine, %	Thyroxine, %	Iodine, %	Iodine, %
16	0.016	0.040	0.196	0.200
65	0.016	0.043	0.197	0.209
130	0.019	0.040	0.213	0.211
325	0.014	0.018	0.203	0.202

iodine proceeds as follows. The first peak results from reduction of iodine in the 5-position, the second peak results from the reduction of iodine in the 3′- and 5′-positions, and the third peak results from reduction of the iodine atom in the 3-position.

Table I summarizes the differential pulse polarographic behavior of iodate and six iodine-containing compounds found in thyroid. The correlation coefficients given in Table I indicate that a linear relationship exists between concentration and peak height for all three species of interest.

Thyroid is hydrolyzed by heating with barium hydroxide in a polyethylene vial. This procedure is more convenient than the common practice of using bottles or centrifuge tubes fitted with air condensers. After hydrolysis with barium hydroxide, liothyronine and thyroxine usually are isolated by extraction into 1-butanol, after which the alcohol is removed by evaporation. In this work, this time-consuming procedure was eliminated by use of an ion-exchange column that retained liothyronine and thyroxine. Then the two hormones were separated from each other by chromatography on mixed-bed resins and elution with solutions containing 1-propanol adjusted to a desired pH with buffers. The liothyronine and thyroxine standards also were subjected to the chromatographic procedure to compensate for possible adsorption on the columns and light-catalyzed degradation.

The constituents of the eluting solution must not be polarographically active in the region of interest, because traces always coelute with the iodoamino acids and can interfere. For this reason, succinic acid, instead of the polarographically active maleic acid, was used. Furthermore, to eliminate metal impurities such as zinc and to aid in the removal of dissolved oxygen, it was necessary to add disodium ethylenediaminetetraacetate and sodium sulfite to the supporting electrolyte.

The polarograms obtained in the reduction of iodoamino acids vary considerably with minor changes in the composition of the supporting electrolyte and the pH. To compensate for these variables, a quantitation procedure was employed using the method of standard addition. The volume of the supporting electrolyte containing the sample need not be known since a known weight of the standard is dissolved in this solution so that no volume change occurs as a result of the standard addition. This procedure simplifies the analysis and calculations. If several samples are to be analyzed, however, it may be advantageous to perform quantitations based on the calibration curve. In the latter case, accurate dilutions to volumes would be necessary. Figures 7 and 8 are polarograms obtained during the analysis of thyroid powder.

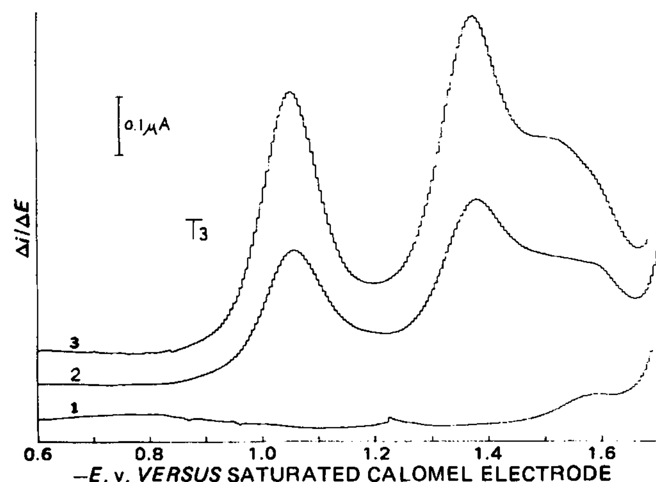

Figure 7—*Differential pulse polarogram of liothyronine sample. Key: 1, reagent blank; 2, liothyronine as derived from thyroid; and 3, after addition of liothyronine standard.*

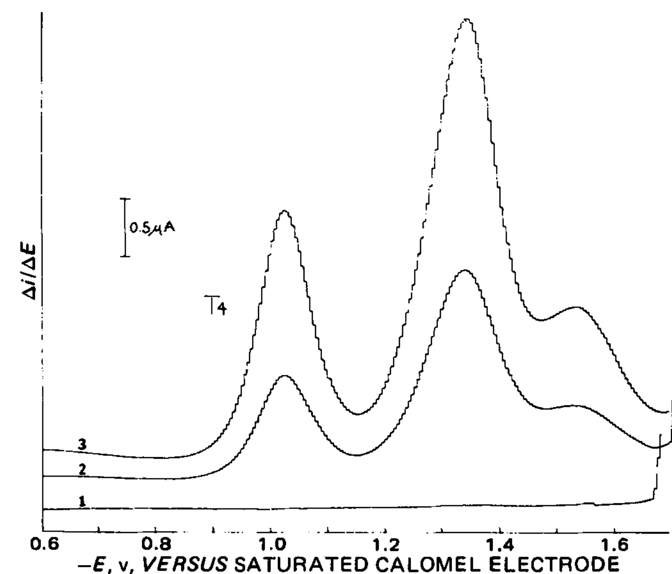

Figure 8—*Differential pulse polarogram of thyroxine sample. Key: 1, reagent blank; 2, thyroxine as derived from thyroid; and 3, after addition of thyroxine standard.*

Thyroid and thyroid tablets ranging in strength from 16 to 325 mg were analyzed for total iodine, liothyronine, and thyroxine by the proposed method. In addition, total iodine was determined by the USP XIX procedure. The results are given in Tables II and III. The iodine results agree with the USP analysis, and the values of liothyronine and thyroxine found in thyroid agree with literature results (1).

The procedures described in this work are relatively straightforward and are specific for the analysis of thyroid since intact liothyronine and thyroxine are determined.

REFERENCES

(1) J. E. Moody, Jr., J. R. Hohmann, Jr., and G. B. Kaplan, *J. Pharm. Sci.*, **57**, 634 (1968).

(2) R. Bilous and J. J. Windheuser, *ibid.*, **62**, 274 (1973).

(3) G. Knapp, H. Spitzy, and H. Leopold, *Anal. Chem.*, **46**, 724 (1974).

(4) I. M. Kolthoff and J. J. Lingane, "Polarography," 2nd ed., vol. II, Interscience, New York, N.Y., 1955, pp. 772–776.

(5) M. Brezina and P. Zuman, "Polarography in Medicine, Biochemistry and Pharmacy," rev. English ed., Interscience, New York, N.Y., 1958, pp. 216–221.

(6) D. C. M. Adamson, A. P. Domleo, J. F. Jeffries, and W. A. C. Shaw, *J. Pharm. Pharmacol.*, **4**, 760 (1952).

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Nonlinear Model for Acetazolamide

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Received March 20, 1978, from the *School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514.* Accepted for publication August 31, 1978. *Present address: School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261.

Abstract □ Intravenous bolus injections of ^{14}C-labeled acetazolamide were made in rabbits. Plasma, urine, and washed red blood cell concentrations were measured, the latter indicating bound drug. AUTOAN and NONLIN were used to fit the plasma data to a linear two-compartment model. However, utilization of the urine and red blood cell data suggested that a nonlinear model was more appropriate. The developed nonlinear system uses a one-compartment model with two tissue-binding parameters. The system simultaneously fits three equations describing drug in the plasma, in the body, and bound to red blood cells. Six parameters were estimated. The initial plasma concentration and the maximum amount bound to tissue protein (minus red blood cell protein) correlated with dose. The dissociation constant from this protein fraction suggested that it is composed mainly of the enzyme, carbonic anhydrase. The dissociation constant for the red blood cell fraction suggested that the drug binds to other protein in addition to carbonic anhydrase. The elimination constants were quite similar, indicating little variation from one animal to another. Utilization of the concepts of site and mechanism of action in this model should be of considerable help in relating drug concentration to pharmacological response.

Keyphrases □ Acetazolamide—nonlinear pharmacokinetic model, plasma, urine, and red blood cell data, intravenous bolus injection of ^{14}C-acetazolamide □ Pharmacokinetics—nonlinear model proposed for acetazolamide □ Models—nonlinear pharmacokinetic model proposed for acetazolamide

The efficacy and safety of drug therapy are commonly thought to be functions of the drug concentration at the site of action, but measurement of the drug concentration in remote tissues is usually difficult or impossible. Measurement of drug in the plasma is convenient and relatively easy, however, and plasma drug levels, although recognized to be vaguely related to effect, are used to evaluate therapy.

The common utilization of plasma levels to develop pharmacokinetic models and equations describing drug behavior in the body likewise depends largely on plasma measurements and thus yields equations that describe plasma levels quite accurately but that may not give useful estimates of drug concentrations in other tissues.

Recently, attention has been focused on equations that describe the time course of drug effect and that relate effect to plasma or peripheral compartment concentrations (1, 2). Pharmacokinetics must move in this direction to achieve the goal of enhancing effective and safe drug usage. This paper deals with such research on a well-known drug, acetazolamide.

Acetazolamide was selected since it was reported to be excreted entirely by the kidney in unchanged form (3, 4), its activity being uncomplicated by active metabolites. Furthermore, the mechanism of action is the inhibition of carbonic anhydrase (3, 5). The target organs of primary interest are the kidney, where the drug induces diuresis (3, 6), and the eye, where it causes reduction in intraocular pressure (5, 7–9).

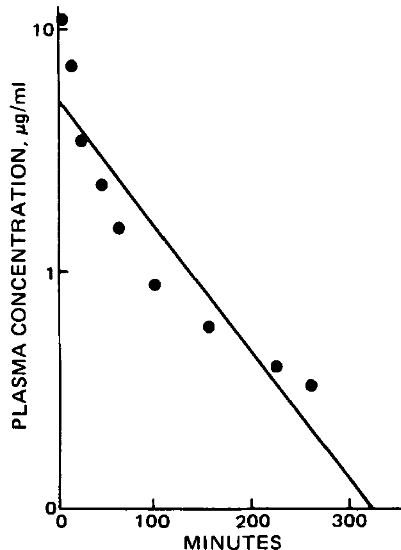

Figure 1—*Semilog plot of plasma acetazolamide concentration* versus *time.*

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