Metal Complexes of Thiouracils III: Polarographic Studies and Correlations among Complex Stabilities, Thiouracil Structures, and Biological Activities

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Abstract □ Although potentiometric studies of cupric ion–thiouracil mixtures were unable to permit the estimation of stability constants of the resultant complexes due to their instantaneous precipitation, the stoichiometric titer provided evidence for the formation and precipitation of either the mixed ligand complex of cupric ion with thiouracil and hydroxide ions (V) or the dimer, containing two cupric ions and two thiouracil anions (VII), and the complex of one cupric and two thiouracil ions (IV). Synthesis, isolation, and characterization of precipitated complexes gave evidence of the formed mixed ligand complex V that lost a molecule of water on drying to give bis(2-thiouracil)-μ-oxodicopper$^{\text{II}}$ (X). Polarographic studies showed a one-electron change attributable to the reduction of the singly charged complex of a cupric ion and a thiouracil anion (III) to an uncharged complex of a cuprous ion and a thiouracil anion at the dropping mercury electrode. The one-electron change could not be attributed to the reduction of the latter complex to copper metal since thiouracil reduction of cupric ion does not occur in acidic solution. Analyses of the $\Delta E_{1/2}$ data for the difference between complex half-wave potential and the half-wave potential of cupric-ion reduction to the cuprous state provided estimates of approximately 0.1 for the ratio of the dissociation constant of the singly charged complex of a cupric ion and a thiouracil anion (III) to the dissociation constant of the uncharged complex of a cuprous ion and a thiouracil anion. It was not possible to determine the absolute values of the cupric-ion complexes' stability constants from polarography. The relative tendency of metal ions to complex with thiouracils, $\text{Cu}^{+2} \gg \text{Pb}^{+2} > \text{Cd}^{+2} \gg \text{Ni}^{+2} \sim \text{Zn}^{+2}$, is consistent with the "natural order" of such transition metals, their relative ionic radii, their electronic structure, and the concept that "soft acids" prefer to complex with "soft bases." The presence of a free sulfhydryl group at the 2-position of pyrimidines appears necessary both for metal complexation and for antithyroid activity. Substituents on the ring nitrogens of thiouracil or alkyl substitution on the sulfur, which prevented the possibility of hydrogen tautomerism to the sulfur atom, destroyed complexation ability and biological activity. Substituents at the 5-position with high electronegativity decreased the pK′a of 2-thiouracils, reduced the stability constants of the metal complexes, and minimized the antithyroid activities.

Keyphrases □ Thiouracil–metal complexes—polarography, correlations among complex stabilities, thiouracil structures, and biological activities □ Polarography—metal complexes of thiouracils □ Structure–activity relationships—thiouracil–metal complexes, correlations among complex stability, thiouracil structure, and biological activity □ Potentiometric titration—metal complexes of thiouracils

Potentiometric titration studies of complexes of variously 5- and/or 6-substituted 2-thiouracil anions (II) with divalent metal ions have permitted the determination of the stability constants of the singly positively charged 1:1 complexes (III) and the uncharged 1:2 complexes (IV) formed in homogeneous solutions with the divalent metal ions: $\text{Pb}^{+2} > \text{Cd}^{+2} \gg \text{Ni}^{+2} \sim \text{Zn}^{+2}$ (1), where the relative order of stabilities of the complexes for these metal ions is as given. The metal ions reacted with the sulfide ion in the 2-position. No complexation of thiouracils with Fe^{+3}, Fe^{+2}, Co^{+2}, Ca^{+2}, or Mn^{+2} was observed potentiometrically (1) or spectrophotometrically (2). The high insolubility of the cupric complexes did not permit the evaluation of their stability constants. Spectral techniques were not sufficiently sensitive to do other than demonstrate that cupric complexes had the highest stabilities (2). Complexes of 1:2 stoichiometry (IV) containing a cupric ion and two thiouracil anions were synthesized as well as the cyclic dimers containing two divalent metal ions and two thiouracil anions (VII), formed from the dimerization of the zwitterion (VI) which resulted from the ionization of the 4-hydroxyl of the singly positively charged 1:1 complex of a divalent metal ion and a thiouracil anion (III). It was also shown that the logarithm of the stability constant for the positively charged 1:1 complex (III) of Cd^{+2} and a substituted 2-thiouracil anion (II) decreased linearly with decreasing pK′a of the thiouracil acid. Structures of the various compounds are given in Scheme I. When sulfhydryl formation in thiouracil was blocked by prohibiting tautomerization, as with 6-methyl-*N*,*N*′-diethyl-2-thiouracil, or by alkylation of the sulfur, no complexation with metal ions was observed.

These subsequent studies were made to utilize polarographic techniques in an attempt to determine the stability constants of the more stable and highly insoluble cupric complexes of the various thiouracils.

In addition, an attempt was made to rationalize the mechanistic bases of the relative complex stabilities and to consider possible correlations of these stabilities with the antithyroid activities of the substituted 2-thiouracil compounds.

EXPERIMENTAL

Materials—The sources, preparation, and characterization of the 2-thiouracil, 6-*n*-propyl-2-thiouracil, 6-methyl-2-thiouracil, 5,6-dimethyl-2-thiouracil, 5-methyl-2-thiouracil, 5-carboethoxy-2-thiouracil, 6-methyl-*N*,*N*′-diethyl-2-thiouracil, and 2-ethylmercapto-4-hydroxypyrimidine, 2-thiouracil disulfide monohydrate, and the trihydrated disodium salt of 2-sulfinyl-4-hydroxypyrimidine were as given previously (1, 2). The solutions of analytical grade cupric nitrate were standardized with the mercury, mercury–edetic acid electrode in the manner previously described (1).

Potentiometric Titrations—The equipment and standardization procedures were described previously (1). Sample solutions for titration were prepared with constant concentrations of the substituted thiouracil (2×10^{-3} *M*) and with 2.00, 1.60, 1.00, and 0.40×10^{-3} *M* cupric nitrate. In addition, mixtures of $0.20\text{–}2.00 \times 10^{-3}$ *M* 2-thiouracil in 2×10^{-4} *M* increments were prepared with these stated cupric nitrate concentrations. The ionic strength was maintained constant at 0.006 *M* by substituting 0.03 *M* sodium perchlorate for equal volumes of 0.01 *M* cupric nitrate, since the ratio of the molar concentrations of sodium perchlorate to cupric nitrate is 1:3 for equal ionic strengths. Precipitation was immediately observed in all solutions on mixing. These solutions were titrated immediately after preparation with 0.1000 *N* NaOH. Typical curves for titrations of 25 ml. of such preparations are shown for

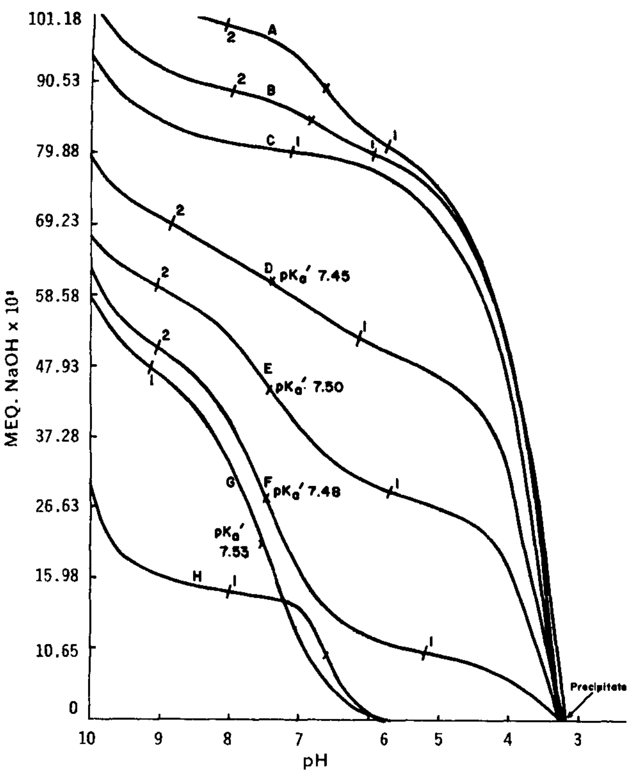

Figure 1—*Potentiometric titration curves of aqueous mixtures of cupric nitrate and 2-thiouracil containing precipitated complex, with* $\mu = 0.006$ *at 25.0°. Twenty-five-milliliter solutions were* 2.00×10^{-3} M *in 2-thiouracil and:* (*A*) 2.00×10^{-3} M, (*B*) 1.80×10^{-3} M, (*C*) 1.60×10^{-3} M, (*D*) 1.00×10^{-3} M, (*E*) 6.00×10^{-4} M, (*F*) 2.00×10^{-4} M, *and* (*G*) *zero* M *in cupric nitrate. Curve H is the titration of 25.0 ml. of* 4.00×10^{-4} M *cupric nitrate. The titer of alkali between inflections 1 and 2 is:* (*D*) 1.57×10^{-2}, (*E*) 2.90×10^{-2}, *and* (*F*) 4.30×10^{-2} *meq.*

cupric nitrate and 2-thiouracil mixtures containing precipitated complexes (Fig. 1). Similar preparations were left standing overnight under nitrogen and were then filtered; four-fifths of the filtrate was potentiometrically titrated in a similar manner. Typical curves are shown for the aged and filtered cupric nitrate and 2-thiouracil mixtures in Fig. 2.

Polarographic Studies—The polarographic studies were performed on a recording polarograph[1] equipped with a water-jacketed H cell maintained at 21.0, 30.0, 35.0, 40.0, or 45.0°. All potentials were measured *versus* a saturated calomel electrode with an agar salt bridge and were corrected for possible potential error. A saturated agar plug with excess potassium nitrate (3*a*) was used in the salt bridge and was changed whenever the solid potassium nitrate became depleted. The potassium nitrate was necessary since potassium chloride would have interfered by producing the anodic wave of the chloride ion. All test solutions were deoxygenated with water-saturated nitrogen gas (passed through a sparger immersed in water) for at least 10 min. and were maintained in a nitrogen atmosphere (nitrogen gas flowing over the solution) during the analysis. The capillary constant ($m^{2/3}t^{1/6} = 2.215$) was determined by running a supporting electrolyte solution (0.2 M $NaClO_4$) for a long, accurately measured period of time (2042.8 sec.), weighing the mercury delivered (4.9185 g.), and counting the number of drops determined from the number of pen oscillations. The values of $t^{1/6}$ and $m^{2/3}$ were calculated from the experimentally determined seconds per drop (t) and the milligrams of mercury dropped per second (m). The height of the mercury column was held constant at 58.5 cm. The current and voltage were always standardized before each curve was run.

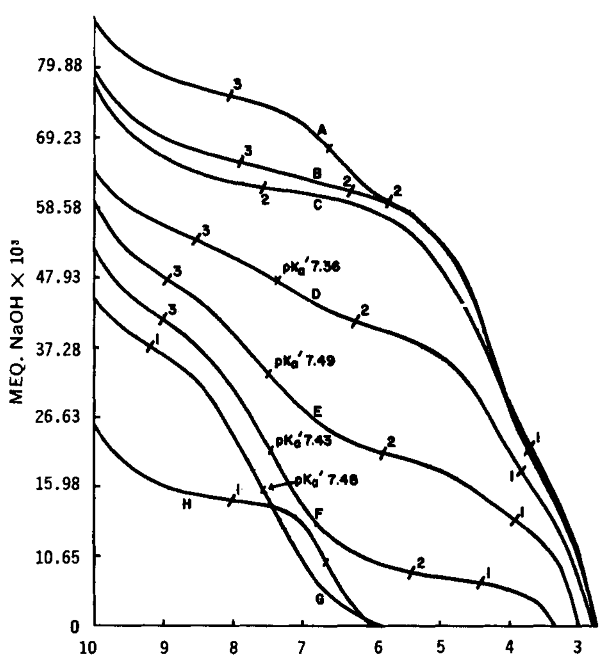

Figure 2—*Potentiometric titration curves of filtered, day-old cupric nitrate-2-thiouracil mixtures, with* $\mu = 0.006$ *at 25.0°. Twenty-milliliter aliquots were* 2.00×10^{-3} M *in 2-thiouracil and:* (*A*) 2.00×10^{-3} M, (*B*) 1.60×10^{-3} M, (*C*) 1.40×10^{-3} M, (*D*) 1.00×10^{-3} M, (*E*) 6.00×10^{-4} M, (*F*) 2.00×10^{-4} M, *and* (*G*) *zero* M *in cupric nitrate. Curve H is the titration of 25.0 ml. of* 4.00×10^{-4} M *cupric nitrate. The titer of alkali between inflections 2 and 3 is:* (*D*) 1.22×10^{-2}, (*E*) 2.50×10^{-2}, *and* (*F*) 3.52×10^{-2} *meq.*

Polarography of Cupric-Ion Solutions—Aliquots (0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, and 5.00 ml.) of a 0.0100 M cupric nitrate solution (prepared in 0.2 M $NaClO_4$) were added to 0.25 ml. of 0.2% tyloxapol[2] and 5.00 ml. of standardized perchloric acid in a 50-ml. volumetric flask and diluted to the mark with 0.2 M $NaClO_4$. These solutions were purged with nitrogen, and the polarograms were recorded at 21.0°. The final perchloric acid concentrations were 0.100, 0.0100, 0.00100, and 0.000100 M. The specific compositions of the solutions are given in Table I. The final concentration of tyloxapol[2] maximum suppressor (0.001%) was that recommended in the literature (4–6) and was found to give reversible waves by its adherence to the expression for the equations of polarographic waves of the familiar symmetrical form (3*b*):

$$E_{\text{DME}} = E_{1/2} - \frac{2.303RT}{nF} \log \frac{i}{i_d - i} \qquad \text{(Eq. 1)}$$

It also follows that:

$$E_{3/4} - E_{1/4} = \frac{-2.303RT}{nF} \log \frac{3.00}{1/3} \qquad \text{(Eq. 2)}$$

where $E_{3/4}$ is the potential, i, at $^3/_4$ of the observed diffusion current, i_d, in μamp.; $E_{1/4}$ is the potential, i, at $i_d/4$; R is the gas constant in cal./deg.-mole; T is the absolute temperature; F is the faraday (23,060cal./abs. v. g. eq.); and n is the number of electrons involved in the electrode reaction. The values of $-(2.303RT \log 9)/F$ at different temperatures are: 0.0557 (21°), 0.0573 (30°), 0.0583 (35°), 0.0592 (40°), and 0.0602 (45°). All diffusion currents were measured at the half-wave potential. The peak of the pen oscillation was used to calculate all potentials and currents (7).

Polarography of Cupric-Ion-Thiouracil Mixtures—Solutions containing 1.0 ml. of 0.01 M cupric ion, 0.25 ml. of 0.2% tyloxapol[2], 5-ml. aliquots of standard perchloric acid (2.648, 2.00, 1.000, 0.800, 0.500, 0.100, 0.0100, and 0.00100 M), and varying volumes of 0.005 or 0.0025 M thiouracil derivatives (2-thiouracil, 6-*n*-propyl-2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, 5,6-dimethyl-2-thiouracil, and 5-carboethoxy-2-thiouracil) were diluted to 50 ml. with 0.2 M $NaClO_4$. The 0.005 and 0.0025 M

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[1] Sargent model XV.

[2] Triton X-100, Rohm and Haas Co.

Table I—Effect of Daily Dose (in mg./100 g. Body Weight) of Substituted 2-Thiouracils on Rat Thyroid Weight (in mg./100 g. Body Weight) after 2 Weeks of Drug Diet

6-*n*-Propyl-2-thiouracil[a]		5,6-Dimethyl-2-thiouracil		5-Methyl-2-thiouracil		6-Methyl-*N*,*N*'-diethyl-2-thiouracil	
Dose[b]	Thyroid Weight[c]	Dose[d]	Thyroid Weight[c]	Dose[b]	Thyroid Weight[c]	Dose[b]	Thyroid Weight
0.00	5.7 ± 1.4	0.00	6.6 ± 0.9	0.00	4.5 ± 0.7	0.00	4.5 ± 0.8
1.29	25.0 ± 2.3	0.97	14.6 ± 1.1	0.95	11.1 ± 1.5	—	—
2.52	28.0 ± 4.2	1.93	13.2 ± 0.9	1.98	13.8 ± 2.5	—	—
4.61	25.5 ± 2.7	3.86	11.8 ± 1.7	3.82	12.6 ± 1.6	7.31	6.9 ± 1.7

[a] The literature values (27) for 2-thiouracil effect on thyroid weights are almost identical with 6-*n*-propyl-2-thiouracil where the respective daily dose and thyroid weight were: 0.04, 7.6; 0.13, 7.6; 0.4, 8.6; 1.2, 11.8; 3.6, 21.4; and 10.0, 23.4. [b] The amount of drug ingested per day was determined by weighing the diet supplied each day and correcting for the amount uneaten. [c] These are given as the mean thyroid weights of five rats ± the standard deviation among these weights. [d] In this one case the dose was estimated from the average daily consumption of 3.86 g./100 g. of body weight.

thiouracil solutions were prepared in 0.2 *M* $NaClO_4$ and, as the volume of thiouracil solution was decreased, an equal volume of 0.2 *M* $NaClO_4$ was substituted to maintain the ionic strength at 0.2 *M*. The solutions were purged with nitrogen as previously described, and the polarograms were recorded at controlled temperatures (21.0, 30.0, 35.0, 40.0, and 45.0°). A 1.00-ml. aliquot of 0.2 *M* $NaClO_4$ was substituted for the cupric ion in the blank solutions of ligand which were run for each test solution. The curve for the blank was subtracted from the test solution curve; the half-wave potential, the $E_{3/4} - E_{1/4}$ value, and the diffusion current were determined from the difference curve. A typical example of the determined difference curve from typical polarograms of solutions of ligand and cupric ion and of ligand alone is given in Fig. 3. Each test solution was run at least three times, and the potential and current measurements were averaged. The peak of the pen excursion was used to calculate all potentials and currents (7) and to give such plots as in Fig. 3.

Polarography of Solutions Containing Cuprous Ion in the Presence of 2-Thiouracil and 2-Thiouracil Disulfide—Cuprous chloride was purified by dissolution of 5 g. of cuprous chloride (light green in color) in 100 ml. of concentrated hydrochloric acid to give a black solution containing $CuCl_2^-$. The addition of 300 ml. of water gave a light-green solution from which white crystals of pure cuprous chloride precipitated (8). The following qualitative tests were run on the precipitated cuprous chloride. A solution of ammonium hydroxide (28%) added to the cuprous chloride gave a colorless solution which slowly turned dark blue $[Cu(NH_3)_2]^{+2}$. Concentrated hydrochloric acid added to the cuprous chloride gave a colorless solution ($CuCl_2^-$) which rapidly turned dark. Addition of potassium iodide to the colorless $CuCl_2^-$ solution produced a white precipitate (cuprous iodide).

Polarograms of the following solutions were run as previously described. Solution A contained 1.979 mg. CuCl, 1.0 ml. of 0.2 *M* $NaClO_4$, 0.25 ml. of 0.2% tyloxapol, 5.0 ml. of 2.648 *M* $HClO_4$, and 43.75 ml. of 0.005 *M* 2-thiouracil (in 0.2 *M* $NaClO_4$). Solution B contained 1.554 mg. 2-thiouracil disulfide, 0.25 ml. of 0.2% tyloxapol, 5.0 ml. of 2.648 *M* $HClO_4$, and 44.75 ml. of 0.2 *M* $NaClO_4$. Solution C contained 1.979 mg. CuCl, 3.148 mg. 2-thiouracil disulfide, 10 ml. of 2.648 *M* $HClO_4$, 0.5 ml. of 0.2% tyloxapol, and 89.5 ml. of 0.2 *M* $NaClO_4$.

Polarography of Presumed Cuprous Complex of 2-Thiouracil—The presumed cuprous chloride complex of 2-thiouracil was synthesized according to the literature (9). The procedure for the synthesis was the dissolution of 1.3 g. of 2-thiouracil in 150 ml. of hot water with the addition of 40 ml. of 1.0 *M* CuCl in concentrated hydrochloric acid solution. The formed yellow crystals were washed with water and then acetone and dried in a vacuum oven at 50°.

Two solutions of the presumed complex were prepared. The first solution contained 1.883 mg. of this material, 0.25 ml. of 0.2% tyloxapol, and 5.0 ml. of 3.0 *M* $HClO_4$ and was diluted to 50 ml. with 0.2 *M* $NaClO_4$. The second solution contained 1.876 mg. of the material, 0.25 ml. of 0.2% tyloxapol, 5.0 ml. of 3.0 *M* $HClO_4$, and 14.75 ml. of 0.005 *M* 2-thiouracil (in 0.2 *M* $NaClO_4$) and was diluted to 50.0 ml. with 0.2 *M* $NaClO_4$. Both solutions were run on the polarograph at 21.0°. The solutions were purged with nitrogen as usual, and a blank solution containing everything but the presumed complex was run in both cases.

Synthesis of Bis(2-thiouracil)-μ-dihydroxodicopper^{II} (IX)—A solution containing 0.062 mole of cupric ion in 100 ml. of water was added slowly, with stirring, to 400 ml. of a warm, aqueous solution (about 1.0 l.) containing 0.062 mole of 2-thiouracil. Very fine, light-yellow crystals slowly formed which were removed by filtration, washed with water and acetone, and dried at 120° overnight. The elemental analysis was performed on material dried to constant weight. UV assay (2) of the 2-thiouracil content was 62.2%; the calculated value for $C_8H_8Cu_2N_4O_4S_2$ is 61.7%. The procedure for the assay was to put an accurately weighed amount of complex into hot 1.0 *M* $HClO_4$ for 1 day and then read the UV spectrum *versus* a 1.0 *M* $HClO_4$ blank. The concentration of 2-thiouracil was determined from the molar absorptivity (ϵ = 13,700) at 273 nm. IR spectrum, $\bar{\nu}$ in cm.$^{-1}$ (mineral oil mull): 3090, 1640, 1600, 1540, 1280, 1160, 1070, 1015, and 825. The weight loss when dried at 100° under vacuum for 2 weeks was 4.57%. The calculated value for $C_8H_8Cu_2N_4O_4S_2$ is 4.3%.

Anal.—Calc. for $C_8H_6Cu_2N_4O_3S_2$: (X) C, 24.18; H, 1.52; Cu, 31.98; N, 14.10; S, 16.14. Found: C, 24.54, 24.06; H, 1.62, 1.66; Cu, 32.84; N, 14.33; S, 19.48, 17.51.

Synthesis of Bis(6-*n*-propyl-2-thiouracil)-μ-oxodicopper^{II}—The procedure for the preparation of bis(6-*n*-propyl-2-thiouracil)-μ-oxodicopper^{II}, which is similar to X except for the 6-*n*-propyl substituted on the 2-thiouracil, from 6-*n*-propyl-2-thiouracil was the same as from 2-thiouracil. The yellow precipitate was dried to constant weight at 120°. The procedure for the spectrophotometric an-

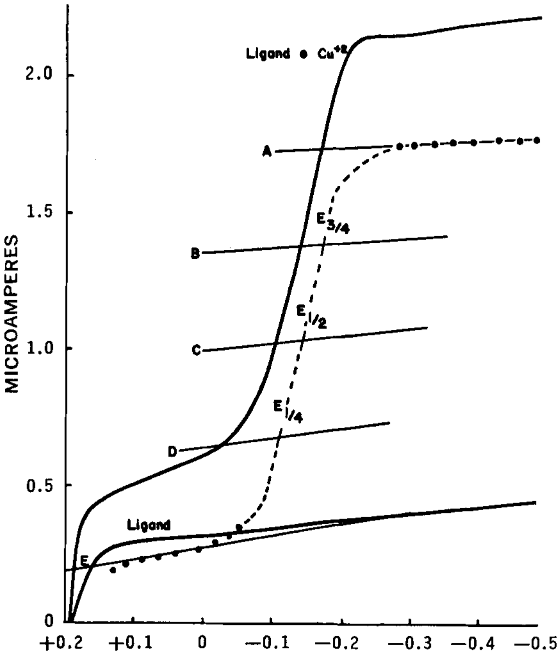
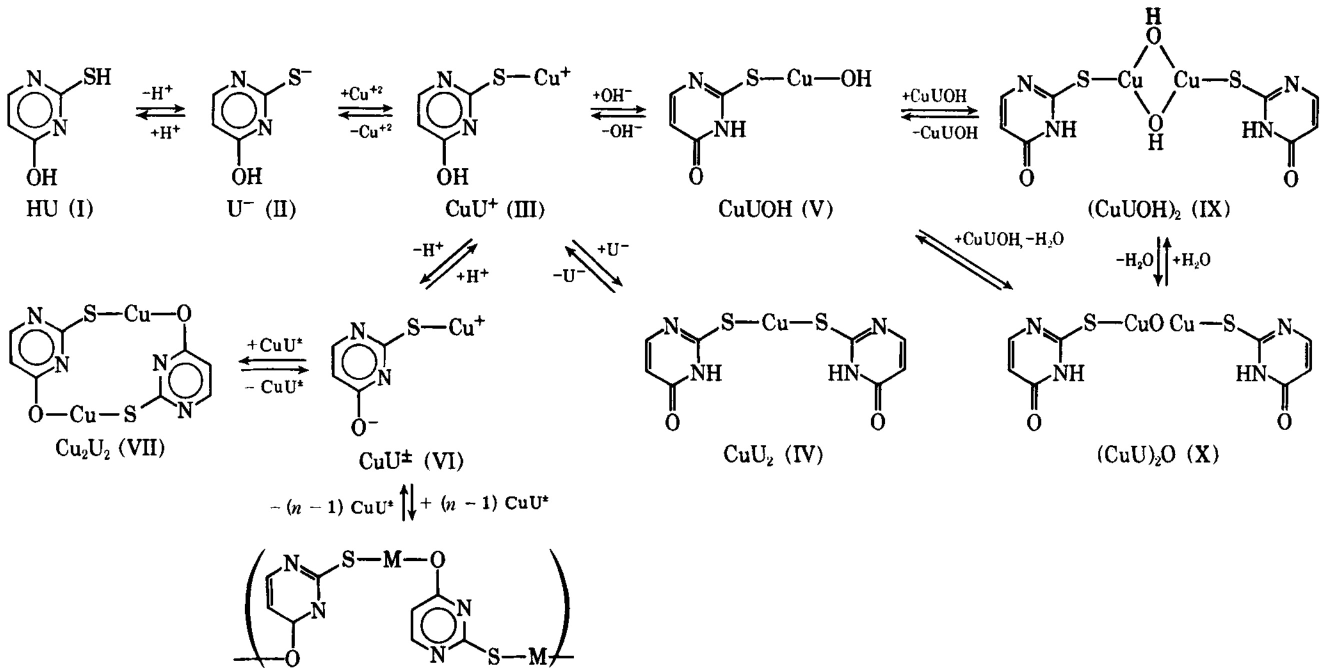


Figure 3—*Polarogram of a test solution of 2.00 × 10^{-4}* M *cupric nitrate in the presence of 1.875 × 10^{-3}* M *2-thiouracil and 0.0800* M *$HClO_4$ at 21.0°. Segments A and E are portions of the dashed difference curve obtained by subtraction of the ligand curve polarogram for the blank without cupric ion from the polarogram for the ligand plus cupric-ion test solution. Lines B, C, and D are at three-fourths, one-half, and one-fourth of the vertical distance between A and E and intersect the dashed difference curve at the* $E_{3/4}$, $E_{1/2}$, *and* $E_{1/4}$ *as read on the abscissa in volts.*



Scheme I—Possible relations among the cupric-ion complexes of thiouracils in solution. Polarographic evidence exists in support of $Cu(U)^+$ *(III) in acidic solution. Potentiometric titrations of precipitating solutions provide evidence for the presence and precipitation of CuUOH (IX) with lessened amounts of* $Cu(U)_2$ *(IV). The existence of* $(CuU)_2O$ *(X) is consistent with spectral and elemental analyses. These can result from dehydration of the mixed ligand precipitate CuUOH (IX) or the presence in solution of* $(CuUOH)_2$ *(IX) as evidenced by the normal existence of a twin hydroxo bridge in solutions of cupric ion (19). The potential existence of* $Cu(U)^{\pm}$ *(VI),* $Cu_2(U)_2$ *(VII), and* Cu_nU_n *(VIII) is argued on the basis of analogy to the complexation of thiouracil with other metal ions (1, 2).*

alysis of the percent of 6-*n*-propyl-2-thiouracil was also the same. IR spectrum, $\bar{\nu}$ in cm.$^{-1}$ (mineral oil mull): 3040, 1640, 1540, 1490, 1440, 1270, 1210, 1170, 1020, 955, and 835.

Anal.—Calc. for $C_{14}H_{18}Cu_2N_4O_3S_2$: Cu, 26.39; 6-*n*-propyl-2-thiouracil, 70.7. Found: Cu, 26.82; 6-*n*-propyl-2-thiouracil, 70.6.

Determination of Antithyroid Activities of Various Substituted 2-Thiouracils—Twenty white male Carworth CFN rats (120–200 g.) were evenly divided into four groups with five rats per group. One group was used as a control and was fed Rockland rat diet. The other three groups were fed the same diet containing 0.025, 0.050, and 0.100 wt. % of the substituted thiouracil for 2 weeks. Both diet and water were supplied *ad libitum*. The amount of drug ingested per day was determined by weighing the diet supplied each day and then weighing the amount uneaten at the next feeding period. The feeding cup was designed so that the feed could not be contaminated with feces or urine. No uneaten feed was observed out of the cup.

The doses were chosen with the hope that a dose–response relationship between drug doses and thyroid weight could be obtained. Body weights were measured just before sacrifice after 2 weeks of the drug diet. The weights of thyroid glands were determined on a torsion balance. The effects of 6-*n*-propyl-2-thiouracil, 5-methyl-2-thiouracil, and 5,6-dimethyl-2-thiouracil on thyroid weights were determined. The effect of a Rockland rat diet containing 0.100% 6-methyl-*N*,*N'*-diethyl-2-thiouracil on thyroid weight of five rats was also determined against a control group. The mean value of the thyroid weights for an average daily dose of each substituted 2-thiouracil studied is given in Table I.

THEORETICAL BASES FOR EVALUATIONS OF POLAROGRAPHIC DATA

Possible Nature of Polarographic Reduction of Complexes if Molar Electron Change, *n*, is 2—It has been shown (3*c*, 10, 11*a*) that the potential, E_{DME}, at the dropping mercury electrode (DME) for the diffusion-controlled rapid and reversible reduction of a complex of a divalent metal ion such as Cu^{+2} with *p* univalent ligand anions, U, to the metallic state (amalgam) and with $n = 2$ for the reaction:

$$\mathrm{Cu(U)}_p^{(n-p)+} + ne^- + \mathrm{Hg} \rightleftarrows \mathrm{Cu^0(Hg)} + p(\mathrm{U}^-) \quad \text{(Eq. 3)}$$

can be given by:

$$E_{\mathrm{DME}} = E_a^0 + (RT/nF)\ln a_{\mathrm{Hg}} + (RT/nF)\ln K_c + (RT/nF)\ln(f_ck_a/f_ak_c) - (RTp/nF)\ln f_{\mathrm{U}}[\mathrm{U}^-] - (RT/nF)\ln i/(i_{\mathrm{D}} - i) \quad \text{(Eq. 4)}$$

where the concentration of the ligand, $[\mathrm{U}^-]$, is relatively large compared to the concentration of the metal ion so that the concentrations of the ligand in the bulk solution and at the electrode surface are the same. The symbol F is the faraday; R is the molar gas constant; T is the absolute temperature; a_{Hg} is the activity of the mercury on the surface of the dropping electrode; f_a is the activity coefficient for the concentration of copper metal in the amalgam formed on the surface of the dropping electrode; f_c and f_{U} are, respectively, the activity coefficients for the concentrations at the electrode surface of the complex metal ion and the complexing agent U^-; k_a and k_c are proportional to the square roots of the diffusion coefficients of the metal ion in the amalgams and the complex metal ion in the solvent, respectively; K_c is the dissociation constant for the metal-ion complex $\mathrm{Cu(U)}^{(n-p)+}$; and n is the number of electrons in the reduction and is 2 in the case of reduction from the cupric state to the metal. The E_a^0 is the ordinary standard potential (3*d*) for the reduction of the simple (or aquo) cupric ion at the dropping mercury electrode:

$$\mathrm{Cu}^{+2} + 2e^- + \mathrm{Hg} \rightleftarrows \mathrm{Cu^0(Hg)} \qquad E_a^0 = -0.003 \textit{ versus } \text{normal calomel electrode} \quad \text{(Eq. 5)}$$

where the half-wave potential for the simple (or aquo) cupric ion can be expressed by:

$$(E_{1/2})_s = E_a^0 + (RT/nF)\ln a_{\mathrm{Hg}} - (RT/nF)\ln(f_ak_s/f_sk_a) \quad \text{(Eq. 6)}$$

where f_a and f_s, respectively, are the activity coefficients for the concentration of the amalgam formed on the surface of the mercury drops and the concentration of the reducible metal ions in the layer of the solution at the surface of the drops; and k_a and k_s are proportional to the square roots of the diffusion coefficients of the metal ion in the amalgams and in the body of the solution, respectively.

Equation 4 is of the form:

$$E_{\mathrm{DME}} = (E_{1/2})_c - (RT/nF) \ln i/(i_{\mathrm{D}} - i) \qquad \text{(Eq. 7)}$$

and is for a polarographic wave symmetrical about its midpoint and the half-wave potential coincides with its inflection point and center of symmetry. This $E_{1/2}$ can be defined by all but the last term on the right side of Eq. 4 and will obviously be a constant at any given $[\mathrm{U}^-]$ when $[\mathrm{U}^-]$ is in high excess of $[\mathrm{Cu}^{+2}]$. Since Eq. 4 is only valid for reversible reactions characterized by Eq. 7, the latter equation, or such variations of the latter as Eq. 2, can be used to test for this reversibility by demonstrating that $(E_{3/4} - E_{1/4})$ values are $1/n$ times the values of $-(2.303RT \log 9/F)$ given in the *Experimental* section for various temperatures. The value of n necessary to fulfill this requirement, if possible, is the number of electrons involved in the electrode reaction.

When Eqs. 4 and 7 are equated, it is apparent that:

$$\Delta E_{1/2} = (E_{1/2})_c = E^0 + (RT/nF) \ln a_{\mathrm{Hg}} + (RT/nF) \ln K_c + (RT/nF) \ln (f_c k_a/f_a k_c) - (RTp/nF) \ln f_{\mathrm{U}}[\mathrm{U}^-] \qquad \text{(Eq. 8)}$$

Thus the difference between Eqs. 8 and 6, or the shift of the half-wave potential caused by complex formation, can be expressed by:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = (RT/nF) \ln K_c - (RTp/nF) \ln f_{\mathrm{U}}[\mathrm{U}^-] + (RT/nF) \ln (f_c k_s/f_s k_c) \qquad \text{(Eq. 9)}$$

where, for the complete reduction of the complex of the divalent cupric ion to metallic copper, $n = 2$. The ratio $f_c k_s/f_s k_c$ can be approximated as unity on the assumptions of equivalent diffusivities of the simple and complex metal ions, *i.e.*, k_s and k_c, and of concentrations equal to activities, *i.e*, $f_c = f_s \sim 1$, so that the last term on the right may be negligible.

It follows that if $n = 2$, as determined from either the slope, RT/nF, of the plot of E_{DME} *versus* $\ln [i/(i_{\mathrm{D}} - i)]$ (Eq. 1) or the $-(2.303RT \ln 9)/nF$ values of $(E_{3/4} - E_{1/4})$, plots in accordance with Eq. 9 of the difference between the half-wave potentials for the polarographic reduction of the complex, $(E_{1/2})_c$, and the half-wave potentials for the reduction of the simple ion to metallic copper $(E_{1/2})_s$ against the logarithm of the concentration of the ligand anion, $\ln [\mathrm{U}^-]$, on the assumption of $f_{\mathrm{U}} \sim 1$, should permit the evaluation of the dissociation constant, K_c, of the $\mathrm{Cu(U)}p^{(2-p)+}$ complex from the intercept $(RT/nF) \ln K_c$ as well as the evaluation of p from the slope $-(RTp/nF)$.

Instead of employing K_c explicitly, $(RT/nF) \ln K_c$ in Eq. 9 may be replaced by $(E_c^0 - E_s^0)$, where E_c^0 and E_s^0 are the respective standard potentials of the ionic reactions:

$$\mathrm{Cu(U)}_p^{(2-p)+} + 2e \rightleftarrows \mathrm{Cu}(s) + p(\mathrm{U}^-) \qquad \text{(Eq. 10)}$$

and:

$$\mathrm{Cu}^{+2} + 2e \rightleftarrows \mathrm{Cu}(s) \qquad \text{(Eq. 11)}$$

so that:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = E_c^0 - E_s^0 - (RTy/nF) \ln f_{\mathrm{U}}[\mathrm{U}^-] \qquad \text{(Eq. 12)}$$

Possible Nature of Polarographic Reduction of Complexes if Molar Electron Change, *n*, is Unity—It has been shown (3*c*, 10, 11*a*) that the potential at the dropping mercury electrode for the rapid and reversible reduction of a complex of a divalent metal ion such as Cu^{+2}, with p univalent ligand anions, to a lower soluble oxidation state such as Cu^+ complexed with q univalent ligand anions and with $n = 1$, as for the reaction:

$$\mathrm{Cu(U)}_p^{(2-p)+} + ne^- \rightleftarrows \mathrm{Cu(U)}_q^{(1-q)+} + (p - q)(\mathrm{U}^-) \qquad \text{(Eq. 13)}$$

can be given by:

$$E_{\mathrm{DME}} = E_c^0 - \left(\frac{RT}{nF}\right) \ln \frac{k_0' f_r'}{k_r' f_0'} - \left(\frac{p - q}{n}\right) \frac{RT}{F} \ln f_{\mathrm{U}}[\mathrm{U}^-] - (RT/nF) \ln [i/(i_{\mathrm{D}}' - i)] \qquad \text{(Eq. 14)}$$

where the concentration, $[\mathrm{U}^-]$, of the ligand is relatively large compared to the concentration of the metal ion, so that the concentrations at the electrode surface and in the bulk solution are the same. The f_0' and f_r' are the activity coefficients for the oxidized and reduced states of the complexed metal ions, respectively, at the electrode surface; k_0' and k_r' are proportional to the square roots of the diffusion coefficients of the dissolved complexed metal ions in the oxidized and reduced forms, respectively; p and q represent the stoichiometric number of ligands bound to the oxidized, Cu^{+2}, and reduced, Cu^+, metal, respectively; E_c^0 is the simple standard potential of the reaction of Eq. 13; and n is the number of electrons in the reduction and is unity in the case of the reduction of the cupric complex ion to the cuprous complex ion. All other symbols are as previously given.

Equation 14 is of the form of Eq. 1 where:

$$(E_{1/2})_c = E_c^0 - \frac{RT}{nF} \ln \frac{k_0' f_r'}{k_r' f_0'} - \left(\frac{p - q}{n}\right) \frac{RT}{F} \ln f_{\mathrm{U}}[\mathrm{U}^-] \qquad \text{(Eq. 15)}$$

so that $(E_{3/4} - E_{1/4})$ values as per Eq. 2 may be used to test for the reversibility of the underlying reaction (*e.g.*, Eq. 13) and to estimate the n number of electrons involved in the electrode reaction.

The potential at the dropping mercury electrode for the rapid and reversible reduction of the simple or aquo divalent metal ion to the lower soluble oxidation state, *i.e.*, for the reaction:

$$\mathrm{Cu}^{+2} + ne^- \rightleftarrows \mathrm{Cu}^+ \qquad \text{(Eq. 16)}$$

is:

$$E_{\mathrm{DME}} = E_s^0 - \frac{RT}{nF} \ln \frac{i}{i_{\mathrm{D}} - i} - \frac{RT}{nF} \ln \frac{k_0 f_r}{k_r f_0} \qquad \text{(Eq. 17)}$$

where f_0 and f_r are the activity coefficients for the oxidized and reduced states of the simple or aquo metal ions, respectively, at the electrode surface; k_0 and k_r are proportional to the square roots of the diffusion coefficients of the dissolved simple metal ions in the oxidized and reduced forms, respectively; and E_s^0 is the simple standard potential of the reaction of Eq. 15, where n is the number of electrons in the reduction and is unity in the case of the reduction of the cupric ion to the cuprous ion.

Equation 17 is of the form of Eq. 1 where:

$$(E_{1/2})_s = E_s^0 - (RT/nF) \ln (k_0 f_r)/(k_r f_0) \qquad \text{(Eq. 18)}$$

It follows that on subtraction of Eq. 17 from Eq. 14:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = E_c^0 - E_s^0 - \frac{(p - q)}{n} \frac{RT}{F} \ln f_{\mathrm{U}}[\mathrm{U}^-] - \frac{RT}{nF} \ln \frac{k_0' f_r'}{k_r' f_0'} \frac{k_r f_0}{k_0 f_r} \qquad \text{(Eq. 19)}$$

which relates the change in the half-wave potential observed for the reduction of a cupric-ion complex to a cuprous-ion complex to the change in the standard potential of the cupric ion to that of the cuprous ion. The "primes" are used for parameters derived from complexes, and the "nonprimes" are used for the simple ions

It has not been possible to measure the diffusion coefficient, k_r, of the free cuprous ion because of its ease of disproportionation to cupric ion and copper metal. Since the ionic radii of the cuprous ion and the sodium ion are 0.96 and 0.95 Å, respectively, the charge densities of the two ions may be presumed to be similar. Thus, the probable size of the hydration sphere and, hence, the probable diffusion coefficient of the cuprous ion may be assumed to be similar to that of the sodium ion, *i.e.*, 1.35×10^{-5} cm.^{2} sec.$^{-1}$ (3*e*). Since cuprous ion is heavier than sodium ion and since it may exist in solution as the aquo ion, the value of k_r that is proportional to the square root of the diffusion coefficient may be overestimated on the basis of this assumption. If the Stokes–Einstein law is valid with respect to ionic species, the ratio of the diffusion coefficients of cuprous to sodium ion should be equal to the inverse ratio of the cube roots of their atomic weights, *i.e.*, $(22.9)^{1/3}/(63.5)^{1/3}$ (11*b*), so that the diffusion coefficient of cuprous ion may be estimated as 1.0×10^{-5} cm.^{2} sec.$^{-1}$. Thus, the ratio k_r/k_0 of the square roots of the diffusion coefficients of cuprous ions (1.0 or 1.35×10^{-5} cm.^{2} sec.$^{-1}$) and cupric ions [0.72×10^{-5} cm.^{2} sec.$^{-1}$ (3*e*)] is 1.1 or 1.3. If both the ratio of the square roots of the diffusion coefficients of the oxidized and reduced complexes and the ratio of the activity coefficients $f_r'f_0/f_0'f_r$ are taken as approximately unity, the calculated value of RT/nF ($\ln k_0'k_r/k_r'k_0$) will not exceed 5 mv., which is the experimental error in estimating an $E_{1/2}$ value. Thus, the last term on the right-hand side of Eq. 19 may be considered negligible and be ignored so that:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = E_c^0 - E_s^0 - \frac{(p - q)RT}{nF} \ln f_U[U^-] \qquad \text{(Eq. 20)}$$

may be considered as a satisfactory relation for the change between the half-wave potential observed for the reduction of a cupric-ion complex to a cuprous-ion complex and the change in the standard potential of the metal ion.

If the reversible reduction of the uncharged 1:2 cupric complex $Cu(U)_2$, (IV), to a lower soluble oxidation state occurs at the dropping mercury electrode, then in accord with Eq. 13, $p = 2$, $n = 1$, $q = 1$, and:

$$Cu(U_2) + e^- \rightleftarrows CuU + U^- \qquad \text{(Eq. 21)}$$

with a standard potential of E_c^0.

If the standard potential for the reduction of cupric ion to cuprous ion, $n = 1$ in Eq. 16, is E_s^0, the difference:

$$E^0 = E_c^0 - E_s^0 \qquad \text{(Eq. 22)}$$

can be written for the difference between Eqs. 16 and 21 as if it were the hypothetical reaction:

$$Cu(U)_2 + Cu^+ \rightleftarrows CuU + U^- + Cu^{+2} \qquad \text{(Eq. 23)}$$

since both Eqs. 16 and 21 are one-electron reactions. The equilibrium constant for Eq. 23 is:

$$K = K_0/K_r = \frac{[CuU][U^-][Cu^{+2}]}{[Cu^+][Cu(U)_2]} \qquad \text{(Eq. 24)}$$

which is the ratio of the dissociation constant of the oxidized complex to the reduced, *i.e.*:

$$K_0 = \frac{[Cu^{+2}][U^-]^2}{[Cu(U)_2]} \qquad \text{(Eq. 25)}$$

$$K_r = \frac{[Cu^+][U^-]}{[CuU]} \qquad \text{(Eq. 26)}$$

It follows from Eqs. 22–24 that:

$$E^0 = E_c^0 - E_s^0 = \frac{RT}{F} \ln K = \frac{RT}{F} \ln K_0/K_r \qquad \text{(Eq. 27)}$$

and substitution of Eq. 27 into Eq. 20 results in:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = \frac{RT}{F} \ln K_0/K_r - \frac{RT}{F} \ln f_U[U^-] \qquad \text{(Eq. 28)}$$

Since:

$$[U^-] = K_a'[HU]/[H^+]\gamma^\pm \qquad \text{(Eq. 29)}$$

appropriate substitution into Eq. 28, if $f_U \sim 1$, results in:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = \frac{RT}{F} \ln \frac{K_0}{K_r} \left(\frac{[H^+]\gamma^\pm}{K_a'}\right) - \frac{RT}{F} \ln [HU] \qquad \text{(Eq. 30)}$$

This difference in half-wave potentials for the reduction of the complex and the metal ion will be a function of the thiouracil-ion concentration (Eq. 28) which, in turn, is dependent on hydrogen-ion and undissociated thiouracil concentrations (Eqs. 29 and 30). If the initially added total concentration of thiouracil, $[HU]_T$, in highly acidic solutions is greatly in excess of the cupric-ion concentration, then the concentration of undissociated thiouracil, [HU], may be approximated by the total concentration of thiouracil added, $[HU]_T$.

If it is the reversible reduction of the charged cupric complex $Cu(U)^+$ to a lower soluble and uncharged oxidation state that occurs at the dropping mercury electrode, then in accord with Eq. 13, $p = 1$, $n = 1$, $q = 1$, and:

$$Cu(U)^+ + e^- \rightleftarrows CuU \qquad \text{(Eq. 31)}$$

It follows from Eq. 20 that:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = E_c^0 - E_s^0 = E^0 \qquad \text{(Eq. 32)}$$

and this difference in half-wave potentials will be independent of thiouracil-ion concentrations. Equation 32 can be written for the difference between Eqs. 16 and 31 as if it were the hypothetical reaction:

$$Cu(U)^+ + Cu^+ \rightleftarrows CuU + Cu^{+2} \qquad \text{(Eq. 33)}$$

since both Eqs. 16 and 31 are one-electron reactions. The equilibrium constant for Eq. 33 is:

$$K = K_0'/K_r = \frac{[CuU][Cu^{+2}]}{[Cu(U)^+][Cu^+]} \qquad \text{(Eq. 34)}$$

which is the ratio of the dissociation constant of the charged oxidized complex:

$$K_0' = \frac{[Cu^{+2}][U^-]}{[Cu(U)^+]} \qquad \text{(Eq. 35)}$$

to that of the reduced K_r of Eq. 26.

It follows that:

$$E^0 = E_c^0 - E_s^0 = \frac{RT}{F} \ln K = \frac{RT}{F} \ln \frac{K_0'}{K_r} \qquad \text{(Eq. 36)}$$

and substitution of Eq. 36 into Eq. 32 results in:

$$\Delta E_{1/2} = (E_{1/2})_c - (E_{1/2})_s = \frac{RT}{F} \ln \frac{K_0'}{K_r} \qquad \text{(Eq. 37)}$$

and this difference in half-wave potentials for the reduction of the complex and the metal ion will not be a function of the thiouracil-ion concentration.

The value of $(E_{1/2})_s$ for the reduction of cupric ion to cuprous ion is not experimentally determinable, because cuprous ion is more readily reducible than cupric ion and a two-electron change always occurs (3*c*). However, the $(E_{1/2})_s$ can be calculated (3*c*) at any temperature from Eq. 18, where E_s^0 is the standard potential of the reversible reduction of Eq. 16 where k_0/k_r is the ratio of the diffusivity constants of the two ions and may be estimated as 1.1 to 1.3 as stated previously. This ratio may be considered constant for small temperature changes, since the temperature effect on a diffusion constant is only 2% per degree (3*e*). The standard potential, E_s^0, can be calculated from the known standard free energy or entropy and enthalpy values at 25° in the literature (12) in accordance with:

$$\Delta F^0 = \Delta H^0 - T\,\Delta S^0 = -nFE_s^0 \qquad \text{(Eq. 38)}$$

where the standard enthalpy and entropy values taken are assumed to be constant for the temperature range used. The potentials of the saturated calomel electrode (SCE) at various temperatures are also available from the literature (13, 14). The values of E_s^0 calculated from Eq. 38 and corrected against the saturated calomel electrode and the $(E_{1/2})_s$ values estimated from Eq. 16 at several temperatures are, respectively: 21°: 0.1512, −0.0878; 25°: 0.1514, −0.0837; 35°: 0.1521, −0.0806; 40°: 0.1526, −0.0755; and 45°: 0.1530; −0.0744. The value of $(E_{1/2})_s$ at 25° agrees very well with the −0.079-v. value calculated in the literature (3*e*), even though the literature value was not corrected for the difference in diffusion coefficients of cupric and cuprous ions. The literature value of the half-wave potential for the reduction of cuprous to free copper is +0.143 v. *versus* the saturated calomel electrode at 18.0° (15) so that, at potentials more negative than +0.143 v., a two-electron reduction would have to be expected if free cuprous ion is produced from the uncharged reduced complex of one cuprous ion and one thiouracil anion, CuU.

Table II—Effects of Cupric Ion and Perchloric Acid Concentrations on Diffusion Current and Wave Potentials for the Polarographic Reduction of Cupric Nitrate[a] at 21°

$[Cu^{+2}]$	$[HClO_4]$	i_D[b]	$E_{1/2}$[c]	$-(E_{3/4} - E_{1/4})$	m[d]
0.0010	0.1	9.00	0.0398	0.0327	2
—	0.01	9.07	0.0413	0.0307	2
—	0.001	9.12	0.0413	0.0323	1
—	0.001	8.90 ± 0.10	0.0413 ± 0.0010	0.0289 ± 0.0020	4
0.0008	0.001	7.33	0.0402	0.0336	2
0.0006	0.01	5.45 ± 0.03	0.0414 ± 0.0010	0.0293 ± 0.0020	3
0.0005	0.0001	4.44 ± 0.03	—	0.0325 ± 0.0004	3
0.0004	0.001	3.55 ± 0.03	—	0.0311 ± 0.0010	3
0.0003	0.01	2.74 ± 0.02	0.0427 ± 0.0012	0.0318 ± 0.0005	8
0.0002	0.100	1.84 ± 0.02	0.0397 ± 0.0015	0.0339 ± 0.0020	5

[a] All solutions were 0.2 M in $NaClO_4$ and contained 0.001% tyloxapol. The plus or minus values are estimates of the standard deviations. [b] The i_D values in microamperes were obtained by doubling the current, i, measured at the $E_{1/2}$. [c] The average value of the half-wave potential in volts *versus* SCE is 0.0411. [d] Number of trials on which the stated mean values are based.

RESULTS

Potentiometric Titrations of Various Thiouracil–Cupric-Ion Mixtures—The addition of an amount of cupric nitrate as low as 2×10^{-4} M to a 2×10^{-3} M aqueous solution of a 2-thiouracil (2-thiouracil, 6-*n*-propyl-2-thiouracil, 6-methyl-2-thiouracil, 5-methyl-2-thiouracil, and 5,6-dimethyl-2-thiouracil) caused an immediate drop in pH from about 5.5 to about 3.0 and the immediate formation of a precipitate, the amount of which increased with time. Attempts at potentiometric titration of such mixtures with smaller concentrations of thiouracils in the presence of minimal amounts of cupric nitrate were unsuccessful in maintaining a homogeneous solution. Since the complexes of cupric ion and the thiouracils had such low solubilities, it was technologically infeasible to use the potentiometric method which demands instantaneous equilibration in homogeneous solution for the estimation of complexation constants (1, 2) of cupric-ion–thiouracil complexes. However, some pertinent information was obtained from these titrations in the presence of the precipitate.

A typical set of potentiometric titration curves is given in Fig. 1 for solutions of 25.00 ml. of 2×10^{-3} M 2-thiouracil (25.0°, $\mu = 0.006$). Various amounts of cupric nitrate have been added to each of these solutions so that the original concentration ranged from 2.00×10^{-3} M to 0.00 M $Cu(NO_3)_2$ for curves A to G of Fig. 1. The plots are given in terms of the milliequivalents of sodium hydroxide necessary to achieve an observed pH value for each of these solutions. As indicated, precipitation was observed instantaneously on mixing except for the particular case (curve G) of the thiouracil in the absence of cupric ion where no precipitation was observed throughout the titration. The titration curve for 25.00 ml. of 4.00×10^{-4} M cupric nitrate is given as curve H. Two apparent inflections, labeled as 1 and 2 in Fig. 1, were observed in almost all cases of the cupric-ion–thiouracil mixtures except for curve G.

A typical set of potentiometric titration curves is given in Fig. 2 for the mixtures of Fig. 1 which were allowed to stand 1 day and were filtered prior to titration. On the addition of standard alkali, precipitation was immediate and continued with further titration. The titration curves of the aged mixtures (Fig. 2) indicated three apparent inflections (*i.e.*, 1, 2, and 3) and thus indicated the presence of at least three species in contrast to the two species of the immediately titrated mixtures (Fig. 1).

The resultant titration curves, A, B, and C (Figs. 1 and 2), showed a greater alkali consumption up to pH 6, a region where the hydrolysis of free cupric ion does not occur (curve H, Figs. 1 and 2), than the titration of the same amount of ligand alone (curve G, Figs. 1 and 2). It would be measured up to inflection 1 of the curves in Fig. 1 and up to inflection 2 of Fig. 2.

The two most acidic species disclosed by the titrations have apparent pK′a values of 3.1 and 4.4 and are readily seen in the curves A–E of Fig. 2 from the titer up to the inflection.

A comparison of the aged solutions with the same curves of Fig. 1 (where inflection 1 appears at the same pH labeled as inflection 2 in Fig. 2 and the net titer to these inflections did not appear to change on aging) indicates that an amount of the original species of apparent pK′a 4.4 (estimated from pH where half the milliequivalents to inflection 1, Fig. 1, are consumed) was transformed on aging to a mixture of species of apparent pK′a values 3.1 and 4.4.

The total milliequivalents of alkali consumed in the titration of the nearly equimolar mixtures to a pH of about 6 (*i.e.*, curves A and B to inflection 1 in Fig. 1 or to inflection 2 in Fig. 2) closely correspond to, but were less than, twice the milliequivalents of sodium hydroxide necessary to neutralize the same concentration of 2-thiouracil alone (curve G, Figs. 1 and 2).

If it were exactly double, the precipitated cupric–thiouracil complex would be indicated to be of 1:1 stoichiometry and the precipitate could be the net result of equal numbers of cupric ions displacing two protons per molecule of equal numbers of thiouracil molecules or the equivalent process of displacing one proton per thiouracil molecule and reacting with one hydroxyl ion. If the added milliequivalents of sodium hydroxide necessary to reach the pH 6 inflection (curves A and B to inflection 1 in Fig. 1 and to inflection 2 in Fig. 2) had been greatly in excess of twice the milliequivalents necessary to neutralize the ligand, it could be proposed that additional sodium hydroxide reacted with the precipitated complex and destroyed it or that the metal hydroxide $Cu(OH)_2$ was precipitated simultaneously. If all the metal had been reacted to form hydroxide, the milliequivalents of sodium hydroxide to these inflections would have been three times the milliequivalents of sodium hydroxide necessary to neutralize the ligand alone. If each metal ion had reacted with two thiouracil molecules, the milliequivalents of sodium hydroxide to these inflections would have been equal to the milliequivalents of sodium hydroxide necessary to neutralize the ligand alone (1). The fact that the milliequivalents of sodium hydroxide to these inflections lay between one and two times the milliequivalents necessary to neutralize the ligand alone implies that cupric-ion–thiouracil complexes precipitate that have mixed stoichiometry of 1:1 and 1:2.

Further confirmation is obtained from the titer between inflections 1 and 2, curves A and B of Fig. 1, and between inflections 2 and 3, curves A and B of Fig. 2, where cupric ion may be in slight excess. The apparent pK′a was comparable to the titration of cupric ion alone (curve H) and is most probably due to the precipitation of excess cupric ion. The titer in the other curves, D, E, and F, between inflections 1 and 2, Fig. 1, and between inflections 2 and 3, Fig. 2, can be assigned to the titration of excess ligand since the apparent pK′a of 7.49 is the same as that for the titration of the ligand thiouracil alone (curve G, Figs. 1 and 2).

The milliequivalents of sodium hydroxide consumed between inflections 1 and 2, Fig. 1, or inflections 2 and 3, Fig. 2, corresponded well to the molar excess of 2-thiouracil over cupric ion in the titrated mixtures. These facts were again highly indicative of a 1:1 stoichiometry of cupric-ion–thiouracil complexes in the precipitated complexes on titration.

Polarography of Cupric-Ion–Thiouracil Mixtures—A typical difference curve for the polarograms of test solutions of cupric ion–thiouracil mixtures and of blank solutions of thiouracils is given in Fig. 3. The typical polarogram of the ligand alone as given shows that the ligand is not reduced under the polarographic conditions.

The polarographic diffusion current, i_D, in microamperes (obtained by doubling the diffusion current, i, measured at the half-wave potential, $E_{1/2}$, in volts) and the voltage difference between the $^3/_4$ and $^1/_4$ wave potentials ($E_{3/4} - E_{1/4}$) are given for 21° and various cupric-ion and perchloric acid concentrations in Table II. The average value of $E_{1/2}$ *versus* SCE is 0.0411 v., similar to the value

Table III—Effects of 6-*n*-Propyl-2-thiouracil and Various Perchloric Acid Concentrations on the Polarographic Values in Volts of $(E_{3/4} - E_{1/4})$ and $[(E_{1/2})_c - (E_{1/2})_s]$[a] for 2.00×10^{-4} *M* Cupric Ion[b] at 21°

10^3[6-*n*-Propyl-2-thiouracil]	$-(E_{3/4} - E_{1/4})$	[HClO$_4$]	$[(E_{1/2})_c - (E_{1/2})_s] \pm \sigma$
4.375	−0.0582	0.0500	−0.1324 ± 0.0008
3.875	−0.0573		−0.1350 ± 0.0018
3.375	−0.0593		−0.1324 ± 0.0041
2.875	−0.0572		−0.1266 ± 0.0031
2.375	−0.0612		−0.1255 ± 0.0030
1.875	−0.0592		−0.1199 ± 0.0026
4.375	−0.0582	0.0800	−0.1266 ± 0.0013
3.875	−0.0582		−0.1319 ± 0.0009
3.375	−0.0533		−0.1305 ± 0.0005
2.875	−0.0572		−0.1258 ± 0.0019
2.375	−0.0572		−0.1193 ± 0.0022
1.875	−0.0553		−0.1151 ± 0.0004
1.375	−0.0571		−0.1119 ± 0.0025
4.375	−0.0552	0.100	−0.1248 ± 0.0012
3.875	−0.0612		−0.1288 ± 0.0027
3.375	−0.0543		−0.1223 ± 0.0020
2.875	−0.0612		−0.1067 ± 0.0137
2.375	−0.0572		−0.1135 ± 0.0085
1.875	−0.0601		−0.0789 ± 0.0001
1.375	−0.0602		−0.1045 ± 0.0024
4.375	−0.0572	0.200	−0.1206 ± 0.0035
3.875	−0.0562		−0.1266 ± 0.0028
3.375	−0.0582		−0.1209 ± 0.0040
2.875	−0.0572		−0.1185 ± 0.0043
2.375	−0.0613		−0.1198 ± 0.0030
1.875	−0.0578		−0.1112 ± 0.0036
1.375	−0.0563		−0.1066 ± 0.0014

[a] The difference in half-wave potentials of the complexed and simple cupric ions against SCE. The average value of $(E_{1/2})_s$ for cupric ion alone in acid was 0.0411 v. This value should be added to the $[(E_{1/2})_c - (E_{1/2})_s]$ values given to obtain the determined $(E_{1/2})_c$ values for the complexed cupric ions. [b] Ionic strength is constant at 0.20 *M* using sodium perchlorate. All solutions contained 0.001% tyloxapol as maximum suppressor. The diffusion current was constant at 1.48 μamp.

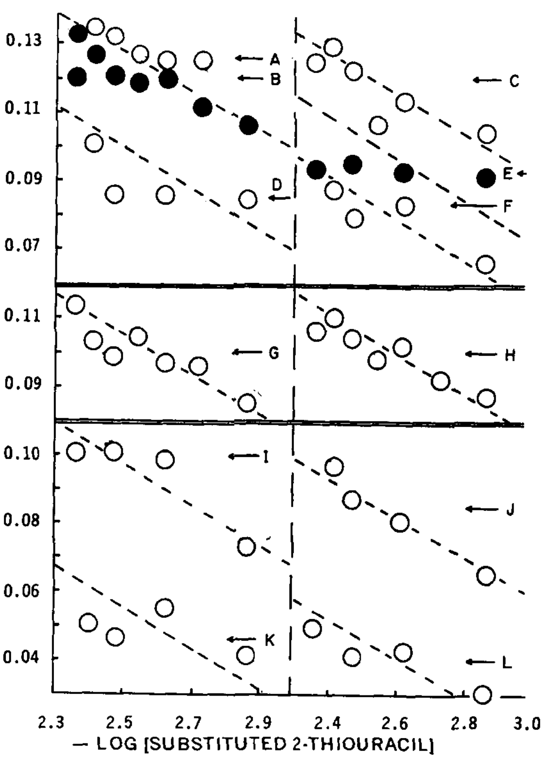


Figure 4—*Plots of the negative difference,* $-\Delta E_{1/2}$, *in the half-wave potentials,* $(E_{1/2})_c$ *for the polarographic reduction of the cupric-ion complex of 2-thiouracil,* 6-n-*propyl-2-thiouracil, and 5,6-dimethyl-2-thiouracil to a cuprous-ion complex and the half-wave potentials,* $(E_{1/2})_s$, *for the polarographic reduction of the simple cupric ion to its cuprous form against the negative logarithm of the substituted 2-thiouracil concentration,* [*HU*]. *The dashed lines are drawn with the theoretical slopes* 2.303RT/F *consistent with the expression* $\Delta E_{1/2} = (2.303RT/F) \log (K_0/K_r) ([H^+]/K_a') - (2.303RT/F) \log [HU]$ *for the reduction of* $Cu^{II}(U)_2$ *of dissociation constant* K_0 *to* Cu^IU *of dissociation constant* K_r. *The arrows estimate a mean value of* $\Delta E_{1/2}$, *when values for* − *log* [*HU*] *greater than 2.8 are ignored, consistent with the expression* $\Delta E_{1/2} = 2.303RT/F \log K_0'/K_r$ *for the reduction of* $Cu^{II}(U)^+$ *of dissociation constant* K_0' *to* Cu^IU *of dissociation constant* K_r. *The curves, substituted 2-thiouracil compounds, temperature,* [*HClO*$_4$], *and mean* $(E_{3/4} - E_{1/4})$ *values are, respectively: A, 6-propyl-2-thiouracil, 21°, 0.05, −0.068; B, 6-propyl-2-thiouracil, 21°, 0.20, −0.058; C, 6-propyl-2-thiouracil, 21°, 0.10, −0.058; D, 6-propyl-2-thiouracil, 40°, 0.08, −0.053; E, 6-propyl-2-thiouracil, 40°, 0.05, −0.049; F, 6-propyl-2-thiouracil, 35°, 0.10, −0.062; G, 5,6-dimethyl-2-thiouracil, 21°, 0.08, −0.060; H, 5,6-dimethyl-2-thiouracil, 21°, 0.05, −0.060; I, 6-propyl-2-thiouracil, 40°, 0.10, −0.059; J, 6-propyl-2-thiouracil, 40°, 0.20, −0.062; K, 2-thiouracil, 30°, 0.08, −0.065; and L, 2-thiouracil, 30°, 0.10, −0.064. The original* [Cu^{+2}] *was* 2×10^{-4} M.

in the literature. The diffusion current was proportional to the copper-ion concentration, where the proportionality constant was 9140 μamp./mole/l. at 21° for the conditions specified in the *Experimental* section. Since twice the value of $-(E_{3/4} - E_{1/4})$ calculated by Eq. 2 and listed in Table II was close to 0.060, this strongly indicates that the simple cupric ions [actually the tetraquo cupric ions $[Cu(H_2O)_4]^{+2}$ in agreement with Kolthoff and Lingane (3e)] reduced directly to the metallic state at the dropping electrode by a two-electron transfer, since the cuprous-ion product of a one-electron transfer is incapable of a stable existence at the potential at which cupric ions are reduced. This is further confirmed by the fact that the polarogram of a solution of simple cupric ions shows only the single wave corresponding to the overall reaction $Cu^{+2} \rightarrow Cu(Hg)$.

Data typical of that obtained from polarographic studies on 2-thiouracil, 6-*n*-propyl-2-thiouracil, and 5,6-dimethyl-2-thiouracil (see typical curves given in Fig. 3) when various concentrations of a thiouracil are mixed with a given concentration of cupric ion at the stated various perchloric acid concentrations are given for 1.38–4.38 × 10^{-3} *M* 6-*n*-propyl-2-thiouracil and 2.00 × 10^{-4} *M* cupric ion at 21° in Table III. The ionic strength was maintained constant at 0.20 *M*, using sodium perchlorate with 0.001% tyloxapol as a maximum suppressor. The observed diffusion current for the experimental polarographic conditions specified in the *Experimental* section was 1.48 μamp. and was invariant with the various thiouracil and perchloric acid concentrations. Thus, $(E_{3/4} - E_{1/4})$ values of Table III were within 10 mv. of the theoretical value calculated by Eq. 2, *i.e.*, 0.056 for a one-electron change.

The polarographic curves became irreversible with increasing $(E_{3/4} - E_{1/4})$ values at hydrogen-ion concentrations lower than 0.01 *M*. Thus, the data considered were limited to those studies of 0.05 *M* or greater in perchloric acid.

The studied solutions of 6-methyl- and 5-methyl-2-thiouracils gave polarographic results similar to those compounds already discussed, except that the polarographic waves became much steeper and thus more irreversible when these solutions were permitted to stand *in the polarographic cell.* Since this occurs only for solutions permitted to stand in the polarographic cell, it could conceivably be attributed to adsorption or interaction with the mercury drop. The $E_{1/2}$ values for 6-methyl- and 5-methyl-2-thiouracils were taken from the first polarographic curves obtained so that aberrations due to this subsequent irreversible behavior could be avoided. The polarographic curve for 5-carboethoxy-2-thiouracil was irreversible, as indicated by values of $(E_{3/4} - E_{1/4})$ in the range of from −0.08 to −0.09, values significantly in excess of the theoretical one-electron change (*i.e.*, −0.06) for Eq. 2.

The fact that $(E_{3/4} - E_{1/4})$ values for the polarographic reduction of the cupric-ion complexes of these substituted thiouracils demonstrated a one-electron change (Eq. 2) denies the reduction to copper as per Eq. 3. It also denies the validity of Eq. 9 for the determination of the *p* numbers of complexed ligands and the dissociation constant of a $Cu(U)_p^{(2-p)+}$ complex. It follows that the difference $(\Delta E_{1/2})$

between the half-wave potential $(E_{1/2})_c$ for a cupric complex reduction to a cuprous complex and the half-wave potential $(E_{1/2})_s$ for cupric-ion reduction to cuprous ion should be consistent with either Eq. 28 (and 30) for the reduction of the $Cu(U)_2$ complex to CuU or with Eq. 37 for the reduction of the $Cu(U)^+$ complex to CuU. In the former case, $\Delta E_{1/2} = [(E_{1/2})_c - (E_{1/2})_s]$ should be linearly related to the logarithm of the substituted thiouracil-ion concentration (Eq. 28), $[U^-]$, and the logarithm of the undissociated thiouracil concentration, [HU] (Eq. 30). In the latter case (Eq. 37), the $\Delta E_{1/2}$ should be independent of both thiouracil-ion and thiouracil acid concentrations.

The derivations of Eqs. 28 and 30 required that the ligand concentration be large relative to the metal-ion concentration for the prerequisite Eq. 14 to be valid. The sensitivity of the polarograph required a cupric-ion concentration of 2.00×10^{-4} *M*, which put a lower limit of about 2.00×10^{-3} *M* on the ligand concentration, $[U^-]$. Thus, this same value for the undissociated acid concentration, [HU] (equivalent to a log [HU] of −2.7 as plotted in Fig. 4), may be too small to fulfill the excess ligand requirement of substituted thiouracil-ion concentration, $[U^-]$, over cupric concentration. The high acidities necessary for the polarographic studies (Tables III and IV) diminish the $[U^-]$ concentration available (Eq. 29) even when the [HU] concentration is in such excess that it can be approximated by the total concentration of added substituted 2-thiouracil, $[HU]_T$.

The upper limit of thiouracil concentration possible was its intrinsic solubility, which is about 5×10^{-3} *M* (1). These limitations severely restrict the range of thiouracil concentrations available for use to determine the validity of a linear dependency of $\Delta E_{1/2}$ on thiouracil acid concentration from plots such as that given in Fig. 4 in accordance with Eq. 30. Slopes of plots of $-\Delta E_{1/2}$ *versus* −log [HU] consistent with Eq. 30 would have the theoretical -2.303 RT/F values at the several temperatures of −0.0584 (21°), −0.0592 (25°), −0.0602 (30°), −0.0611 (35°), −0.0621 (40°), and −0.0631 (45°).

The scatter in the plots of Fig. 4 are less than many in the literature (3*e*), and the error in each point is close to the ±2.5 mv. which is normal for the polarograph used[1] (16).

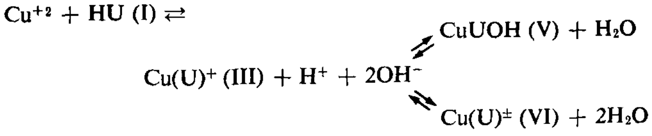
On the premise that there is no valid demonstration from Fig. 4 that the $\Delta E_{1/2}$ values are dependent on undissociated thiouracil acid concentration, and thus the concentration of the thiouracil-ion ligand, the mean $-\Delta E_{1/2}$ values estimated for log [HU] > −2.7 are listed in Table IV for various thiouracils, temperatures, and perchloric acid concentrations. There appears to be no significant variation of $\Delta E_{1/2}$ with the perchloric acid concentrations studied, although there is a pronounced tendency for the $-\Delta E_{1/2}$ values to decrease or the $\Delta E_{1/2}$ values to increase with increasing temperatures. In accordance with Eq. 37, this implies that the K_0'/K_r ratio tends to increase with increasing temperature and the dissociation constant, K_0', of the $Cu^{II}U^+$ complex tends to increase with increasing temperature relative to the dissociation constant, K_r, of the reduced Cu^IU complex.

Polarography of Cuprous Ion in Solutions of 2-Thiouracil and 2-Thiouracil Disulfide—The polarograms of Solution A (specifically described in the *Experimental* section), containing solid cuprous chloride, 0.2648 *M* $HClO_4$, and 4.375×10^{-3} *M* 2-thiouracil, gave a reversible wave $(E_{3/4} - E_{1/4} = -0.063)$ and an $E_{1/2}$ value of −0.153 mv., which was the same as was found for a solution containing cupric ion and 2-thiouracil under the same conditions. Only a portion of the white cuprous chloride appeared to dissolve when slightly heated. The rest slowly turned to orange crystals. The anodic polarogram was also recorded to look for the wave due to oxidation of free or complexed cuprous to cupric ion, but none was found.

The polarogram of Solution B (see *Experimental* section), containing 2-thiouracil disulfide in acid solution, produced no wave and was similar to that for 2-thiouracil under the same conditions (Fig. 3).

The polarogram of Solution C (see *Experimental* section), containing solid cuprous chloride and 0.995×10^{-4} *M* in 2-thiouracil disulfide and 0.2648 *M* in perchloric acid, gave a wave with an $E_{1/4}$ value of −0.078 mv. and an apparent $E_{1/2}$ value of −0.090 v., which was close enough to the $E_{1/2}$ value of −0.079 v. given in the literature (3*e*) for the reduction of free cupric to cuprous ion. The cuprous chloride did not appear to dissolve and what remained turned orange.

Polarography of Presumed Cuprous Complex of 2-Thiouracils—Solutions of the presumed cuprous complex of 2-thiouracil, prepared as specified in the *Experimental* section, produced an $E_{1/2}$ value of −0.078 v. *versus* SCE, which was the same as the $E_{1/2}$ value given in the literature for the reduction of cupric to cuprous ion (3*e*).



Scheme II

Polarograms of the presumed cuprous–2-thiouracil complex in the presence of added thiouracil gave $(E_{3/4} - E_{1/4})$ values of −0.058 v., indicative of a one-electron transfer, and an $E_{1/2}$ value which was the same as that for cupric ion in the presence of the same 2-thiouracil concentration.

Addition of concentrated ammonia to fresh dilute acid solutions of the presumed cuprous complex of 2-thiouracil gave an instantaneous blue color, typical of cupric ion.

DISCUSSION

Possible Structures of Precipitates from Alkaline Titrated Solutions of Cupric-Ion–Thiouracil Complexes—The large immediate drop in pH when solutions of cupric nitrate and thiouracil were mixed (Figs. 1 and 2) demonstrated that a strong complex (or complexes) was formed with a large displacement of hydrogen ion. The resultant complex(es) immediately precipitated, and the amounts of precipitates increased with time and while alkali was added.

More alkali was consumed up to pH 6 (a region where the hydrolysis of free cupric ion does not occur as shown by curve H, Figs. 1 and 2) as characterized by the milliequivalents of alkali consumed up to inflection 1 of curves A, B, and C of Fig. 1 (and the corresponding inflection 2 of the filtered, aged, cupric-ion–thiouracil mixtures of Fig. 2) than was consumed when the equivalent amount of ligand alone was titrated (curve H, Figs. 1 and 2). The fact that the milliequivalents of alkali consumed were greater than 1.5 times the milliequivalents of ligand required that a greater portion of the alkaline-reacted complexes by either a mixed ligand complex of the form CuUOH (V in Scheme I) or some other complex which could donate two protons during the alkaline titration. This latter complex could form the zwitterion $Cu(U)^{\pm}$ (VI) on the neutralization of the second proton from the potential hydroxyl group at the 4-position of the 1:1 complex, $Cu(U)^+$ (III). Both of these potential complexes readily explain the consumption of two hydroxyls per molecule of complexed thiouracil, HU, when Scheme II is considered. The detailed structures of the complexes are given in Scheme I.

Either the mixed ligand complex CuUOH (V) could precipitate or the zwitterion $Cu(U)^{\pm}$ (VI) could dimerize to give the neutral insoluble chelate $Cu_2(U)_2$ (VII) or the insoluble linear polymer $Cu_n(U)_n$ (VIII). Analogous arguments have been given previously (1, 2) for the thiouracil complexes of other metal ions.

The fact that the milliequivalents of alkali consumed to inflection 1 of curves A, B, and C of Fig. 1 (and inflection 2 of curves A, B, and C of Fig. 2) were greater than the milliequivalents of ligand present (curve H of Figs. 1 and 2) but were less than twice this number of milliequivalents (*i.e.*, approximately 17% less than twice) demands the postulation of formation and possible precipitation of a complex (or complexes) that consume only one hydroxyl ion per thiouracil molecule in addition to the postulated formation and possible precipitation of complexes that consume two hydroxyl ions per complexed thiouracil. The former could be formed in the following manner:

$$Cu^{+2} + 2HU + 2OH^- \rightleftarrows Cu(U)_2\ (IV) + 2H_2O \quad (Eq.\ 39)$$

Further evidence in support of this postulate was obtained from titration studies when there was excess free or uncomplexed ligand in those mixtures where the thiouracil concentration exceeded the cupric-ion concentration (curves D, E, and F of Figs. 1 and 2). If only CuUOH (V) or $Cu(U)^{\pm}$ (VI) and its polymers (VII and/or VIII) had been formed (Scheme II) and possibly precipitated on alkaline titration, the titers between inflections 1 and 2 of curves D,

Table IV—$\{-\Delta E_{1/2} = -[(E_{1/2})_c - (E_{1/2})_s]\}$ Values[a] for the Apparent Reduction of the Singly Positively Charged 1:1 Complex of Cupric Ion to the Uncharged 1:1 Complex of Cuprous Ion with Various Substituted 2-Thiouracil Anions

[HClO$_4$]	2-Thiouracil				6-*n*-Propyl-2-thiouracil				5,6-Dimethyl-2-thiouracil			5-Methyl-2-thiouracil[b]		6-Methyl-2-thiouracil		5-Carboethoxy-2-thiouracil[c]		
	21°	30°	35°	45°	21°	35°	40°	45°	21°	35°	40°	21°	35°	21°	35°	21°	35°	40°
0.265	0.066	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.200	0.075	0.050	0.035	0.020[d]	0.120	0.082	0.085	0.087	0.120	—	—	0.067	—	0.067	—	—	—	—
0.100	0.070	0.040	0.030	—	0.120	0.083	0.100	0.100	0.100	—	—	0.072	—	0.070	—	—	—	—
0.080	0.073	0.047	0.033	—	0.126	0.086	0.086	0.085	0.100	—	—	0.076	—	0.090	—	—	—	—
0.050	0.080	0.055	0.043	—	0.126	—	0.093	0.093	0.130	0.092	0.087	0.092	0.084	0.092	0.093	0.048	0.032	0.026
Mean	0.073	0.048	0.035	0.020[d]	0.123	0.084	0.091	0.091	0.113	0.092	0.087	0.077	0.084	0.076	0.093	0.048	0.032	0.026
$10^2K_0'/K_r$[e]	5.60	15.9	26.8	48[d]	0.78	4.22	3.48	3.62	1.15	3.16	3.98	4.80	4.22	5.04	3.02	16	30	38

[a] These are estimates of the mean $-\Delta E_{1/2}$ values for several substituted 2-thiouracil concentrations ranging from 2.38 to 4.38×10^{-3} *M* in the presence of 2.00×10^{-4} *M* Cu^{+2}. The $\Delta E_{1/2}$ values for substituted 2-thiouracil concentrations less than 2×10^{-3} *M* were ignored, since the validity of the equations relating $\Delta E_{1/2}$ to the ratio of the dissociation constants K_0/K_r or K_0'/K_r is based on the premise that the concentration of ligand is relatively large compared to the concentration of metal ion. The $\Delta E_{1/2}$ values are the differences between the half-wave potentials $(E_{1/2})_c$ for the polarographic reduction of the cupric-ion complex of the substituted 2-thiouracils and the half-wave potentials $(E_{1/2})_s$ for the polarographic reduction of the simple cupric ion to its cuprous form. [b] The polarographic wave became steeper with more negative $E_{1/2}$ values on repeated polarographic studies on the same solution so that only initial curves were used and values from curves where $(E_{3/4} - E_{1/4})$ were very much less than −0.5 were discarded. [c] The polarographic waves were stable but very irreversible and gave $(E_{3/4} - E_{1/4})$ values of about −0.09 v. [d] The polarographic curves may have contained two waves very close together but were treated as a single curve. Thus, these data are not considered accurate and are given for comparative purposes only. [e] These values are related to the $\Delta E_{1/2}$ values by $\Delta E_{1/2} = (2.303RT/F) \log K_0'/K_r$, where K_0' is the dissociation constant of the charged complex $Cu^{II}(U)^+$ and K_r is the dissociation constant of the reduced complex Cu^IU. In several instances when $\Delta E_{1/2}$ values at substituted 2-thiouracil concentrations less than 2×10^{-3} *M* were included, these values indicated a possible linear relation to the logarithms of the thiouracil concentrations. This could be rationalized on the premise that the complex $Cu^{II}(U)_2$ of dissociation constant K_0 was polarographically reduced to Cu^IU of dissociation constant K_r and that $\Delta E_{1/2} = (RT/F) \ln K_0/K_r\ (RT/F) \ln [U^-]$ or $\Delta E_{1/2} = (RT/F) \ln (K_0/K_r)([H^+]/K_a') - (RT/F) \ln [HU]$. Calculated $-\log K_0/K_r$ values for the several instances where such linear regressions appeared to be possible were ([HClO$_4$], °C, $-\log K_0/K_r$) for 2-thiouracil: 0.0265, 21°, 10.38; 0.080, 21°, 10.11; 0.200, 30°, 9.99; and 0.100, 35°, 9.30; for 6-*n*-propyl-2-thiouracil: 0.100, 21°, 11.61; 0.080, 35°, 10.35; 0.200, 40°, 10.51; and for 5,6-dimethyl-2-thiouracil: 0.200, 21°, 11.27.

E, and F of Fig. 1 (and the corresponding titers between inflections 2 and 3 of curves D, E, and F of Fig. 2) would be equal to the difference between the millimoles of ligand (5.00×10^{-2}) and the millimoles of cupric ion in the titrated mixtures. This was not so. In every such instance the titration of excess, free, uncomplexed ligand [the portion of the curve used for these calculations is characterized by the thiouracil pK'a of approximately 7.5 (1) and is clearly labeled in Figs. 1 and 2] was less than would be expected if each complexed thiouracil molecule had consumed two hydroxyls as a result of the formation of a 1:1 complex of a thiouracil with cupric ion. These facts supported the assumption of the partial formation and possible precipitation of a $Cu(U)_2$ complex (IV) during the titration where the amount of free, uncomplexed thiouracil available to be titrated was lower than expected if only 1:1 complexation had occurred.

There was no tendency for such precipitated complexes of cupric ion and thiouracil to be disrupted at relatively high concentrations of hydroxyl ions, at least up to a pH of 10. The sharpness of inflection 1 in curve C of Fig. 1 and inflection 2 in curve C of Fig. 2 is indicative of complexes of high stability, low solubility, and low rates of dissolution.

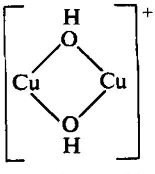
The fact that filtered, aged mixtures of cupric ion and thiouracil showed the appearance of a new titratable species (see the new inflection 1 in Fig. 2 where inflection 2 of Fig. 2 and inflection 1 of Fig. 1 are similar) argues for the presence of precipitated materials on aging that consume alkali more readily. The fact that the overall consumption of titer was similar in both Figs. 1 and 2 argues for only a small precipitation of complex on the initial mixing of thiouracil and cupric-ion solutions.

Confirmation of Structure of Complexes and Precipitates of Cupric-Ion Complexes of Thiouracils—The plausible relations among the possible structures of complexes and precipitates from the interactions of cupric ions and thiouracils are given in Scheme I. This scheme is based on the possible analogy to the schemes given for thiouracil complexes with other metal ions (1, 2) and the information deduced from the potentiometric titrations and polarographic studies given in this paper. Polarography gave definitive evidence for the presence of $Cu(U)^+$ (III) complexes in perchloric acid solutions greater than 0.05 *M*. The precipitation of a relatively stable $Cu(U)_2$ (IV), CuUOH (V), $Cu_2(U)_2$ (VIII), or $Cu_n(U)_n$ (VIII) complex is highly probable and consistent with the data.

The complex precipitated from the addition of equal moles of cupric ion to 2-thiouracil (and also to 6-*n*-propyl-2-thiouracil) had a carbonyl group at the 4-position of the pyrimidine ring, as indicated by an intense absorption band at 1640 cm.$^{-1}$, typical of the carbonyl absorption of the ligand (17, 18). This fact and the elemental analyses exclude $Cu_2(U)_2$ (VII) or $Cu_n(U)_n$ (VIII) as the structure of the complex synthesized under the stated conditions. The formation of CuUOH (V) is consistent with this information and the fact that a 1:1 cupric-ion-thiouracil complex is the major species precipitated during the potentiometric titrations of mixtures. The isolated precipitates, after extensive drying to lose 1 mole of water on the assumption of a CuUOH empirical formula, had elemental analyses corresponding to $(CuU)_2O$ (X). This could be readily explained by the dehydration of the precipitates of Structure V or IX. Solutions of cupric ion have been shown (19) to exist with a twin hydroxo bridge structure (Scheme III).

This information is consistent with, and analogous to, the structure of bis(2-thiouracil)-μ-dihydroxocopper^{II} (IX in Scheme I), which on loss of a mole of water would give X and should possess the IR spectrum and elemental analysis observed. The extreme insolubility of the synthesized complex frustrated efforts to obtain molecular weights and NMR spectra.

The fact that the added hydroxide ion prefers to add at the charged copper site of III instead of removing the proton of the potential 4-hydroxyl argues for a higher positive charge density at the 2-position when cupric ion is complexed than when Cd^{+2} or Pb^{+2} are the complexing species. In these latter cases, precipitates formed under similar conditions had the elemental analyses and spectra consistent with an $M_2(U)_2$ structure analogous to VII or VIII (1, 2). The fact that mixtures of $Cu(U)_2$ and CuU(OH) may be formed on alkaline titration of solutions whereas it is the latter that is more readily precipitated from 1:1 cupric-ion-thiouracil mixtures in acidic solutions implies the greater insolubility of CuU(OH). However, as alkali is added and the pK'a of thiouracil is approached, the rate of hydroxide-ion increase is less than that of thiouracil-anion increase and the precipitation of $Cu(U)_2$ may be favored. Since these complexes are highly insoluble, proper reequilibration may be difficult to obtain. The new inflection point observed in the titration curves of Fig. 2 may possibly be assigned to the manifestation of the titration of a 4-hydroxyl group of the pyrimidines, an hydroxyl group that results from a time-dependent enolization of the normal 4-carbonyl.



Scheme III

Nature and Strength of Polarographically Determined Cupric and Cuprous Complexes of Thiouracils in Acid Solution—The fact that the polarographic reduction of the cupric-ion complexes of 5- and/or 6-substituted thiouracils at the dropping mercury electrode demonstrated a one-electron change of reasonable reversibility supports the premise of reduction of the complexed cupric species only to the cuprous state and not to the free metal. This can occur if the resultant thiouracil complexes of cuprous ion were of high insolubility and/or were of high stability and thus highly resistant to polarographic reduction and dissociation. It is also possible that the resultant $Cu^{I}U$ complex was immediately precipitated on formation and possibly adsorbed on the mercury drop.

The prevention of the reduction of cuprous ion by complexation is not new and occurs in the presence of cyanide ion (20). Cuprous ion is a very soft acid (21) and would be expected to complex strongly with the "soft base" sulfur (21) to inhibit the reduction of cuprous ion to copper metal.

The inability to pick up a polarographic wave attributable to cuprous-ion complexes for mixtures of 2-thiouracil or 2-thiouracil sulfide with cuprous ion and for a presumed synthesized cuprous-ion–2-thiouracil complex is further evidence that such complexes are not facilely polarographically reducible. The chemical tests and polarographic waves observed were only attributable to contaminations by cupric ion or their thiouracil complexes.

Although in several instances there was a tendency for $-\Delta E_{1/2}$ to decrease with decreasing thiouracil concentration [HU] (Table III) or with increasing $-\log$ [HU] values (Fig. 4), the evidence was not conclusive, especially when the unreliability of data at lower thiouracil concentrations is considered since $[U^-]$ must highly exceed $[Cu^{+2}]$. Typical plots for various cupric-ion complexes of substituted 2-thiouracils in accordance with Eq. 30 are given in Fig. 4. The dashed lines are drawn with the theoretical $-2.303RT/F$ slopes. However, when the unreliable data for $[HU] < 2 \times 10^{-3}$ M are not considered in the estimation of a possible regression, no decision as to the validity of Eq. 30 can be made. In addition, if the validity of the regression is assumed, it is necessary to postulate the preferential polarographic reduction of a $Cu^{II}(U)_2$ complex rather than a $Cu^{II}(U)^+$ complex to a $Cu^{I}U$ complex with a ratio of dissociation constants of K_0/K_r of the magnitude of 10^{-10}–10^{-12} (see footnote *b* of Table IV). This would imply that the cuprous complex $Cu^{I}U$ is extremely highly dissociated (*i.e.*, 10^{11} times greater) compared to the reducible $Cu^{II}(U)_2$ species. This is of an incomprehensible magnitude. In addition, the $Cu^{II}(U)^+$ complex should be formed prior to the $Cu^{II}(U)_2$ and should be preferred in the acidic solutions studied (1, 2). Assuredly, the positively charged $Cu^{II}(U)^+$ complex should be preferentially reduced at a dropping mercury electrode over a neutral $Cu^{II}(U)_2$ complex.

The ratios of dissociation constants of $Cu^{II}(U)^+$ to $Cu^{I}U$ calculable from Eq. 37 and given in Table IV are of orders of magnitude of 0.1, which is much more reasonable than the ratios (approximately 10^{-11}) calculable from Eq. 30 on the premise of preferential reduction of $Cu^{II}(U)_2$. The fact that the dissociation constant of the 1:1 cupric complex, $Cu(U)^+$, is less than that of the 1:1 cuprous complex, CuU, is consistent with the larger positive charge of the cupric ion.

There is an alternative explanation for a one-electron change on polarographic reduction in cupric-ion–thiouracil mixtures and for linear plots of $\Delta E_{1/2}$ *versus* log [HU] consistent with equations of the form of Eqs. 28 and 30, where $(E_{1/2})_s$ would be for the reduction of cuprous ion to metallic copper. This polarographic wave could be attributed to the reduction of a cuprous-ion complex of thiouracil, $Cu^{I}U$, or of a thiouracil disulfide formed instantaneously on mixing, where the reduction of cupric ion is effected by the oxidation of thiouracil to thiouracil disulfide. However, sufficient evidence has been presented previously (2) that this cupric-ion reduction by thiouracil does not occur in the acid solutions used for the polarographic studies.

Relative Tendency of Metal Ions to Complex with Thiouracils—The order of decreasing stability of metal complexes of thiouracils (1, 2) is: $Cu^{+2} \gg Pb^{+2} > Cd^{+2} \gg Ni^{+2} \approx Zn^{+2}$. Metal ions that do not appear to complex thiouracils (1, 2) are Fe^{+3}, Fe^{+2}, Co^{+2}, Ca^{+2}, and Mn^{+2}.

The "natural order" of stability constants (22, 23) for the first-row transition metals, $Fe^{+3} < Co^{+2} < Ni^{+2} < Cu^{+2} > Zn^{+2}$, shows the highest complexation stability at cupric ion, is a direct consequence of their ionization potentials and ionic radii (24), and follows the order of the heats of hydration (23).

When ligands approach cupric ion, the energy of the normally degenerate (having equal energies) 3*d* electron orbitals is changed, causing some orbitals to become lower in energy and some higher (23). The nine 3*d* electrons of cupric ion will preferentially fill the lower energy orbitals first and will give a decrease in the total energy, called the crystal field stabilization energy. The crystal field stabilization energy is theoretically a maximum in a d^8 ion in an octahedral field but, in the case of cupric ion, the d_{z^2} and $d_{x^2-y^2}$ orbitals are degenerate. The degeneracy is removed by Jahn-Teller stabilization, causing the $3d^9$ configuration to become most stable (23). Jahn-Teller stabilization results from the fact that if the ground level of a system is composed of degenerate energy states, a distortion of the system must occur to remove the degeneracy and make one energy state lower. In the case of cupric ion, there are two degenerate energy states and one is raised and the other is lowered in energy. This change in energy states results in an axial distortion of the octahedral cupric ion to a square-planar configuration (23).

The 3*d* orbitals of sulfur are empty and may accept electrons by back-donation from the partially filled 3*d* orbitals of cupric ion to form π-bonds, thus adding to the strength of the complex. The complexes of cadmium and lead may owe part of their relatively strong bonding to back-donation of electrons from their filled 4*d* and 5*d* orbitals, respectively, to the empty 3*d* orbitals of sulfur (25).

Pearson (21) classified metal ions and ligands in terms of "soft" and "hard" acids and bases. In this classification, those metal ions that complex by ionic attraction are called "hard" acids while those metal ions that complex primarily by formation of covalent bonds are called "soft."

Sulfur anions bound to conjugated systems, as in thiouracil and thiophenol anions, rank near the top as soft bases. According to Pearson's classification, a soft base forms the strongest bonds with soft acids. Cadmium and silver ions (21) are typical soft acids; calcium, manganese, and ferric ions are typical hard acids and would not be anticipated to complex readily with thiouracil anions which are soft bases. Lewis acids, which fall into a borderline class, are ferrous, cobaltous, nickel, cupric, zinc, and lead ions, but only the last three appear to complex with thiouracils. The stability of ferrous and cobaltous-ion complexes would be expected to be lower than that found for nickel, since their ionic radii are larger and heats of hydration are lower. Lead ion is also a borderline case and probably owes its relatively high stability constant with thiouracils to its large size and polarizability.

Possible Correlations of Thiouracil Structures with Antithyroid Activities—The fact that uracil, the oxygen analog of thiouracil, has no antithyroid activity (26) demonstrates that a sulfur at the 2-position of the pyrimidine nucleus is needed to promote antithyroid activity.

Substitution on the sulfur of 2-thiouracil abolishes its biological activity (27). The data of Table I, where 6-methyl-*N*,*N*′-diethyl-2-thiouracil had no or negligible activity at a higher dose level relative to those of the nonnitrogen substituted compounds, strongly indicate that the antithyroid activity of thiouracil compounds demands tautomerizable hydrogens on thiouracil nitrogens that can form ionizable sulfhydryl groups.

Substitution of a nitrogen for the carbon at the 6-position of 2-thiothymine(5-methyl-2-thiouracil) gave 2-thio-6-azathymine, which had appreciable antithyroid activity (28). When the *N*-1 and *N*-3 nitrogens of 2-thio-6-azathymine were substituted with methyl groups, all antithyroid activity was lost (28). This is consistent with the lack of antithyroid activity of 6-methyl-*N*,*N*′-diethyl-2-thiouracil (Table I).

Since thiouracils with alkyl substituted sulfur atoms and with nitrogen substituents that prohibit hydrogen transfers to form a thiol do not complex (1, 2) with the tested metal ions and do not have antithyroid activity, it is implied that complexation or the molecular parameters that control complexation are important to the antithyroid activity of thiouracils. It is consistent with this premise that the presence of an electronegative carboethoxy group at the 5-position of thiouracil, which reduces the electron density at the sulfur, reduces the stability of metal complexes (1, 2) and drastically reduces the antithyroid activity of 2-thiouracil (27). Other thiouracil derivatives substituted at the 5-position with electronegative groups also have reduced antithyroid activity. Such compounds are 5-cyano-2-thiouracil and 5-carboxy-2-thiouracil (27) and would be expected to reduce the electron density at the sulfur atom. The other 5- and/or 6-alkyl substituted 2-thiouracils have similar pK′a values (1) 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.

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GLC Determination of Sulthiame in Plasma

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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-

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[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.