Metal-ion Catalysis in Some Reactions of Hexacyanoferrate(III) Ions. Part I. Copper Catalysis in the Oxidation of Cysteine and Related Thiols

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The formation of disulphide compounds from cysteine, *N*-acetylcysteine, and 3-mercaptopropanoic acid, by oxidation with hexacyanoferrate(III) ions, is catalysed by small amounts of copper salts, present as impurities. The rate law for the copper-catalysed oxidation is established. The mechanism of reaction involves thiol complexes of copper(I—III) and an intermediate, thought to be the radical species $RSSR^{-\cdot}$, or its protonated form $RSSRH^{\cdot}$.

OXIDATION of thiols by hexacyanoferrate(III) ions has been used [1] as an analytical procedure for many years. With adequate control of reaction conditions,[2,3] the reactions are both fast and of accurate stoicheiometry for thiols and for mercapto-groups in, for example, albumin. This successful application of the oxidation reaction is in marked contrast to the confused state of knowledge [4] of the kinetics. The most important source of confusion arises from neglect of catalysis by trace amounts of copper and previous mechanistic studies [5-9] are therefore inadequate. The study was begun with 2-amino-3-mercaptopropanoic acid (cysteine), and was extended to include two related thiols, *N*-acetylcysteine and 3-mercaptopropanoic acid, when the significance of metal catalysis became apparent. Although these compounds have a common pattern of reactivity, the rates of reaction are different and detailed study could sometimes be made more easily with one than another.

EXPERIMENTAL

Reagents.—Potassium hexacyanoferrate(III) (B.D.H. AnalaR) was purified by recrystallisation, first from hot water and then by precipitation with methanol from aqueous solutions at *ca.* 25 °C. The fine orange crystals were dried over silica gel and stored in the dark to avoid possible decomposition to aquopentacyanoferrate(III) ions.[10] Potassium hexacyanoferrate(II) (B.D.H. AnalaR) was repeatedly crystallised from hot water, dried over silica gel, and stored in the absence of light. The product was almost pure white.

Cysteine hydrochloride monohydrate and *N*-acetylcysteine (both B.D.H. Biochemical Grade) were used as supplied. 3-Mercaptopropanoic acid (Evans, 'assay 100%') was distilled (130 °C at 22 mmHg) before use. The refractive index at 20 °C was 1·4920 (lit.,[11] 1·4910). Cystine (B.D.H. Biochemical Grade) was dissolved in aqueous ammonia, the solution filtered, and the reagent precipitated with acetic acid to remove [12] contamination by copper(II) cystinate. 3,3′-Dithiodipropanoic acid was prepared by oxidising an aqueous solution (0·8 mol dm^{-3}) of 3-mercaptopropanoic acid with the stoicheiometric amount of bromine, and purified by recrystallisation from ethanol, m.p. 153—153·5 (lit.,[13] 154 °C).

All other reagents were of AnalaR quality and were used as supplied. The water used as solvent was doubly distilled in glass apparatus. The copper content of both the solvent and reagents was checked by atomic absorbance spectroscopy, with the following results (accurate to *ca.* ±10%):

Reagent	10^6 (Cu/mol)/(reagent/mol)
Potassium hexacyanoferrate(III)	12
Potassium hexacyanoferrate(II)	23
Cysteine	12
N-Acetylcysteine	2·0
3-Mercaptopropanoic acid	0·6
Sodium acetate	0·5
Acetic acid ('glacial')	Below detection
Water (double distilled)	$1{\cdot}5 \times 10^{-8}$ mol dm^{-3}

Buffer Solutions for Kinetic Measurements.—All kinetic measurements were carried out in acetate buffer solutions. Lower 'background' rates of reaction were attained when

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[1] L. Flatow, *Biochem. Z.*, 1928, **194**, 132; H. L. Mason, *J. Biol. Chem.*, 1930, **86**, 623.

[2] A. E. Mirsky, *J. Gen. Physiol.*, 1940–41, **24**, 709; M. L. Anson, *ibid.*, **25**, 355.

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[5] J. J. Bohning and K. Weiss, *J. Amer. Chem. Soc.*, 1960, **82**, 4724.

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[10] B. M. Chadwick and A. G. Sharpe, *Adv. Inorg. Chem. Radiochem.*, 1966, **8**, 83.

[11] L. C. Cheney and J. R. Piening, *J. Amer. Chem. Soc.*, 1945, **67**, 731.

[12] P. Ray and I. Bhaduri, *J. Indian Chem. Soc.*, 1950, **27**, 297.

[13] G. G. Stoner and G. Dougherty, *J. Amer. Chem. Soc.*, 1941, **63**, 987.

the buffers were prepared from glacial acetic acid and a concentrated (5 mol dm^{-3}) solution of sodium hydroxide, than when sodium acetate was used. The sodium hydroxide solutions were first shaken with, and stored over, low-density magnesium dioxide in Polythene bottles. This procedure has been claimed [14] to remove trace-level contamination by transition-metal ions.

Kinetic Measurements.—The decrease in hexacyanoferrate(III)-ion concentration with time was monitored at 410 nm, using a Shimadzu QR50 spectrophotometer which was modified to permit continuous recording of absorbance or transmittance. The extinction coefficient was confirmed as 994 dm^3 mol^{-1} cm^{-1} in our solutions, in good agreement with other results.[9] The validity of Beer's law in the present concentration range, and the absence of significant absorption by other reagents and products, were also checked.

Initial rates of reaction were measured because there was a complex dependence on the concentration of the reaction products. They were obtained from gradients of (absorbance against time) curves over the first 10% of reaction. Such curves were extrapolated reliably to the value corresponding to the initial solution composition. Alternatively, the absorbance–time data were fitted to cubic equation (1)

$$\text{Absorbance} = A + Bt + Ct^2 + Dt^3 \qquad (1)$$

by a linear-regression computer program and the initial rate extracted as the coefficient B. However this method was less convenient and did not give significantly different, or better, results. As earlier workers on these reactions, we found that the empirical equation (2) was obeyed; its

$$k''t = 1/[Fe(CN)_6{}^{3-}]_t - 1/[Fe(CN)_6{}^{3-}]_0 \qquad (2)$$

meaning is discussed below. It does not imply second-order dependence on $[Fe(CN)_6{}^{3-}]$. The constant k'' was used as a convenient measure of behaviour throughout the course of the reactions.

Reactions were normally studied at ambient temperature (19—24 °C). The temperature variation within a particular set of reactions was not greater than 1 K, corresponding in our cases to *ca.* ±1·5% in rate. We and other [9] workers find that a variation in rate of up to 50% may occur with different stock solutions from the same starting materials, whereas duplicate runs using the same solutions usually agreed in rate to within ±5%. This study therefore derives conclusions, whenever possible, from measurements on a single set of solutions and made within 4 h. Intercomparisons were then possible between such sets of measurements.

Reaction Products.—The extent of reduction of hexacyanoferrate(III) ions was checked by determining residual hexacyanoferrate(III) after complete reaction (1 h for cysteine, 6—8 h for the other thiols) in solutions which initially contained thiol (4·00 × 10^{-3} mol dm^{-3}), hexacyanoferrate(III) (5·00 × 10^{-3} mol dm^{-3}), and acetate buffer (pH 4·3), but which were otherwise identical with solutions used for kinetic experiments. The results [mol hexacyanoferrate(III) reduced per mol thiol] were (within ±0·01):

Cysteine	0·99
N-Acetylcysteine	0·97
3-Mercaptopropanoic acid	0·98

The difference from unity is adequately explained by oxidation of hexacyanoferrate(II) by molecular oxygen (which was not excluded). Under similar conditions this amounts to *ca.* 0·5% h^{-1} for a 5 × 10^{-4} mol dm^{-3} hexacyanoferrate(II) solution. The absence of any detectable intermediate from hexacyanoferrate(III) ions was confirmed by the observation that the isosbestic point of mixtures of hexacyanoferrate-(III) and -(II) ions (at 280·9 nm) was accurately maintained throughout the reaction for all three thiols. Thus there was little formation of hexacyanoferrate–thiol complexes during the reaction, in contrast to the hexacyanoferrate(III)–sulphite ion reaction.[15] The disulphide compounds cystine and 3,3′-dithiodipropanoic acid precipitated slowly from reacted solutions and were identified (i.r. spectra and m.p.) by comparison with authentic specimens. All samples of cystine were slightly blue, due to unidentified minor side reactions.

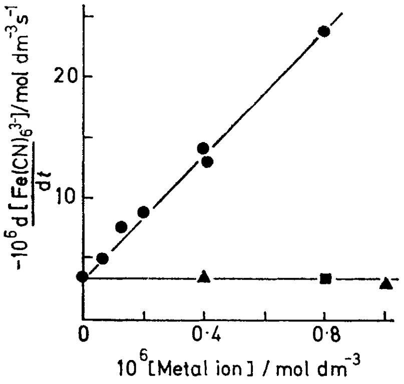

FIGURE 1 Variation of initial rate of reaction of *N*-acetylcysteine (2 × 10^{-3} mol dm^{-3}) with: (●), [Cu^{II}]; (▲), [Fe^{III}]; (■), [Fe^{II}]. $[Fe(CN)_6{}^{3-}]_0$ = 4·0 × 10^{-4} mol dm^{-3}, pH 4·05

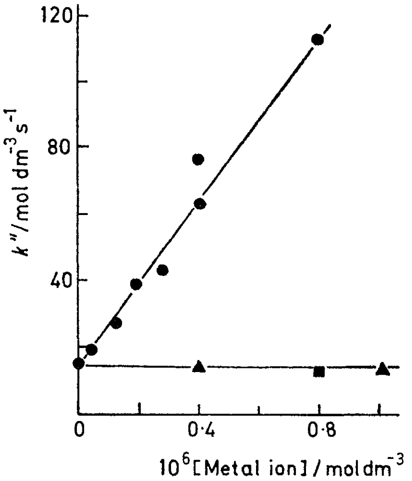

FIGURE 2 Variation of k'' for *N*-acetylcysteine (2·0 × 10^{-3} mol dm^{-3}) with: (●), [Cu^{II}]; (▲), [Fe^{III}]; (■), [Fe^{II}]. $[Fe(CN)_6{}^{3-}]_0$ = 4·0 × 10^{-4} mol dm^{-3}, pH 4·05

RESULTS AND DISCUSSION

Dependences of the Rate of Reaction.—Copper and iron ion concentrations. Initial rates of reaction of potassium hexacyanoferrate(III) and *N*-acetylcysteine are shown in Figure 1 with known amounts of copper(II), iron(II), or iron(III) ions added; they display linear dependences on the copper-ion concentration and independence of

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[14] J. R. Kolczynski, E. M. Roth, and E. S. Shanley, *J. Amer. Chem. Soc.*, 1957, **79**, 531.

[15] J. M. Lancaster and R. S. Murray, *J. Chem. Soc.* (*A*), 1971, 2755.

that of iron. The same dependences are present throughout the reactions since k'' (Figure 2) varies in the same way. Similar results were found with 3-mercaptopropanoic acid and cysteine.

For N-acetylcysteine (and presumably also for the other compounds), the rate observed in absence of added copper(II) ion is due to that ion present as impurity in the reactants. A series of reactions similar to that of Figure 1, but at lower concentration, was carried out using reactants (the copper analyses of which are given above) with initial concentrations [hexacyanoferrate-(III) ($5{\cdot}0 \times 10^{-4}$ mol dm^{-2}), N-acetylcysteine ($5{\cdot}0 \times 10^{-3}$ mol dm^{-3}), pH 4·05] which gave convenient initial rates for copper(II) ion concentrations below $8{\cdot}3 \times 10^{-8}$ mol dm^{-3}. The results gave a good fit to equation (3),

$$-\mathrm{d}[\mathrm{Fe(CN)_6}^{3-}]/\mathrm{d}t = 84[\mathrm{Cu}^{2+}] + 4{\cdot}6 \times 10^{-6} \qquad (3)$$

the last term being consistent with a copper content, as impurity, of $5{\cdot}5 \times 10^{-8}$ mol dm^{-3}. Calculated impurity levels were:

Reagent	Concentration mol dm^{-3}	10^8 [Copper] mol dm^{-3}
$K_3Fe(CN)_6$	$5{\cdot}0 \times 10^{-4}$	0·6
N-Acetylcysteine	$5{\cdot}0 \times 10^{-3}$	1·0
$MeCO_2H$	0·16	None detected
$Na(MeCO_2)$	0·04	2·0
Water		1·5
	Total	5·1

Thus no other significant contribution to the rate remains to be explained. The difficulty in attaining reproducible reaction rates is due to varying contamination by copper(II) ions. The linear dependence of rate on copper contration, and limits given [16] for other methods, suggests that this reaction may prove useful in kinetic analysis for copper.

In two previous papers [8,9] there are indications that metal-ion catalysis plays an important role in the oxidation of cysteine. In one [8] copper appeared to be excluded; in the other,[9] the authors found *equal* effects with $\mathrm{Fe^{II}}$, $\mathrm{Cu^{II}}$, $\mathrm{Sn^{II}}$, and $\mathrm{Co^{II}}$, and cautioned against any simple interpretation in advance of further experiment. We have not found iron to be an effective catalyst despite some evidence to the contrary.[17]

Precipitation of copper(II) and hexacyanoferrate(II) ions should not have occurred in any of our rate measurements. The solubility product is reported [18] as 10^{-17} mol^3 dm^{-9} so there is no reason to suppose that a heterogeneous process is involved.

Thiol concentration. All three thiols showed a greater than first-order dependence of the initial rate of reaction on thiol concentration. Figure 3 shows this for N-acetylcysteine and 3-mercaptopropanoic acid. Equation

$$\frac{-\mathrm{d}[\mathrm{Fe(CN)_6}^{3-}]/\mathrm{d}t}{[\mathrm{Thiol}]} = k_1 + k_2[\mathrm{Thiol}] \qquad (4)$$

(4) is applicable in all cases, with comparable contributions from both terms. This situation persists throughout the reaction, since the ratio of k'' to the thiol concentration obeys (Figure 4) a similar relation. Earlier results [5] for 3-mercaptopropanoic acid are also fitted much better by this two-term expression than by the (first order) one originally proposed. We confirm the observation of these authors that the disulphide compound produced in the reaction does not alter the oxidation rate.

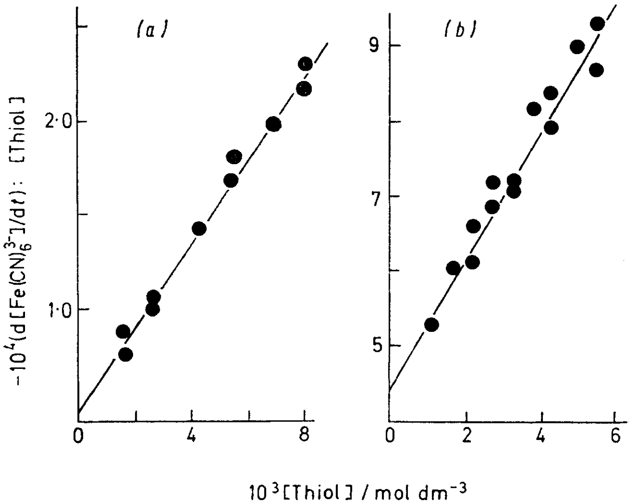

FIGURE 3 Variation of the ratio {(Initial rate of reaction) : [Thiol]} with [Thiol], for $[\mathrm{Fe(CN)_6}^{3-}] = 4{\cdot}0 \times 10^{-4}$ mol dm^{-3} and pH 4·05; (*a*) N-acetylcysteine, $10^4[\mathrm{Fe(CN)_6}^{4-}] = 4{\cdot}0$ mol dm^{-3}; (*b*) 3-mercaptopropanoic acid, $[\mathrm{Fe(CN)_6}^{4-}] = 0$

Concentration of hexacyanoferrate-(III) *and -*(II) *ions.* For all three thiols, initial rates of reaction showed (Figure 5) zero-order and first-order terms in hexacyanoferrate(III)-ion concentration when studied in the absence of hexacyanoferrate(II) ions. If the reaction were of simple second order in $[\mathrm{Fe(CN)_6}^{3-}]$, and our results were correct near the upper end of the range, experimental rates would lie on the broken lines in the Figure. Clearly the reactions, contrary to earlier claims,[5-8] are not second order in $[\mathrm{Fe(CN)_6}^{3-}]$. Further-

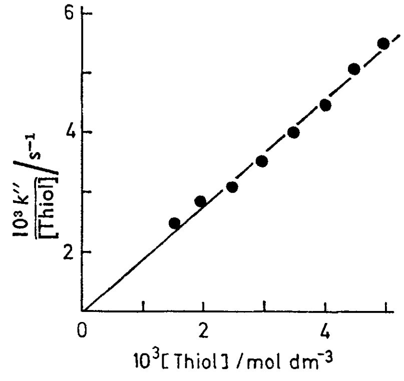

FIGURE 4 Variation of k''/[Thiol] with [Thiol] for N-acetylcysteine. $10^4[\mathrm{Fe(CN)_6}^{3-}] = 5{\cdot}0$ mol dm^{-3}, pH 4·05

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[16] K. B. Yatsimirskii, 'Kinetic Methods of Analysis,' Pergamon, Oxford, 1966; G. E. Batley, *Proc. Roy. Austral. Chem. Inst.*, 1972, **39**, 261.

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[18] A. Bellomo, *Talanta*, 1970, **17**, 1109.

more, addition of hexacyanoferrate(II) ions at constant hexacyanoferrate(III)-ion concentration lowers the rates of reaction; this is true for all three thiols. The observations for cysteine are exemplified by the following initial rates, for which [Cysteine] = $7{\cdot}4 \times 10^{-5}$ mol dm^{-3}, [Fe(CN)$_6^{3-}$] = $4{\cdot}2 \times 10^{-5}$ mol dm^{-3}, and pH 4·05.

10^5[Fe(CN)$_6^{4-}$]/mol dm^{-3}	-10^6d[Fe(CN)$_6^{3-}$]/dt/mol dm^{-3} s^{-1}
0	1·2
2·0	0·77
4·0	0·37

As [Fe(CN)$_6^{4-}$] increased, the term in the rate law which is independent of hexacyanoferrate(III)-ion concentration became smaller and eventually disappeared at [Fe(CN)$_6^{4-}$] *ca.* 4×10^{-4} mol dm^{-3}. An alternative experimental approach involved examination of the dependence of the rate on the hexacyanoferrate-ion concentration during a single reaction as hexacyanoferrate(II) is formed by the reaction. Here too the zero-order term decreased progressively with increasing [Fe(CN)$_6^{4-}$].

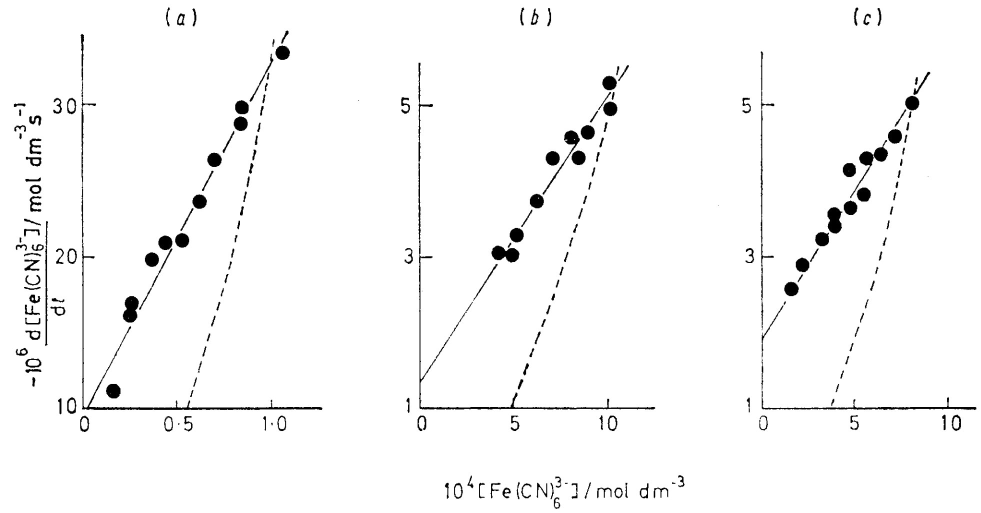

FIGURE 5 Variation of initial rate of reaction with [Fe(CN)$_6^{3-}$], in the absence of Fe(CN)$_6^{4-}$, pH 4·05. The broken lines correspond to second-order dependence: (*a*) cysteine ($0{\cdot}074 \times 10^{-3}$ mol dm^{-3}); (*b*) *N*-acetylcysteine ($4{\cdot}0 \times 10^{-3}$ mol dm^{-3}); (*c*) 3-mercaptopropanoic acid ($1{\cdot}95 \times 10^{-3}$ mol dm^{-3})

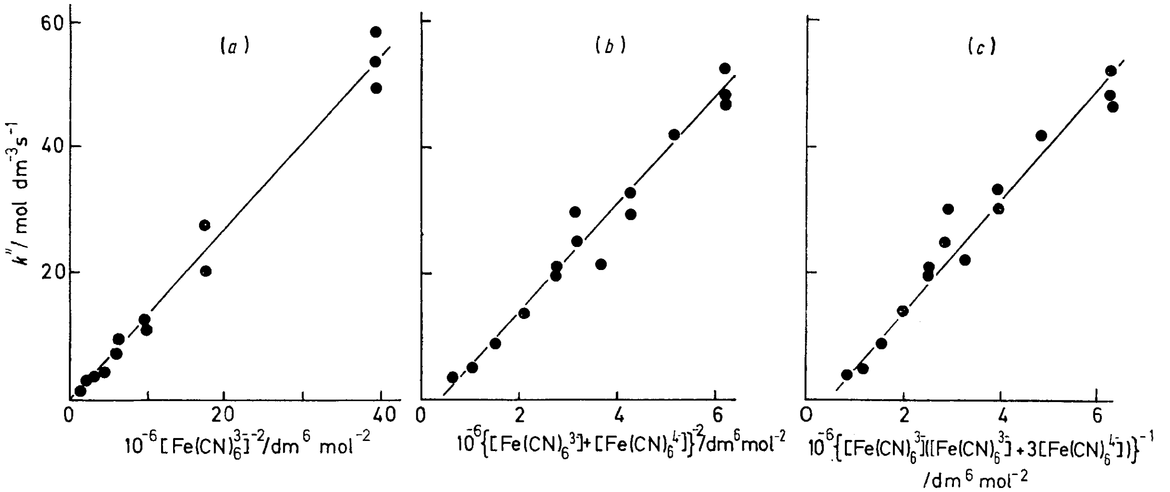

FIGURE 6 Dependence of k'' on [Fe(CN)$_6^{3-}$]$_0$ for *N*-acetylcysteine: (*a*) (−2) power in absence of hexacyanoferrate(II) ions([Thiol] = $1{\cdot}6 \times 10^{-3}$ mol dm^{-3}); (*b*) (−2) power variation with [Fe(CN)$_6^{4-}$] ([Thiol] = $4{\cdot}0 \times 10^{-3}$ mol dm^{-3}); (*c*) alternative representation of data above ([Thiol] = $4{\cdot}0 \times 10^{-3}$ mol dm^{-3})

Previous authors [5-8] erroneously deduced second-order dependence on [Fe(CN)$_6^{3-}$] from the observation that the reciprocal of [Fe(CN)$_6^{3-}$] is linearly related to the time of reaction. We confirm the *observation* and use it to define a parameter k''. The error in the previous deduction arose from failure to observe the dependence on [Fe(CN)$_6^{4-}$]. We shall show (Appendix) that our rate law and associated rate coefficients are consistent with the observation of this linear relation.

$$k'' = -\mathrm{d}[\mathrm{Fe(CN)_6}^{3-}]^{-1}/\mathrm{d}t \qquad (5)$$

With the faster reactions, we sometimes found it more convenient to measure values of k'' than initial rates. In addition, changes in k'' provide a further test of the mechanism. The values of k'' do not, however, have any simple interpretation in terms of a single rate coefficient.

It has been claimed[5,7] that values of k'' obey the relation (6). Over the limited range investigated, our

$$k'' = \{[\mathrm{Fe(CN)_6}^{3-}] + [\mathrm{Fe(CN)_6}^{4-}]\}^{-1} \qquad (6)$$

data are accommodated much better by either equation (7) [Figure 6 (*a*) and (*b*)] or equation (8) [Figure 6 (*c*)].

$$k'' = \{[\mathrm{Fe(CN)_6}^{3-}] + [\mathrm{Fe(CN)_6}^{4-}]\}^{-2} \qquad (7)$$

$$k'' = [\mathrm{Fe(CN)_6}^{3-}]^{-1}\{[\mathrm{Fe(CN)_6}^{3-}] + 3[\mathrm{Fe(CN)_6}^{4-}]\}^{-1} \qquad (8)$$

Acidity. The dependence of reaction rate on acidity was investigated only in the range $3{\cdot}7 < \mathrm{pH} < 4{\cdot}7$, which is available conveniently with acetate buffers, and thus avoided changes due to change of buffer.

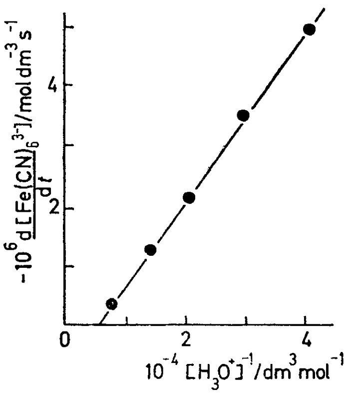

FIGURE 7 Dependence of initial rate of reaction of *N*-acetylcysteine (10^{-3} mol dm^{-3}) on hydrogen-ion concentration. $[\mathrm{Fe(CN)_6}^{3-}]_0 = 4{\cdot}7 \times 10^{-4}$ mol dm^{-3}

For *N*-acetylcysteine, initial rates of reaction varied linearly (Figure 7) with 10^{pH}, *i.e.* approximately with $[\mathrm{H}^+]^{-1}$. Similar observations were made with 3-mercaptopropanoic acid. For cysteine it was easier to measure changes in k'', and a linear dependence of this quantity on 10^{pH} was found for it and for *N*-acetylcysteine. The oxidation of 2-mercaptoethanol has been shown to have a similar dependence of reaction rate on acidity when $3{\cdot}2 < \mathrm{pH} < 4{\cdot}1$, but a much slower variation in the more acid region $1{\cdot}8 < \mathrm{pH} < 3{\cdot}2$.

The published pK_a values for the compounds used in the present study are:

3-Mercaptopropanoic acid[19]	pK_1 4·33, pK_2 10·54
Cysteine[20,21]	pK_1 2·12, pK_2 8·21, pK_3 10·38
Hexacyanoferrate(III)[22]	all > 1
Hexacyanoferrate(II)[23]	pK_3 2·22, pK_4 4·17

These macroscopic pK_a values determine the form of the dependence of the rate of reaction on pH, although the microscopic pK_a value for ionisation of the sulphydryl group is needed for any discussion of the degree of complexing through sulphur. The value for cysteine is *ca.* 8·50.[21,23]

Arrhenius Activation Energies.—Initial rates of reaction were measured for *N*-acetylcysteine and 3-mercaptopropanoic acid at three temperatures between 11 and 26 °C. The results lead to Arrhenius activation energies *ca.* 10 kJ mol^{-1}. These are anomalously low for elementary reactions in solution, and thus suggest that we are dealing with reactions in which an *exothermic* equilibrium process precedes the rate-determining step. Such a system will have lowered apparent activation energy.

Effect of Oxygen on Rates of Reaction.—In previous work[5,7,8] on thiol oxidation by hexacyanoferrate(III) ions, there has been careful exclusion of oxygen. It is not clear that this practice was based on observed necessity. With *N*-acetylcysteine, we find no oxygen effect. Thus, initial rates of reaction of hexacyanoferrate(III) ions ($3{\cdot}0 \times 10^{-4}$ mol dm^{-3}) and *N*-acetylcysteine ($1{\cdot}0 \times 10^{-3}$) were $(8{\cdot}7 \pm 0{\cdot}2) \times 10^{-7}$ mol dm^{-3} s^{-1} when measured with exclusion of oxygen and $8{\cdot}6 \times 10^{-7}$ mol dm^{-3} s^{-1} when the solutions were saturated with oxygen (1 atm) prior to starting the reaction. The dependence of reaction on hexacyanoferrate(III)-ion concentration was also of just the same form in the absence of oxygen as that in solutions at equilibrium with the atmosphere. Because of this experience, we did not exclude oxygen in the other reactions.

Mechanism of Reaction.—Since the three thiols studied show very similar dependences on the variables examined, they will be represented simply as RSH. Interpretation of the rate measurements requires a knowledge of the form in which copper(II) is stored in the reaction medium. The literature concerning the interaction of copper with thiols is confused and apparently contradictory. There is no doubt that under anaerobic conditions copper(II) is reduced to copper(I) by most thiols including cysteine,[24-26] and that in some cases[25-28] relatively stable mixed copper(I)–copper(II) complexes are formed in the process. On the other hand, there is convincing evidence[27,29] that, in the presence of oxygen, copper(II) forms a stable 1 : 2 complex with cysteine in alkaline solution, and that copper is present in the II oxidation state until oxygen concentrations and/or thiol concentra-

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[19] J. T. Spence and H. H. Y. Chang, *Inorg. Chem.*, 1963, **2**, 319.

[20] D. D. Perrin and I. G. Sayce, *J. Chem. Soc.* (*A*), 1968, 53.

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[28] W. E. Blumberg and J. Peisach, *J. Chem. Phys.*, 1968, **49**, 1793.

[29] D. Cavallini, C. De Marco, S. Duprè, and G. Rotilio, *Arch. Biochem. Biophys.*, 1969, **130**, 354.

tions fall to low values (*cf.* refs. 28—30). In the case of iron catalysis of 2-mercaptoacetate oxidation, it is clear [31] that the processes which reduce iron in absence of oxygen [equation (9)] are not major components of the reaction in the presence of oxygen.

$$2\mathrm{Fe^{III}}\,(\mathrm{RS\ complex}) = 2\mathrm{Fe^{II}}\,(\mathrm{RS\ complex}) + \mathrm{RSSR} \qquad (9)$$

The equilibrium between hydrated copper(II) and the 1 : 1 and 1 : 2 complexes may be described by equations (10) and (11), from which $[\mathrm{Cu}^{2+}] = \lambda[\mathrm{H}^+]^2$, $[\mathrm{Cu(SR)}^+] = \lambda K_1K_a[\mathrm{H}^+][\mathrm{RSH}]$, and $[\mathrm{Cu(SR)_2}] = \lambda K_1K_2K_a{}^2[\mathrm{RSH}]^2$.

$$[\mathrm{Cu(SR)}]^+ \rightleftharpoons \mathrm{Cu}^{2+} + \mathrm{RS}^- \qquad (10)$$

$$[\mathrm{Cu(SR)_2}] \rightleftharpoons [\mathrm{Cu(SR)}]^+ + \mathrm{RS}^- \qquad (11)$$

Here K_1 and K_2 are the stability constants of the 1 : 1 and 1 : 2 complexes respectively, K_a is the ionisation constant of RSH, and λ is given by equation (12).

$$\lambda = [\mathrm{Cu^{II}}]_\mathrm{T}/([\mathrm{H}^+]^2 + K_1K_a[\mathrm{H}^+][\mathrm{RSH}] + K_1K_2K_a{}^2[\mathrm{RSH}]^2) \qquad (12)$$

$[\mathrm{Cu(SR)}^+]$ and $[\mathrm{Cu(SR)_2}]$ each refer to the sum of the concentrations of the possible isomeric complexes.

In order that, under our conditions, virtually all the copper(II) is present in the form of the 1 : 1 complex, it is necessary that $\log K_1 \gg 7{\cdot}5$ and $\log K_2 \ll 7{\cdot}5$. The reported stability constants for cysteine and substituted cysteines [32,33] are suspect because of the complicating redox reaction, but examination of the values for cysteine and penicillamine with other bivalent cations [34] suggests that the first inequality would almost certainly be satisfied. If the second inequality also holds, the inverse first-order dependence of the rate of cysteine oxidation on hydrogen-ion concentration is consistent with reaction occurring *via* the small equilibrium concentration of the 1 : 2 complex. Uncomplexed cysteine is in the zwitterionic form throughout the pH range investigated, so that formation of the neutral complex $[\mathrm{Cu(SR)_2}]$ from $[\mathrm{Cu(SR)}]^+$ and cysteine involves the overall loss of one proton. The situation is more complicated with *N*-acetylcysteine and 3-mercaptopropanoic acid since these compounds have pK_a values in or close to the pH range investigated. To explain the simple dependence of the rate of reaction on the reciprocal of the hydrogen-ion concentration, it is necessary to suppose that both compounds behave as monodentate ligands and that the state of ionisation of the carboxy-group has no significant effect on the stability or reactivity of the copper complexes.

The two-term experimental rate law appears to require two parallel reaction paths leading to formation of the disulphide compound. The form of hexacyanoferrate-(II)-ion dependence in the first term suggests that, for one path, hexacyanoferrate(II) ion lowers the rate of reaction by reforming reactant from an intermediate copper(III) complex which would otherwise fragment to copper(I) and the disulphide compound. Copper(III) complexes with suitable ligands have useful stability, even as analytical reagents.[35] The rates of decomposition of the amino-complexes are substantial,[36] but at least one sulphur-bonded species is reported to be stable.[37] The form of the second term in the rate law is compatible with competition between hexacyanoferrate(II) and hexacyanoferrate(III) ions for an intermediate in the second path.

An obvious possibility for the intermediate is the thio-radical $\mathrm{RS^{\bullet}}$. Radiolysis studies have shown that thio-radicals from such widely different compounds as cysteamine,[38] cysteine,[39] hydrogen sulphide,[40] and a variety of thiols of simple [41] and complex [42] structure are in equilibrium with the corresponding dithio-radical anion $\mathrm{RSSR^{-\bullet}}$. The species formed from cysteamine [37] and glutathione [43] are protonated at pH < 4, and the corresponding cysteine species reacts rapidly [44] with oxygen.

The mechanism which we propose is embodied in equations (13)—(18). Equilibrium (13) lies to the left under our conditions. Reactions (14) and (15) provide one path, and reactions (16)—(18) a parallel path to the same products. Oxidation of copper(I) in reactions (19) and (20) is believed to be rapid.[45] Applying the steady-state approximation to $[\mathrm{Cu^{III}(SR)_2}]^+$ and $\mathrm{RSSRH^{\bullet}}$, and noting that the sequence (16, 18, and 19) does not involve any overall consumption of $[\mathrm{Fe(CN)_6}]^{3-}$, it may be shown that (21) is applicable, where $\Sigma\mathrm{Cu}$ is the total copper concentration, $k_a = 2K_2k_1$, $k_b = k_1/k_2$, $k_c = 2K_2k_3$, $k_d = k_5/k_4$, and $k_1[\mathrm{RSH}]$ has been neglected relative to $[\mathrm{H}^+]$.

Comparison of observed and predicted behaviour. The rate law (21) is consistent with our experimental results. Values of k_a and k_c can be estimated from the gradients and intercepts of Figure 1 and gradients of Figure 3 respectively, assuming that the solutions contained

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copper at levels corresponding to the analyses of their components. The constants k_b and k_d for *N*-acetylcysteine may be estimated from Figure **3**(*a*). The corresponding quantities for 3-mercaptopropanoic acid are approximated similarly. Measurements required to estimate values of k_b and k_d for cysteine have not been made yet. The results of these estimates are as follows.

$$[\mathrm{Cu^{II}(SR)}]^+ + \mathrm{RSH} \overset{K_2}{\rightleftharpoons} [\mathrm{Cu^{II}(SR)_2}] + \mathrm{H^+} \quad (13)$$

$$[\mathrm{Cu^{II}(SR)_2}] + [\mathrm{Fe(CN)_6}]^{3-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{Cu^{III}(SR)_2}]^+ + [\mathrm{Fe(CN)_6}]^{4-} \quad (14)$$

$$[\mathrm{Cu^{III}(SR)_2}]^+ \xrightarrow{k_2} \mathrm{Cu^I} + \mathrm{RSSR} \quad (15)$$

$$[\mathrm{Cu^{II}(SR)_2}] + \mathrm{RSH} \xrightarrow{k_3} [\mathrm{Cu^I(SR)}] + \mathrm{RSSRH^{\bullet}} \quad (16)$$

$$\mathrm{RSSRH^{\bullet}} + [\mathrm{Fe(CN)_6}]^{3-} \xrightarrow{k_4} \mathrm{RSSR} + [\mathrm{Fe(CN)_6}]^{4-} + \mathrm{H^+} \quad (17)$$

$$\mathrm{RSSRH^{\bullet}} + [\mathrm{Fe(CN)_6}]^{4-} \xrightarrow{k_5} \mathrm{RS^-} + \mathrm{RSH} + [\mathrm{Fe(CN)_6}]^{3-} \quad (18)$$

$$[\mathrm{Cu^I(SR)}] + [\mathrm{Fe(CN)_6}]^{3-} \longrightarrow [\mathrm{Cu^{II}(SR)}]^+ + [\mathrm{Fe(CN)_6}]^{4-} \quad (19)$$

$$\mathrm{Cu^I} + [\mathrm{Fe(CN)_6}]^{3-} \longrightarrow \mathrm{Cu^{2+}} + [\mathrm{Fe(CN)_6}]^{4-} \quad (20)$$

$$\frac{-\mathrm{d}[\mathrm{Fe(CN)_6}^{3-}]}{\mathrm{d}t} = \frac{[\mathrm{Fe(CN)_6}^{3-}][\mathrm{RSH}]\,\Sigma\mathrm{Cu}}{[\mathrm{H^+}]} \left\{\frac{k_a}{1 + k_b[\mathrm{Fe(CN)_6}^{4-}]} + \frac{k_c[\mathrm{RSH}]}{[\mathrm{Fe(CN)_6}^{3-}] + k_d[\mathrm{Fe(CN)_6}^{4-}]}\right\} \quad (21)$$

	Cysteine	*N*-Acetyl-cysteine	3-Mercapto-propanoic acid
k_a	$4{\cdot}5 \times 10^4$	$1{\cdot}2 \times 10^3$	$3{\cdot}1 \times 10^3$
k_b		8×10^3	$1{\cdot}6 \times 10^4$
k_c	$1{\cdot}2 \times 10^4$	220	$1{\cdot}3 \times 10^3$
k_3		3·5	30

The linearity of plots giving values of k'' may be interpreted in terms of this mechanism. Since, under our conditions, the concentrations of thiol and (total) copper remain almost constant throughout a given reaction, equation (21) may be integrated in a straightforward manner (see Appendix). Insertion of the values above for the last two compounds gives a predicted variation of hexacyanoferrate(III)-ion concentration with time which could erroneously be accepted as a linear plot of (concentration)$^{-1}$, and thus as obeying a simple second-order relation.

It appears that the oxidation of the present thiols by hexacyanoferrate(III) ions provides a very clear example of a reaction which is stoicheiometrically simple, but which involves several competing paths. Although in some cases it may be convenient to treat the hexacyanoferrate ions as relatively uncomplicated ' electron-transfer ' reagents, on account of their relative immunity to ligand exchange, it is not necessary that such transfer involves the bulk form of the substrate.

APPENDIX

Integration of equation (21) leads to (22) where $k_r = k_a/k_b$, $k_s = k_c[\mathrm{RSH}]$, $F = [\mathrm{RSH}]\Sigma\mathrm{Cu}/[\mathrm{H_3O^+}]$, $[\mathrm{Fe(CN)_6}^{3-}]_0 = l$, $[\mathrm{Fe(CN)_6}^{4-}]_0 = m$, and $[\mathrm{Fe(CN)_6}^{3-}]_t = l(1 - x)$.

$$-[k_b(l + m)(k_dk_r + k_s) + k_s]F\,t = \frac{[k_b(l + m)(k_dk_r + k_s) + k_s]xl}{k_r + k_sk_d^{-1}} + k_d(l + m)[1 + k_b(l + m)]\ln(1 - x) + k_b^{-1}k_rk_s\frac{[(k_d - 1) - k_b(l + m)]^2}{k_s + k_r(k_d - 1)} \ln\left\{1 + \frac{[k_r(k_d - 1) + k_s]xl}{k_r(l + k_dm) + k_s(m + k_b^{-1})}\right\} \quad (22)$$

The third term on the right-hand side of (22) is much smaller than the preceding terms, so that this equation may be approximated to (23) where G, p, and q are functions of l, m, and the rate constants. Comparison of (22) with the empirical expression (24) may therefore be made as a comparison of (23) and (25).

$$G\,t = -px - q\ln(1 - x)$$
$$\simeq -px + q[x + (x^2/2) + (x^3/3) +, etc.] \quad (23)$$

$$k''\,t = l^{-1}[(1 - x)^{-1} - 1] \quad (24)$$
$$\simeq l^{-1}(x + x^2 + x^3 +, etc.) \quad (25)$$

For the first three terms of equations (23) and (25), the expressions will be approximately proportional if p/q *ca.* 0·5. If the conditions appropriate to these experiments are taken as $[\mathrm{RSH}] = 10^{-3}$ mol dm^{-3} and $l = 10^{-4}$ mol dm^{-3}, then substitution of the values for k_a to k_d gives the following p/q values.

Compound	10^4m	p/q
N-Acetylcysteine	0	0·38
	1	0·23
3-Mercaptopropanoic acid	0	0·68
	1	0·4

This criterion for p/q is therefore approximately satisfied, within a range about the experimental conditions used.

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