

# Electron-Spin Resonance Study of an Alkaline Solution of Copper(II) Oxalate–meso-Tartrate Complex (The Somogyi Reagent) and Related Complexes

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Electron-spin resonance (e.s.r.) studies of the structure of four reagents commonly used in the carbohydrate field, namely, the Somogyi reagent, the Fehling reagent, the Benedict reagent, and the Reeves reagent, in frozen aqueous solution at 77 K, gave definite indications of dimer in the Fehling reagent only; the Benedict reagent contains a relatively low concentration of dimer as compared to monomer. The presence of an "extra" peak at 3.245 kgauss in the e.s.r. spectrum of the Somogyi reagent at 25 °C may be associated with a paramagnetic, superoxide, ion-ligand  $O_2^-$ ; at 77 K, extra peaks were observed for the Somogyi, Fehling, and Benedict reagents.

Key words: Alkaline; complex; copper, e.s.r.; ligand; paramagnetic; reagent; superoxide; spectra; structure.

## 1. Introduction

Despite considerable work on determination of the structure of coordination complexes [1–4],<sup>1</sup> there are practically no reports on the structure of the blue complex present in the Somogyi reagent. From early work on determination of the structure of the blue complex present in the related Fehling solution [5], the presence of an ionized species in the complex was indicated, and a five-membered, copper-chelate ring was proposed; the latter was also favored by Cotton and Wilkinson [6].

In an e.s.r. and magnetic-susceptibility study of five copper compounds of D-tartaric acid, Ablov and co-workers [7] proposed a polymeric, chain-type structure for copper D-tartrate trihydrate; however, x-ray analysis of the related vanadyl tartrates showed a binuclear structure [3, 4]. The work of Lefebvre [8] on the composition of copper(II) citrate chelates indicates that they have a monomeric or dimeric structure, depending on pH and pCu; recent studies on this subject [9–11] have been interpreted as showing the existence, at pH 11 or higher, of a dimer in which two  $Cu^{2+}$  ions are separated by  $\sim 0.31$  nm.

In general, the e.s.r. spectrum of monomeric species ( $\Delta M = 1$ ,  $g \sim 2$  region) in frozen aqueous solution (at 77 K) of Cu(II) complexes shows a characteristic, single, intense, perpendicular component, ( $g_{\perp}$ ) and less-intense, four-line, parallel components ( $g_{\parallel}$ ); the spectrum of the complex can thus be characterized

by its anisotropic features associated with  $g_{\perp}$ ,  $g_{\parallel}$  and  $A_{\perp}$ ,  $A_{\parallel}$  components [2].

A typical e.s.r. spectrum of the Cu(II) complex in aqueous solution at room temperature shows a four-line, asymmetric pattern due to coupling of an unpaired electron of copper with the nuclear spin of the  $^{63}Cu$  and  $^{65}Cu$  nuclei ( $I_{Cu} = \frac{3}{2}$ );  $^{63}Cu$  and  $^{65}Cu$  have similar nuclear moments [1]. However, e.s.r. spectra of inorganic cupric salts (for example,  $CuCl_2$ ,  $Cu(NO_3)_2$ , or  $CuSO_4$ ) in aqueous solution show only a one-line pattern [12] that readily changes to a four-line spectrum on addition of a base (complex formation).

Recent advances, particularly in the application of modern polarography [9, 10], conductivity measurements [13], and, especially, low-field e.s.r. spectrometry [9, 11, 14, 15] provide tools for structural studies of coordination complexes. For copper or vanadyl complexes of a dimer having  $\Delta M = 2$  transitions, an e.s.r. low-field peak is observed [9, 11, 14, 15] at about  $H_0/2$  or 1.6 kG ( $g \sim 4$ ), whereas a monomer structure ( $\Delta M = 1$  transitions) shows a peak at  $g \sim 2$  ( $H_0/3.3$  kG).

## 2. Experimental Procedure

Ultraviolet and visible spectra were recorded with a Beckman DK-2 or Cary 14 spectrophotometer. Electron-spin resonance spectra were recorded with a Varian Model 4500 EPR spectrometer<sup>2</sup> with 100-kHz field-modulation and detection, operating at  $\sim 9.2$

<sup>1</sup> Figures in brackets indicate the literature references at the end of this paper.

<sup>2</sup> Certain commercial instruments are mentioned in this paper; this does not imply recommendation or endorsement by the National Bureau of Standards.

and  $\sim 9.5$  GHz. The klystron frequency was measured with a transfer oscillator and frequency counter. The magnetic field was measured by a proton gaussmeter monitored by the same frequency counter. All spectra were recorded in duplicate or triplicate, and the measurements were averaged. Replicate readings for the frequency-counter meter agreed within  $\pm 3$  percent.

The solutions were examined in a Varian Model V-4548 aqueous-solution sample-cell at various temperatures (25 to 60 °C) and at 77 K. A measured volume of the inosose solution was introduced directly into a cell by means of a microsyringe equipped with a long needle to facilitate mixing. The inosose solution and the reagents were prepared according to the published procedures indicated in the text.

### 2.1. Magnetic Parameters [16, 17]<sup>3</sup>

For the e.s.r. calculations, the following equations were employed, where  $g_{\text{cal}}$  is the  $g$  value calculated from the spectrum,  $\nu_{\text{K}}$  = klystron frequency, and  $\nu_{\text{p}}$  = proton frequency;  $g_{\text{cal}} = 3041.9 \nu_{\text{K}}/\nu_{\text{p}}$ ;  $\Delta$  (gauss) =  $(\nu_{\text{p}} - \nu_{\text{p}'}) \times 2.3486 \times 10^{-4}$ .  $A$  (in  $\text{cm}^{-1}$ ) =  $g_{\text{cal}} - 4669 \times 10^{-4} \times \Delta$  (in gauss) where there is a separation between peaks. In spectra of liquids (at 25 °C),  $g_{\text{m}}$  is defined as the middle point of the hyperfine lines of the spectrum. For spectra of crystalline materials (at 25 °C or 77 K), identification of  $g_{\parallel}$  and  $g_{\perp}$  was similar to that in the literature [16, 18]. Calculated  $g_{\text{m}}$  values for the solution at 25 °C were in approximate agreement with that calculated by the use of the relationship  $g_{\text{m}} = (2g_{\perp} + g_{\parallel})/3$  [18].

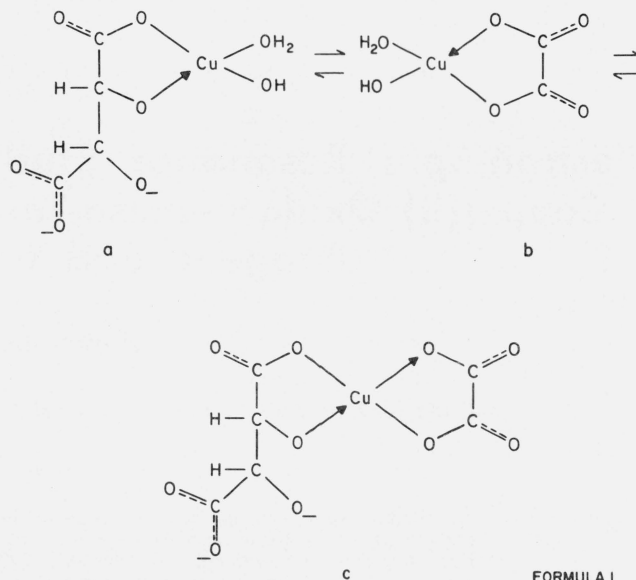
## 3. Results and Discussion

### 3.1. Structure of the Alkaline Copper(II) Oxalate-*meso*-Tartrate Complex (the Somogyi Reagent) and Related Complexes

Figure 1 shows, for four reagents important in the carbohydrate field, the complete e.s.r. spectra (1.0 to 3.5 kG) of supercooled (77 K), polycrystalline samples of the Cu(II) complexes from aqueous solutions. The reagents studied are: the Somogyi reagent [A, alkaline solution of copper(II) oxalate-*meso*-tartrate complex [19, 20]], the Fehling reagent [B, alkaline copper(II) *meso*-tartrate complex [21]], the Reeves reagent [C, copper(II) tetraamine hydrate complex [22]], and the Benedict reagent [D, alkaline copper(II) citrate complex [21]]. Of these spectra, B and, in part, D (fig. 1) have low-field absorption (1.0–2.0 kG region), suggesting the presence of dimeric as well as monomeric species, whereas A and C lack this feature.

The Cu(II) complexes present in the *Somogyi reagent* (in the polycrystalline state at 77 K and at room temperature) can be envisaged as an equilibrium mixture of *meso*-tartrate-oxalate monomeric species

(a, b, and c), with the following as possible structures for the monomeric anions:<sup>4</sup>



Evidence is now presented, for the first time,<sup>5</sup> for the existence of a dimer of the Cu(II) tartrate complex of the *Fehling reagent* at 77 K; a polynuclear, complex structure for this reagent had been suggested without proof [22, 23]. The low-field lines (1.6 kG) of the e.s.r. spectrum of the frozen Fehling solution (B, fig. 1) may be associated with the  $\Delta M = 2$  transitions<sup>6</sup> of a  $\text{Cu}^{2+}$ - $\text{Cu}^{2+}$  ion pair, as has been suggested for other complexes [14, 17]. Hence, the evidence strongly suggests the existence of a dimer in the Fehling solution at 77 K. A possible structure for a dimer present in the Fehling reagent is depicted, where  $a'$  and  $c'$  are *meso*-tartrate hydroxyl-carboxylate ligands that bridge [25] the  $\text{Cu}^{2+}(\text{OH})^-$ - $\text{Cu}^{2+}(\text{OH})^-$  ion-pair  $b'$  to give five-membered, chelate rings (a strongly alkaline reagent facilitates the ionization of the hydroxyl groups of *meso*-tartaric acid). The e.s.r. spectrum of the Fehling reagent at room temperature did not show the low-field absorption as observed elsewhere for Cu(II) sulfosalicylic acid dimer [11].

The e.s.r. spectrum of the *Benedict reagent* at 77 K (spectrum D, fig. 2) shows much broader, weaker, low-field absorption at 1.5 to 2.0 kG; it may also indicate the presence of a dimer. Indications of dimer in Cu(II) citrate complex at different pH's have been found [9, 11]. This sort of existence of dimers is lacking in the *Somogyi reagent* (A) and the *Reeves reagent* (C).<sup>7</sup>

<sup>4</sup>The coordination number of Cu(II) complexes in solution is larger than that of such complexes in the solid state; consequently, an aqueous Cu(II) *meso*-tartrate-oxalate complex may contain two water ligands that provide a maximum coordination number of six.

<sup>5</sup>After completion of this work, Chasteen, N. D. and Belford, R. L., [*J. Inorg. Chem.*, **9**, 169 (1970)] described the e.s.r. spectra of alkaline Cu(II) D, DL, and *meso*-tartrates; the authors found a binuclear copper complex with the racemic or DL-tartrate only. The Fehling reagent used in this study was prepared from a commercial Rochelle salt, a mixture of potassium-sodium-*meso* and D-tartrates.

<sup>6</sup>Reference [24] describes application of high-field e.s.r. spectrometry ( $H_0$  3.6–4.0 kG) to a study of transitions  $\Delta M = 2$ ; the paper discusses a dimer structure for Cu(II) propionate monohydrate, for which a seven-line spectrum was observed.

<sup>7</sup>The Reeves reagent has proved to be a useful tool in conformational analysis [26, 27]; see also, refs. [28, 29], describing other possible structures for Cu(II)-tetraamine complexes  $[\text{Cu}(\text{NH}_3)_4 \cdot (\text{H}_2\text{O})_2]^{2+}$ .

<sup>3</sup>See refs. [2] and [17] for a discussion of the magnetic parameters  $g_{\parallel}$ ,  $g_{\perp}$ ,  $A_{\parallel}$ , and  $A_{\perp}$ , and the transitions for metal complexes.  $\Delta M = 1$  and  $\Delta M = 2$  for monomer and dimer structures, respectively.

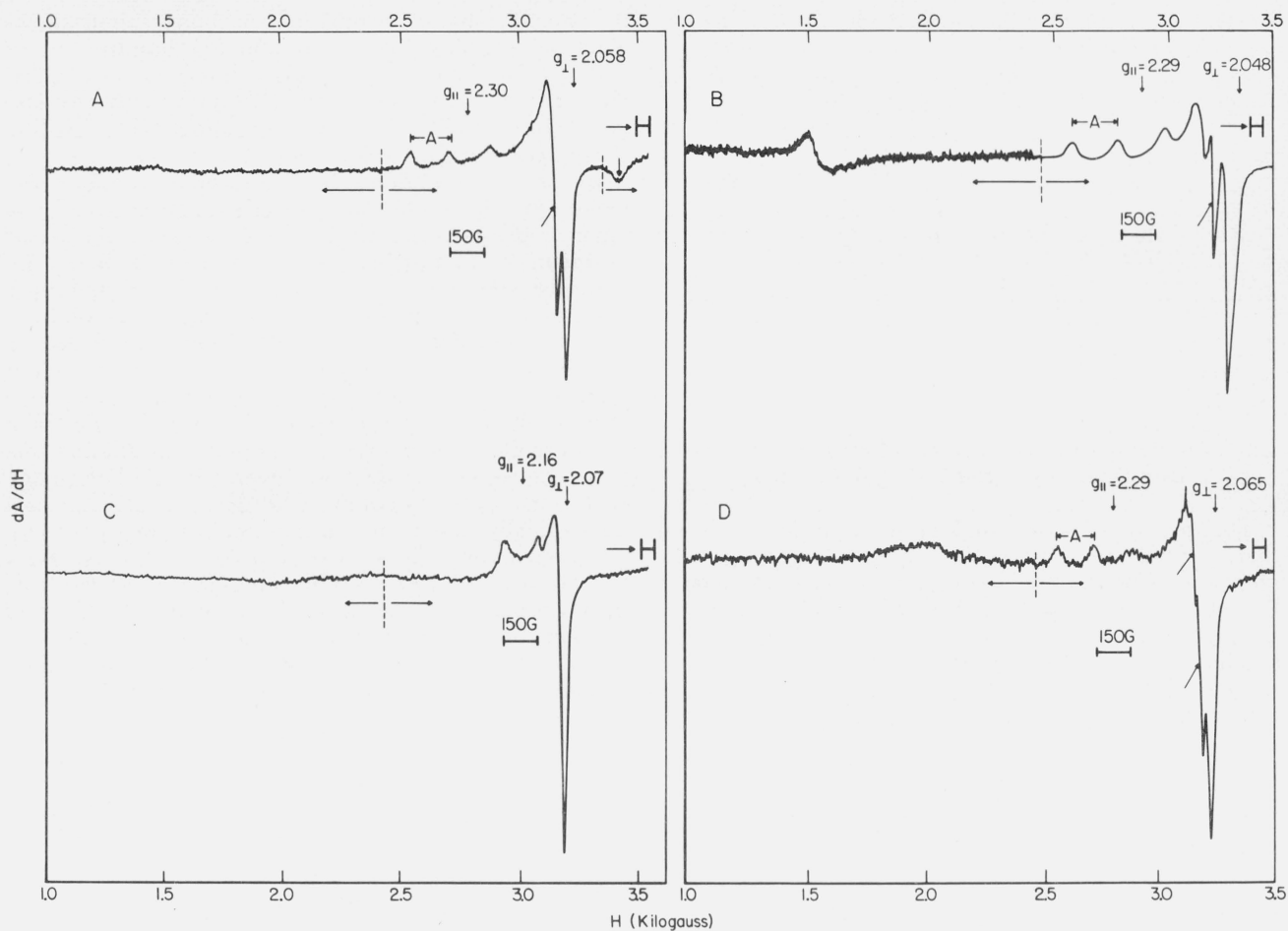
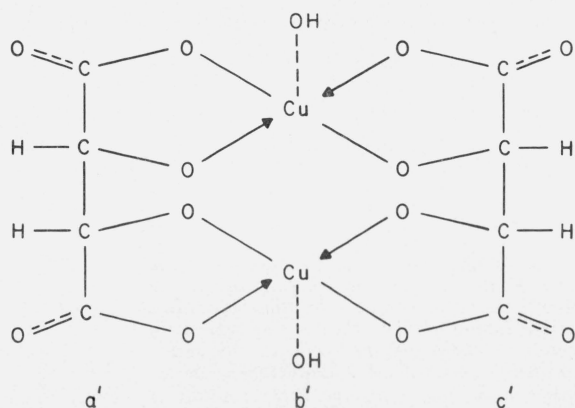


FIGURE 1. E.s.r. spectra of frozen (77 K) aqueous alkaline solutions of Cu(II) complexes: spectrum A, meso-tartrate-oxalate (the Somogyi reagent); B, meso-tartrate (the Fehling reagent); C, citrate (the Benedict reagent); and D, tetraamine hydrate (cuprammonium, the Reeves reagent). All four low-field e.s.r. spectra on the left side of the dotted line were recorded at somewhat greater field-modulation amplitude than the spectra for the  $g \sim 2$  region. A signal at 3.46 kG (spectrum A) was obtained at the same field-modulation amplitude as that used for the low-field spectrum.



FORMULA 2

The hyperfine structure in the e.s.r. spectrum of the freshly prepared Benedict reagent at room temperature slowly disappears as the reagent ages, although the oxidizing power remains. For example, a three-year-old reagent showed only a one-line e.s.r. spectrum having width  $\Delta = 19.3$  gauss and  $g_m = 2.091$ ; the associated structural changes remain unexplained.

Some overlap is observed in all four of the e.s.r. spectra of figure 1. Spectrum C is highly atypical and does not show the four components. The magnetic parameters for the monomeric species are given in table 1; the table also shows the coupling constants ( $a_{Cu}$ ) found for the Cu(II) complexes of the four reagents in solution (at room temperature).

TABLE 1. Magnetic parameters<sup>a</sup> for the Cu(II) complexes of the Somogyi (A), Fehling (B), Reeves (C), and Benedict (D) Reagents at 77 K, and the coupling constants  $a_{\text{Cu}}$  for the reagents at 25 °C

Reagent	$g_{\perp}$	$g_{\parallel}$	$A_{\parallel}$ ( $\text{cm}^{-1} \times 10^{-4}$ )	$a_{\text{Cu}}$ (gauss)
A	$2.058 \pm 0.003$	$2.302 \pm 0.005$	$164 \pm 10$	<sup>b</sup> $67 \pm 1$
B	$2.048 \pm 0.004$	$2.294 \pm 0.003$	$188 \pm 10$	<sup>c</sup> $68 \pm 2$
C	$2.072 \pm 0.001$	.....	.....	<sup>d</sup> $80 \pm 3$
D	$2.065 \pm 0.003$	$2.291 \pm 0.002$	$165 \pm 5$	<sup>e</sup> $47 \pm 2$

<sup>a</sup> Indicated limits of error for the  $g$ ,  $A$ , and  $a_{\text{Cu}}$  values are based on repeatability of measurements of reference points in the spectra.

<sup>b</sup> For spectrum E, figure 3.

<sup>c</sup> For spectrum Y, figure 2.

<sup>d</sup> For spectrum A-1, figure 2.

<sup>e</sup> For spectrum B-1, figure 2.

### 3.2. The Nature of Complexing Ligands (meso-Tartrate and Oxalate) in the Somogyi Reagent

The results of a recent kinetic study [30] indicate that, in order to achieve stability in solution for complexes of Cu(II), it is necessary to have at least a bidentate donor coordinated to the metal ion. In the Somogyi reagent, the bidentate ligands are the organic anions oxalate and meso-tartrate; the former, because of its structure, provides a continuous pathway between

the metal ions of conjugated  $\pi$  bonds that allows electron exchange to occur about a hundred times faster than do the others [31]. However, as will be shown, the complexing power (ability) of the oxalate anion present in the Somogyi reagent is far below that of the meso-tartrate anion present.

In order to obtain more information about the monomer relationship of the oxalate and meso-tartrate ligands present in the Somogyi reagent, the e.s.r. spectrum of an aqueous solution of each at room temperature was separately studied. It was found that, when potassium-sodium meso-tartrate was omitted from the reagent (all other constituents unchanged), a blue solution was obtained having the prominent e.s.r. spectrum depicted in T, figure 2; however, soon after preparation of the solution, crystallization of a bluish white copper oxalate complex started, and this proceeded continuously for several days. The shape of the e.s.r. spectrum of the solid (E-1, fig. 2) suggests a magnetically dilute sample that could have a polycrystalline structure. The solid decomposes on recrystallization from water, but is stable when recrystallized from basic solution. It was also observed that addition of reagent containing potassium-sodium meso-tartrate (U, fig. 2) to the oxalate solution keeps the copper oxalate complex in solution. This result indicates that,

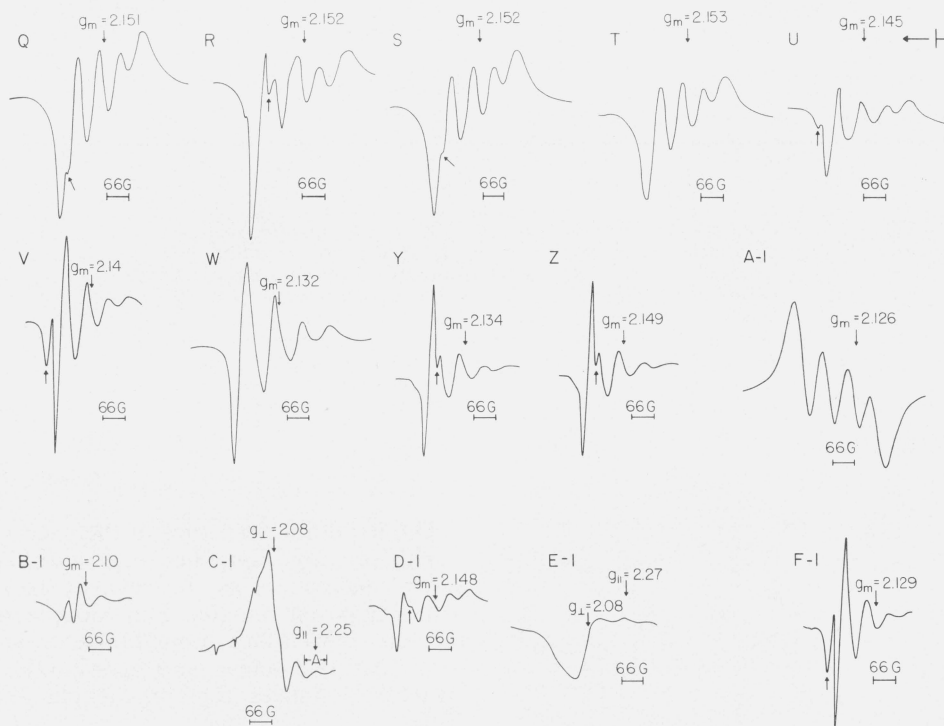


FIGURE 2. E.s.r. spectra of selected Cu(II) complexes at 25 °C: spectrum Q, the Somogyi reagent in which copper sulfate was added as the last constituent; R, the Somogyi reagent containing <sup>65</sup>Cu isotope; S, the Somogyi reagent containing <sup>63</sup>Cu isotope; T, the Somogyi reagent containing oxalate ligand only; U, the Somogyi reagent containing tartrate ligand only; V, the Fehling reagent in ~200mM sodium carbonate; W, the Fehling reagent in ~50mM sodium hydroxide; Y, freshly prepared Fehling reagent; Z, the Somogyi reagent in deuterium oxide; A-1, the Reeves reagent; B-1, the Benedict reagent; C-1, the freeze-dried solid from the Somogyi reagent; D-1, solid G-1 dissolved in deoxygenated water; E-1, the solid from the oxalate solution T; F-1, the Fehling reagent in deuterium oxide.



in the Somogyi solution, the Cu(II) oxalate complex cannot long exist in solution in the absence of a more powerful complexing agent, namely, the *meso*-tartrate anion, but that the latter (alone) can exist. The Cu(II) *meso*-tartrate complex (U, fig. 2) has about one tenth the derivative peak-height of the Cu(II) oxalate complex (T, fig. 3). The e.s.r. spectrum of the Somogyi reagent prepared in deuterium oxide (Z, fig. 2) was difficult to reproduce. Sometimes, it was similar to that for the aqueous solution (for example, spectrum R, fig. 2), and sometimes it was identical with that of the Fehling reagent prepared in deuterium oxide (F-1, fig. 2).

### 3.3. The Nature of the Extra Lines Observed in the E.S.R. Spectra of the Somogyi and Related Reagents

Various workers [9, 32] have observed the presence of extra lines, in addition to four transitions, in a variety of Cu(II) complexes. Recently, Baines and co-workers [33] observed a multilined e.s.r. spectrum for Cu(II) complexes in frozen sodium hydroxide and in a frozen sodium hydroxide-cellulose system. The authors [33] explained the extra paramagnetic species observed as being due to trapped electrons that subsequently formed radicals; however, these are very reactive species that could not be observed at room temperature [34-43].

The e.s.r. spectrum of the Somogyi reagent at room temperature (E, fig. 2) shows an extra line at about 3.245 kG ( $g=2.068$ , indicated by the arrow, E) and this signal slowly increased in magnitude with the "aging" of the reagent caused by exposure to daylight. On freezing, the extra line seems to shift reversibly toward the  $g_{\perp}$  line ( $g=2.072$ ; see arrow in A, fig. 1).

A similar reversible shift on cooling was also observed for the Fehling reagent; the extra line observed at 77 K (indicated by the arrow in B, fig. 1) shifted on warming the sample to room temperature (Y, fig. 2), and this spectrum was identical with that of freshly prepared Fehling reagent. The similarity between spectra E (fig. 3) and Y (fig. 2) is also evident.

Examination of the e.s.r. spectrum Q may lead to confusion regarding the extra line for the  $^{63}\text{Cu}$  isotope, as a slight difference in the nuclear spins of  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  isotopes has been deduced from spectra by the use of Kivelson's method [44, 45]. To eliminate this remote possibility of confusion regarding the extra line for the  $^{63}\text{Cu}$  isotope, separate Somogyi reagents containing  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$  isotopes, respectively, were prepared, and their e.s.r. spectra were examined (spectra R and S in fig. 2 for  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$ , respectively). From the position of their strongest peaks (at about 3.277 kG for  $^{63}\text{Cu}$ , and 3.270 kG for  $^{65}\text{Cu}$ , as compared to 3.275 kG for the Somogyi reagent prepared from the natural-abundance mixture of  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$ ), it was concluded that the extra peak in spectrum Q does *not* arise from  $^{63}\text{Cu}$ , which would be difficult to observe under ordinary conditions [1, 44]. The extra peak was also observed in the spectrum of the Somogyi reagent prepared from the  $^{65}\text{Cu}$  and  $^{63}\text{Cu}$  isotopes, respectively, and measured at 77 K.

Study of the extra lines observed in the e.s.r. spectra of the Somogyi and related reagents at room temperature was approached from several different angles. The possibility that the extra line in the e.s.r. spectrum of the Somogyi reagent arises from a labile, paramagnetic ligand of the Cu(II) complex received support from several pertinent observations: (1) the position and intensity of the extra line was found to be pH-dependent (see fig. 3); (2) the line decreased in size, and finally disappeared, on successive treatment with a reducing compound, namely, inosose (fig. 4, see arrows); and (3) it was lessened in size by bubbling nitrogen through the solution or by using deoxygenated water to prepare the reagent (see spectra C-1 and D-1, fig. 2). Moreover, as already mentioned, this extra line had a tendency to (a) increase in intensity and shift to higher field on "aging" by exposure to daylight or by slight warming, (b) decrease in intensity when kept in the dark, and (c) be somewhat dependent on the order of mixing of the components.

The possibility that the extra line was due to impurity in the reagents was eliminated. A typical e.s.r. spectrum of the Somogyi reagent (E, fig. 3) showed an extra line at about 3.245 kG (see arrow). This reagent was successively titrated with an aqueous base (50 to 200 mM sodium hydroxide), and the change in the shape of the e.s.r. spectrum was monitored. Spectrum G (fig. 3) was obtained at about pH 10.1 (range 9.6 to 10.3); it showed a decrease in intensity of the extra peak (see arrow); at pH 10.9 (range 10.6 to 11.0), the peak appeared as a shoulder (spectrum I, arrow); at pH 11.5 (range 11.2 to 12.1), it shifted to higher field (K, arrow). A further addition of base, to pH 13.2 (range 13.0 to 14.0), produced an increase in intensity of the peak (F, arrow), and, at 100 mM in base, the extra line reached the maximum intensity (H, arrow), resembling the spectrum of the Somogyi reagent containing Cu(II) tartrate only (U, fig. 2).

Also, treatment of the Somogyi reagent with aqueous acetic acid (1 M) caused shift of the original extra line. At pH 8.8 (range 8.3 to 8.9), the line appeared as a shoulder at the strongest peak (J, fig. 3, arrow), and, at pH 7.6 (range 7.5 to 7.8), it disappeared completely and irreversibly, to give a four-line, asymmetric, e.s.r. pattern (L, fig. 3) typical of Cu(II) complex [1]. This spectrum resembles that of the Somogyi reagent containing, as the organic ligand, oxalate only (T, fig. 2); further addition of acetic acid to the probe caused liberation of iodine that interfered with the measurements. However, when the Somogyi reagent, prepared without potassium iodide or iodate, was neutralized to about pH 6 (1 M hydrochloric acid), the e.s.r. spectrum obtained showed a one-line pattern, similar to that for neutral cupric salts [12].

The results of the foregoing experiments definitely prove the lability and pH-dependence of the "extra" paramagnetic species present in the Somogyi reagent; they also indicate the basic character of the species that could not exist below pH 8.0. As judged from the shape of the e.s.r. spectra (H, L, fig. 3, and T, U,

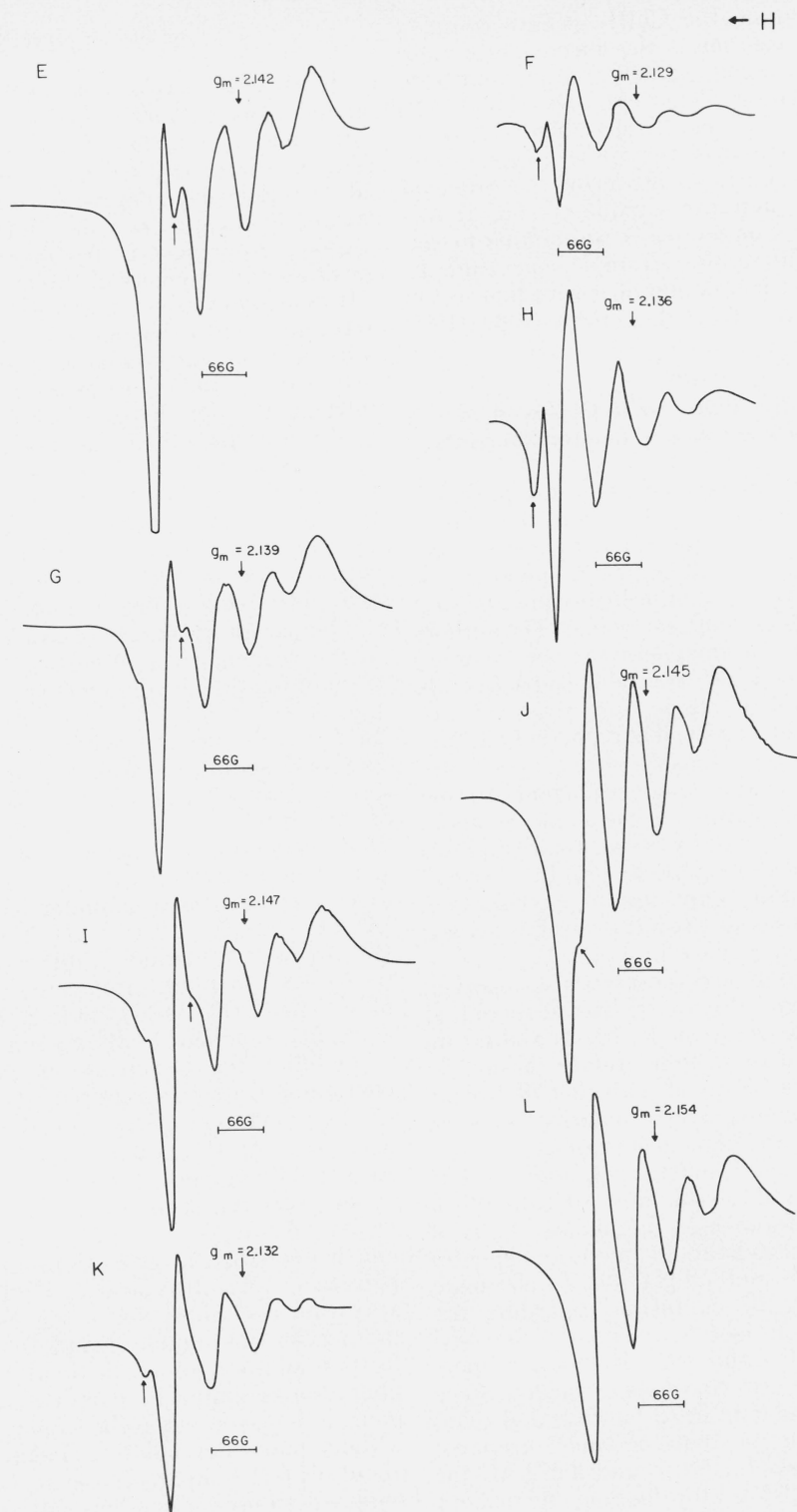


FIGURE 3. E.s.r. spectra of the Somogyi reagent at 25 °C, at various pH's: E, pH 9.4; G, ~ 10.1 (range 9.6 to 10.3); I, ~ 10.9 (range 10.6 to 11.00); K, ~ 11.5 (range 11.2 to 12.1); F, ~ 13.2 (range 13.0 to 14.0); H, ~ 100mM sodium hydroxide; J, pH ~ 8.8 (range 8.3 to 8.9); and L, ~ 7.6 (range 7.5 to 7.8).

fig. 2) at the lower pH, Cu(II) oxalate complex appears to be a preponderant component of the Somogyi reagent; and, at higher pH, the equilibrium apparently favors Cu(II) tartrate complex as the major component.

An analogous pH-dependence was observed for the Fehling reagent; the original, high-field peak in Y (arrow, fig. 2) shifted to below the strongest peak when the reagent was prepared in 200 mM sodium carbonate solution (V, fig. 2, arrow). This line was not visible in 50 mM sodium hydroxide solution, apparently due to overlapping with the strongest peak (spectrum W); moreover, it shifted when the solution was prepared in deuterium oxide (F-1, fig. 2, arrow).

#### b. Effects of Absorbed Oxygen and Reductants

The influence of absorbed oxygen on the position of the extra line for the Somogyi reagent was demonstrated by the following experiment. An aliquot of the Somogyi reagent was freeze-dried to give a paramagnetic solid having the e.s.r. spectrum shown in C-1 (fig. 2); the spectrum was somewhat different from those reported [46] for cupric sulfate pentahydrate. Dissolution of this solid in deoxygenated water<sup>8</sup> under

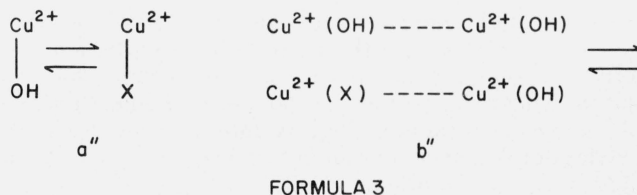
nitrogen gave the Somogyi reagent that had an e.s.r. spectrum (D-1, fig. 2) showing an extra, high-field peak that was considerably lessened (arrow) in comparison with the usual solution spectra (E, fig. 3) or (M, fig. 4).

The influence of the reductant [for example, an inosose (pentahydroxycyclohexanone) [47] or ascorbic acid] on the shape of the e.s.r. spectrum of the Somogyi reagent is shown in figure 4. This experiment may be used for demonstrating that the extra line observed in the e.s.r. spectrum of the Somogyi reagent is the first (among all of the transitions) to respond to treatment with the inosose. Figure 4 shows that a gradual decrease in intensity of the extra lines occurs, until they disappear completely (P, fig. 4, arrow) as a consequence of gradual addition of the inosose to the reagent (see arrows in M, N, O, and P, fig. 4). This experiment indicates the existence of the superoxide type of component in the Somogyi reagent, and only a paramagnetic species of such a type is able to show the observed sensitivity toward inosose (or any other reductant).

#### c. New Possible Ligands in the Somogyi Reagent and the Fehling Reagent

Careful examination of the results in the preceding experiments indicates existence, in the Somogyi reagent, of the superoxide anions  $O_2^{\cdot -}$  and  $\dot{O}_2^-$  in equilibrium with the excess of hydroxyl ions present in the reagent. The concentration of the paramagnetic oxygen-carrying ligand (for example,  $\dot{O}_2^-$  in the Somogyi reagent) is rather small, as judged from the low width and magnitude of the extra derivative peak.

Consequently, the structure proposed for the Somogyi and the Fehling reagent should contain new parts, involving the active-oxygen ligands a'' and b'', for monomeric and dimeric species, respectively.



where  $\text{X} = O_2^{\cdot -} \rightleftharpoons \dot{O}_2^- \rightleftharpoons O_2 \rightleftharpoons OH^-$ . The components  $O_2^{\cdot -}$ , and  $O_2$  are known [48] to be paramagnetic, and one line can be expected in their e.s.r. spectra. To the above equilibrium ( $O_2^{\cdot -} \rightleftharpoons \dot{O}_2^- \rightleftharpoons O_2 \rightleftharpoons OH^-$ ) can be added the contribution from the electron-rich organic ligand (such as  $R-O^-$  with any hydroxy compound or hydroxy carboxylic acid). For example, the observed shift of the extra line for the Fehling reagent in the different basic media (spectra V, W, Y, fig. 2) may be partially explained as due to a change in conformation of the coordinated tartrate ligand [49, 50]; furthermore, the presence of the extra line is frequently noted in spectra of Cu(II) complexes containing hydroxyl or hydroxy carboxylic groups.

One possible source of the active-oxygen-containing

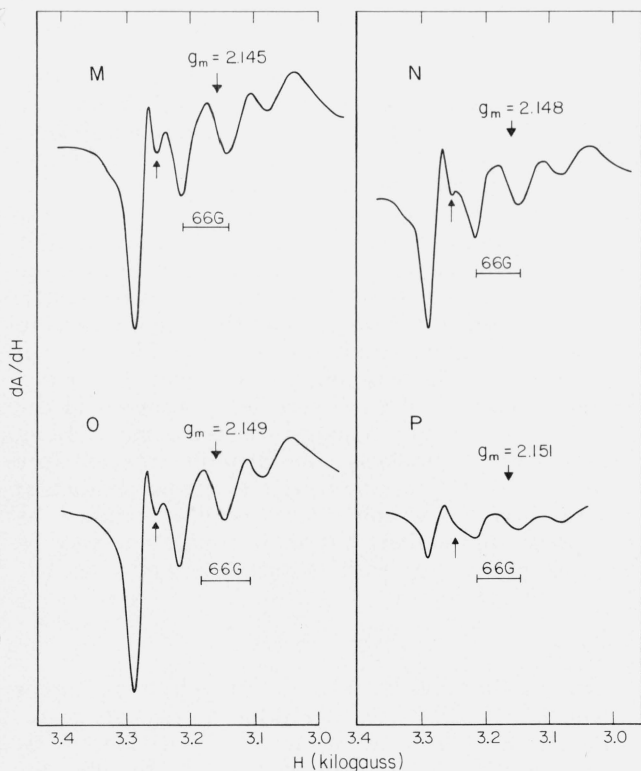
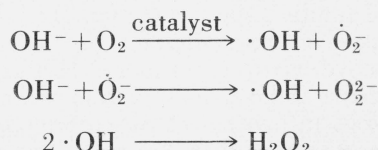


FIGURE 4. E.s.r. spectral changes after titration of the Somogyi reagent ( $\sim 0.4$  ml) with mM inosose: spectrum M, the Somogyi reagent before titration ( $25^\circ$ ); O, after mixing with  $\sim 0.1$  ml of 1 mM inosose ( $25^\circ$ ); N, after mixing with  $\sim 0.2$  ml ( $55^\circ$ ); P, after mixing with  $\sim 0.4$  ml ( $55^\circ$ ).

<sup>8</sup> Distilled water was refluxed with potassium permanganate, and the distillate was cooled under a bubble-stream of purified nitrogen.

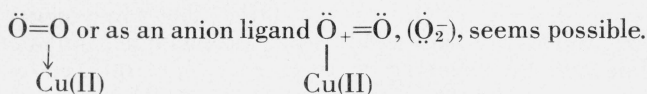
species that could possibly arise in the alkaline Somogyi reagent is shown in the following reactions:



The energy required for the first two reaction-sequences to occur can be supplied by photons [51], or another species equivalent to paramagnetic  $\dot{\text{O}}_2^-$  can be formed by the reaction of a semidone radical ( $\text{R}'$ , see below) with  $\text{O}_2$ ;  $\text{R}' + \text{O}_2 \rightarrow \text{R}'\text{O}_2$ . The catalyst that could possibly initiate this reaction sequence could be  $\text{Cu}^{2+}$  ion, photon [51] (colored Somogyi solution, 690–700 nm) with the possible excitation of  $d$  electrons [52],<sup>9</sup> an indirect influence of the hydrated electron [43], or a trace proportion of the semidone type of radical derived from *meso*-tartaric acid or any hydroxy carboxylic acid. The paramagnetic products would then be stabilized in equilibrium, in an anion vacancy ( $\text{OH}^-$  ligands), after all transitory species (such as  $\cdot\text{OH}$  radicals) had been decomposed.

Calculation of the  $g$  values possible arising from the paramagnetic superoxide ions ( $\dot{\text{O}}_2^-$ ) observed in the frozen solutions at 77 K (see fig. 1) gave the following values: for the *Somogyi reagent* (A, arrow next to  $g_{\perp}$  line),  $g=2.072$ , and the presence of another, unknown, extra line at 3.459 kG with  $g=1.912$  (A, arrow far right) was observed; for the *Fehling reagent*,  $g=2.054$  (B, indicated by the arrow); and for the *Benedict reagent*,  $g=2.068$  and  $g=2.074$  (D, arrows). These values are roughly comparable to the assigned  $g$  values of mononegatively charged oxygen ions,  $g(\text{O}^-)=2.06$ , obtained [39, 40] on radiolysis of strongly alkaline ices at 77 K, or  $g(\text{O}_2^-)=2.089$  at 77 K [Ref. 40, p. 156].

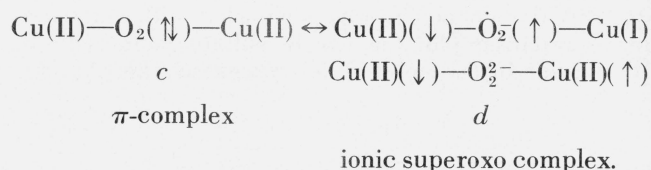
Taking into consideration the chemistry and the catalytic ability of Cu(II) in oxidation–reduction reactions involving molecular oxygen in enzymic [54, 55] and nonenzymic systems [56–58], or in ligand-transfer [59] or electron-transfer [60] oxidations, the idea that Cu(II) complex may contain active oxygen, either as an addition,  $\pi$ -electron-bonded, ethylene type of complex [49, 61, 62]



In the first example, in the  $\text{CuO}_2\text{—Cu}$  ( $\pi$ -electron-bonded complex), the molecule will be diamagnetic, the spins being completely coupled in the metal–metal bond [49, 61, 62, 63]. Analogous oxygen-containing complexes have been reported for a series of transition metals, such as iridium [63–65], platinum [66], cobalt [63, 67, 68], and others [69].

In the second case, in which the active oxygen in the (Cu(II)) complex is present as an anion ( $\dot{\text{O}}_2^-$ ), for example in  $(\text{CuO}_2)^{2+}$  or in  $(\text{CuO}_2\text{Cu})^{2+}$  cations, the spin in the oxygen anion remains uncoupled, and, in the e.s.r. spectrum, these complexes would be expected to give one extra line, in addition to four others. The existence of such peroxy or superoxy complexes has been proposed for certain phenolases [70–72] and for certain metallo-proteins, such as hemoglobin, that was able to carry molecular oxygen [63, 73], or combine with it in certain electron-transfer processes, for example, in the two-step reaction mechanism proposed for oxidase, laccase, and other enzymes [74].

For example, beginning with a diamagnetic Cu(II)–oxygen adduct  $c$  ( $\pi$ -complex), an electron transfer in an alkaline solution may involve an oxygen bridge; as a result, an ionic superoxy complex  $d$  containing a paramagnetic ligand  $\dot{\text{O}}_2^-$  (or a peroxy nonparamagnetic ligand  $\text{O}_2^{2-}$ ) could be formed. This transfer can be visualized as follows:



In addition to these adducts containing either molecular oxygen or an superoxy type of group, and the old oxidants manganese dioxide and lead dioxide, there have recently been discovered germanium peroxide [75], the specific oxidant nickel peroxide [76–78], the perferryl cation  $\text{FeO}_2^{2+}$ , [79] and the  $\text{Cu}^{2+}$ –hydrogen peroxide complex ( $\text{CuO}_2\text{H}^+$ ) [80].

All of the evidence presented here indicates the existence of the superoxy type of ligand in the Cu(II) complex in both the Somogyi and the Fehling reagent at room temperature and in the polycrystalline state at 77 K. However, the possibility is not excluded that a certain proportion of the extra lines observed in the e.s.r. spectra of the Somogyi reagent, the Fehling reagent, and, particularly, the Benedict reagent (see arrows, fig. 1) at 77 K may be due to the perpendicular features from  $^{63}\text{Cu}$  and  $^{65}\text{Cu}$  isotopes modified by quadrupole interaction, but their separation may be obscured by overlap with features derived from the superoxide ion.

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<sup>9</sup> In analogy to electron transfer with an ion pair [52] ( $\text{Fe}^{3+}\text{OH} \xrightarrow{h\nu} \text{Fe}^{2+} + \cdot\text{OH}$ ), the following reaction is also feasible [53]:  $\text{Cu}^{2+}\text{OH} \xrightarrow{h\nu} \text{Cu}^{2+} + \cdot\text{OH}$ .



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