The Oxidation of Cysteine by Cytochrome Oxidase and Cytochrome c

ENZO BOERI*, HERRICK BALTSCHEFFSKY**, ROGER BONNICHSEN and KARL-GUSTAV PAUL

Medicinska Nobel institutet, Biokemiska avdelningen, Stockholm, Sweden

In connection with investigations on the interaction between catalase and cysteine it appeared to be necessary to compare the oxidation of cysteine as catalyzed by catalase with the oxidation catalyzed by the cytochrome system of heart muscle. The latter catalysis, discovered by Keilin in 1930, has been the subject of more recent investigations with other tissues as well. The amperometric technique for the estimation of cysteine and cystine used in the present investigation gave some new aspects since it requires smaller concentrations of the reactants than the manometric technique.

MATERIAL AND METHODS

The heart muscle preparation was obtained essentially according to Keilin and Hartree. Pig hearts were collected within four hours after death, freed from fat, ligaments etc., and ground two times in a meat mill. The mince was washed for four hours with running tap water with occasional squeezing in cheese cloth. Portions of 300 g were rapidly homogenized in a “Turmix” with 500 ml cold 0.02 M phosphate buffer pH 7.4, the homogenizer being previously chilled with ice. Then followed centrifugation for 20 min. at 2,000 r.p.m. in an angle centrifuge. The supernatant was decanted, and the remaining material again homogenized as above. The two supernatants were combined, and cold, molar acetic acid was added during agitation to pH 5.6—5.7. After centrifugation the sediment was washed with cold water and centrifuged again, and suspended in cold 0.25 M phosphate buffer pH 7.4 to a volume of 60—75 ml. The suspension was shaken slowly in a flask until all lumps had disappeared. Sodium nitrate (1 mg/ml) was added. The material was centrifuged for one hour at top speed in a Sorvall centrifuge (about 12,000 r.p.m.). The precipitate was suspended in 0.02 M buffer pH 7.25 to give

* Rockefeller Foundation Fellow. Permanent address: Stazione Zoologica, Naples, Italy.
** Present address: Länsjukhuset, Åbo, Finland.

Acta Chem. Scand. 7 (1953) No. 5
the original volume, and ground in a glass homogenizer. After a second high speed centrifugation in the Sorvall centrifuge the bottom layer was suspended in 0.1 M phosphate buffer pH 7.25. The QO₂ of such preparations when tested with succinate, varied between 100 and 250 μl O₂/mg dry weight/h (Warburg manometers with air as gas phase, pH 7.25, 37°C, 100 osc./min.). In the legends the amounts of heart muscle preparation are given as mg dry weight, corrected for buffer salts.

The preparations were tested for catalase, but no measurable activity was found. Moreover, when catalase was added to give a final concentration of 6.1 x 10⁻⁷ M, no measurable increase in the rate of cysteine oxidation was found.

Different preparations of cytochrome c with iron contents from 0.347 to 0.427 % were used. The cytochromes were obtained from beef hearts with the technique usually employed in this laboratory. As suggested by Tsou the per cent “autoxidizable cytochrome c” was calculated from the spectral changes upon CO treatment at pH 7.4 of reduced cytochrome. His values for molar absorptions were used.

Cysteine hydrochloride Eastman Kodak or Merck was used.

The oxidation tests were performed at the selected temperatures (25°C or 37°C) in test tubes immersed in a water thermostat. The tubes, containing all reactants except the heart muscle preparation, were left in the bath for five minutes to equilibrate temperature. Meantime oxygen from a tank via a washing flask (to equilibrate temperature and humidity) was bubbled through the solution. Then the heart muscle preparation (1/15—1/30 volumes of the original content of the tube) was added. The reaction was stopped by rapidly pouring the content of the tube into the titration vessel, thereby diluting it ten times with a solution containing sodium sulfite and no oxygen. Some of the solution in the vessel was withdrawn to rinse the test tube. The titration was carried out within two minutes in a nitrogen atmosphere at pH 9. As an alternative procedure, giving the same results as above, the reaction was stopped by the addition of sulfuric acid in an amount sufficient to bring the content of the test tube to about pH 3. The acid was neutralized with an equivalent amount of ammonia before the titration. Blanks without enzymes were always made.

The amount of cysteine oxidized by the enzyme system was taken as

\[
\text{cysteine oxidized} = c₀ - cᵢ - c₉
\]

where \( c₀ \) = cysteine present at time zero, \( cᵢ \) = remaining cysteine at time \( t \), and \( c₉ \) = the amount of cysteine oxidized in the blank test. Usually the latter amount was low (< 5 %), especially at 25°C and in the rapid experiments.

Cysteine was determined amperometrically with silver nitrate or copper (II) sulfate according to Kolthoff and Stricks. The latter method was preferred for low cysteine concentrations.

The experiments on the reduction of cytochrome c by cysteine were made in a Beckman spectrophotometer at 550 mμ.
RESULTS

The oxidation of cysteine requires the presence of both cytochrome oxidase and cytochrome c, as already shown by Keilin. Cytochrome c alone has a very low catalytic activity, small in comparison to the activity of the system (heart muscle preparation + cytochrome c). Boiling destroys the catalytic activity of the heart muscle preparation in this reaction (Table 1).

The appearance of cystine in a molar ratio of 1:2 to vanished cysteine fully accounts for the disappearance of the latter under our experimental conditions. Medes had earlier found the same under similar conditions. We used the following procedure to confirm that the oxidation does not go further than to cystine. After the addition of heart muscle preparation and cytochrome c to a cysteine solution sufficient time was allowed to pass to let all cysteine be oxidized. The end of the reaction was well indicated by the disappearance of the absorption bands of ferrocytochrome c. An aliquot of the solution was titrated in the presence of sodium sulfite: during this condition both RSH and RSSR compounds react with silver ions in a molar ratio of 1:1, and with cupric ions in a ratio of 1:2. This is explained by the reaction between RSSR and sulfite (Clark).

\[
\text{RSSR} + \text{SO}_2^- \rightarrow \text{RS}^- + \text{RSSO}_2^-
\]  

Thus a titration with a silver or copper salt in the presence of sulfite does not give a measure of the amount of RSH but of RSH + RSSR. As it is seen in Table 2 the total amount of RSH + RSSR had dropped to half of its original

Table 1. The oxidation of cysteine by the various components of the system. A: \(2.23 \times 10^{-3}\) M cysteine. B: Heart muscle preparation (1.55 mg/ml) in M/15 phosphate buffer pH 7.3.
C: \(4.48 \times 10^{-4}\) M cytochrome c. Temp. 25°, pH 7.50; M/15 phosphate buffer. Reaction time 1 min.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Reactants</th>
<th>Remaining amount of cysteine in µmoles</th>
<th>% oxidized of initial amount of cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 ml. A</td>
<td>6.68</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>3 ml. A + 0.2 ml. B</td>
<td>6.68</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>3 ml A + 0.2 ml. C</td>
<td>6.61</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>3 ml A + 0.2 ml B + 0.2 ml C</td>
<td>2.77</td>
<td>58.6</td>
</tr>
<tr>
<td>5</td>
<td>3 ml A + 0.2 ml B + 0.2 ml boiled O</td>
<td>6.57</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Acta Chem. Scand.* 7 (1953) No. 5
Table 2. Identification of reaction product. Content of reaction vessel: 5 ml \(1.143 \times 10^{-3}\) M cysteine solution + 0.6 ml \(1.88 \times 10^{-4}\) M cytochrome c + 0.83 ml heart muscle preparation of dry weight 21.2 mg per ml. Temp. 25°, pH 7.25.

<table>
<thead>
<tr>
<th>Titration no.</th>
<th>Titrated material</th>
<th>(\mu)moles of (RSH + RSSR) per ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Original cysteine solution</td>
<td>1.143</td>
</tr>
<tr>
<td>2</td>
<td>At the end of the oxidation</td>
<td>0.578</td>
</tr>
<tr>
<td>3</td>
<td>After reduction with Na-amalgam</td>
<td>1.130</td>
</tr>
</tbody>
</table>

value when all the cysteine had been acted upon by (cytochrome oxidase + cytochrome c). Another aliquot of the solution was acidified, thoroughly treated with sodium amalgam, and titrated. The amalgam reduced the cystine back to cysteine, and the original concentration at time zero was restored (after correction for the dilution with acid).

For the oxidation of four moles of cysteine one mole of oxygen is required under the actual conditions. The oxidation of cysteine during one minute in experiment 4 in Table 1 would thus correspond to the \(Q_{O_2}\)-value of 4670 \(\mu\)l \(O_2\)/mg/h. However, the extrapolation, on which this calculation of \(Q_{O_2}\) is based, is not justified in this case, since the reaction is not zero but first order with respect to the disappearance of cysteine (cf. below). Thus \(Q_{O_2}\)-values, calculated from successive periods, will decrease with time. It was consistently

Table 3. Influence of oxygen supply. Initial cysteine concentration: \(2.5 \times 10^{-3}\) M (total amount of cysteine = 12.5 \(\mu\)M). Cytochrome c: \(2.69 \times 10^{-5}\) M. Heart muscle preparation: 0.093 mg per ml of final solution. Total volume 5 ml Temp. 37°, pH 7.50, M/15 phosphate. Reaction time one minute.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Oxygen supplied by</th>
<th>Cysteine oxidized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\mu)moles</td>
</tr>
<tr>
<td>1</td>
<td>Air without shaking</td>
<td>2.10</td>
</tr>
<tr>
<td>2</td>
<td>Air with shaking</td>
<td>2.99</td>
</tr>
<tr>
<td>3</td>
<td>Air bubbling</td>
<td>3.85</td>
</tr>
<tr>
<td>4</td>
<td>Oxygen bubbling</td>
<td>5.29</td>
</tr>
</tbody>
</table>

found that the oxidation of cysteine proceeded faster in the amperometric than in the manometric experiments. The cytochrome oxidase preparation, employed in the experiments in Table 1, gave manometrically \( Q_{o_2} = 227 \) with 0.02 \( M \) succinate and 200 with 0.015 \( M \) cysteine as substrates, both determinations being made at 37°. Here the oxygen uptake was linear with time for the period of the experiment (18 min.). The rate of cysteine oxidation is highly dependent on the oxygen tension (Table 3).

**Kinetics of the reaction.** Fig. 1 illustrates the kinetics of the oxidation of cysteine by (heart muscle preparation + cytochrome c). It appears that the

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Temperature</th>
<th>Average k-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2, 3</td>
<td>25° C</td>
<td>0.038</td>
</tr>
<tr>
<td>4, 5, 6</td>
<td>37</td>
<td>0.082</td>
</tr>
</tbody>
</table>

*Acta Chem. Scand. 7 (1953) No. 5*
reaction is of order one with respect to the concentration of cysteine. Hence in the sequel the first order constant of the reaction

\[ k = \frac{1}{(\text{sec.})(\text{mg preparation/ml})} \times \ln \frac{x_0}{x_t} \]  

(2)

will be employed, where \( x_0 \) and \( x_t \) denote the concentrations of cysteine at times zero and \( t \), respectively. "\( \mu \)"\(^{11} \) of the over-all reaction under these experimental conditions is calculated as 11 800 cal. \( \times \) mole\(^{-1} \) from the values of the constants at 25° and 37° (Table 4). This is an order of magnitude frequently found in biological reactions.

The effect of the concentration of cytochrome oxidase. Fig. 2 shows the oxidation at different concentrations of the heart muscle preparation. For small additions of the preparation the rate of oxidation increases rapidly, while further additions are less effective until at high oxidase concentrations a saturation effect is evident. In Fig. 3 the same data are plotted in the form of the first order constant per mg preparation versus the concentration of the oxidase.

The effect of the concentration of cysteine. According to the results given in Fig. 4, there is an optimal concentration of cysteine at which a maximal number of cysteine molecules are oxidized per unit of time. However, if the relative

*Acta Chem. Scand.* 7 (1953) No. 5
amounts are considered (Fig. 5) it is seen that the lower the cysteine concentration is, the higher will be the fraction of the cysteine molecules, oxidized per unit of time. Fig. 5 shows clearly an inhibition by cysteine, acting already at low cysteine concentrations. This observation is in agreement with earlier results obtained by Potter and Dubois\(^\text{12}\) and Ames and Elvehjem\(^\text{13}\) (cf., however, also Barron and Singer\(^\text{14}\)).

Since the oxidation of cysteine is first order with respect to cysteine without any sign of increased reaction velocity with the gradual disappearance of cysteine from the solution, the inhibition by cysteine must occur rapidly and be only slowly reversible.

The effect of the concentration of cytochrome c. Fig. 6 shows a plot of the inverse of the amount of cysteine oxidized in a 4 min. experiment versus the inverse of the cytochrome c concentration\(^\text{15}\). Fig. 7 is a plot of the first order

---

*Acta Chem. Scand.* 7 (1953) No. 5
constant (Formula 2) versus the concentration of cytochrome c, and shows that there is an accessible concentration of cytochrome c after which a further addition does not increase the rate very much. Fig. 7 permits also the calculation of $k_{0.5}$, i.e. that concentration of cytochrome c at which, under the actual conditions, the oxidation of cysteine proceeds at a half maximal rate. Its numerical value is $0.8 \times 10^{-5} M$. This constant has not the meaning of a Michaelis constant. Employing different experimental conditions Slater\textsuperscript{14} found $k_m = 1.2 \times 10^{-5} M$, and $6.1 \times 10^{-5} M$ with p-phenylenediamine and ascorbic acid as reducing agents. The fact that no hyperbolic relation correlates in our

---

**Fig. 7.** Influence of [cytochrome c]. Ordinate: Value of $k$ (Formula 2). Abscissa: $M$ of cytochrome c. Same experiment as in Fig. 6.

---

**Fig. 8.** Comparison between normal and autoxidizable cytochrome c. Ordinate: log $I_a/I$ at 550 mμ. Abscissa: Time in min. Cytochrome c: $1.88 \times 10^{-5} M$. Curve A: Cytochrome containing 0.348 % Fe, 2 % autoxidizable cytochrome. Curve B: 0.437 % Fe, 100 % autoxidizable. At time zero addition of cysteine to a concentration of $5.7 \times 10^{-4} M$. At ten min. addition of dithionite. 24°, pH 7.25, M/15 phosphate.

*Acta Chem. Scand. 7 (1953) No. 5*
Fig. 9. Effect of pH on the oxidation of cysteine by heart muscle preparation + cytochrome c. Ordinate: Values of k (Formula 2). Abscissa: pH. Cytochrome c: 2.62 × 10^{-5} M. Cysteine: 2.0 × 10^{-3} M (Curve 1), 2.0 × 10^{-2} M (Curve 2). 25°C, M/15 phosphate.

instance the substrate concentration to the reaction velocity might be due to the influence of the inhibiting effect of cysteine.

Another preparation of cytochrome c, spectroscopically normal but completely autoxidizable, proved to be quite inactive under the same conditions. With p-phenylenediamine or succinate as substrates Kellin and Hartree had found the same, and stated, as the reason for the inactivity of autoxidizable cytochrome c, that neither succinic acid nor p-phenylenediamine could reduce it with sufficient velocity. Also with cysteine as reducing agent the autoxidizable cytochrome c is reduced more slowly than is normal cytochrome (Fig. 8). A spectrophotometric assay of the activity of a cytochrome c preparation can be based on this fact.

Fig. 10. Ordinate: log ([cysteine]/[ferricytochrome c]). Abscissa: Time in sec. Cytochrome c: 1.49 × 10^{-8} M (initially all oxidized). Cysteine: 7.45 × 10^{-8} M (initial concentration). M/15 prim. phosphate, brought to desired pH values with 0.10 M NaOH.

Acta Chem. Scand. 7 (1953) No. 5
Fig. 11. Ordinate: Rate of reduction of cytochrome. Abscissa: pH. O experimental values given in Fig. 10. Full drawn line: Theoretical rate of reduction, calculated from pK = 8.20 for the thiol group of cysteine and pK = 9.35 for the transformation of ferricytochrome III to IV, and assuming that the reaction takes place according to
\[
\frac{d [\text{cyt. Fe}^{+ +}]}{dt} = 550 [R S^-] [\text{cyt. III Fe}^{+ +}]
\]

The effect of pH on the rate of the oxidation of cysteine by (heart muscle preparation + cytochrome c) is given in Fig. 9. For two different concentrations of cysteine the optimum was found at about pH 8 under the conditions actually employed.

Some determinations of the influence of pH on the reduction of ferricytochrome c by cysteine were also made (Fig. 10 and 11). The plotting of log ([cysteine]/[ferricytochrome c]) against time gives straight lines at all examined values of pH. pH 8.8 was found to be optimal for this reaction.

DISCUSSION

The aerobic, "oxidatic", oxidation of cysteine involves the components: oxygen-cytochrome oxidase (as heart muscle preparation)—cytochrome c—cysteine. The interactions between the components are complex in nature.

In a system containing oxygen + cysteine no oxidation to cystine occurs in the absence of a catalyst. When e.g. copper ions act as catalyst in this reaction, a pronounced pH maximum is found, the position of which depends to some extent upon the nature of the buffer system. Hydrogen peroxide is formed during this reaction. Correction for the oxidation of cysteine directly by oxygen in our experiments is given by the blank determinations.

In the system oxygen + cytochrome oxidase + cysteine a slow oxidation of cysteine takes place (Table 1). In the case of oxygen + cytochrome c + cysteine it is obvious that the oxidation goes further than what can be accounted for by a stoichiometric reaction between the two latter components. This may be
attributable to an unspecific metal catalysis by the cytochrome c or to the presence of non-cytochrome iron in the preparation.

In a system oxygen + cytochrome oxidase + cytochrome c there is a rapid oxidation of ferrocytochrome. Some indirect information on this reaction may be gained from a comparison of Fig. 9 and Fig. 11, which shows that the optimal pH for the oxidation of cysteine shifts from 8.8 to 8.0 when cytochrome oxidase is introduced into the system. The shift could depend either upon an alkali denaturation of the cytochrome oxidase or upon the presence of an essential group with pK around 7 in it, where only the acidic form is active. The latter hypothesis is supported by the results of Wainio et al.\(^{20}\) who found the optimal pH for the oxidation of cytochrome c by cytochrome oxidase to be 6.67 in 0.033 M phosphate.

Molecular oxygen + ferrocytochrome c do not react except at pH<4 or >13\(^{21}\). There is a not yet sufficiently studied modification of cytochrome c, the "autoxidizable cytochrome c", which is oxidized by molecular oxygen at any pH. It has been shown by Keilin and Hartree \(^6\), Tsou \(^7\), and in the present investigation that autoxidizable cytochrome c is inactive in promoting oxidation of various substrates by (molecular oxygen + heart muscle preparations). The reason, as given by Keilin and Hartree, is that the substrates reduce this modified cytochrome c more slowly than normal cytochrome.

The reaction between ferricytochrome c and cysteine is bimolecular, one mole of cytochrome being reduced by one mole of cysteine. It proceeds only within a confined pH range (Fig. 11). Towards the acid side the region may be limited by the dissociation of the sulfhydryl group of cysteine. The oxidation of cysteine could be depicted as a two-step process:

\[
2\text{RSH} \rightleftharpoons 2\text{RS}^- + 2\text{H}^+ \quad (3)
\]

\[
2\text{RS}^- \rightleftharpoons \text{RSSR} + 2\text{e}^- \quad (4)
\]

There seems to be some uncertainty about the pK of reaction (3). The treatise of Schmidt \(^{22}\) favours the value of pK = 8.18, whereas Cohn and Edsall \(^{23}\) give pK = 10.25. The most recent investigation \(^{24}\) discloses the difficulties in attributing the two pK:s visualized in titration curves, to either of the thiol or the amino groups, respectively. Our results fit with pK = 8.18, but would be difficult to explain if the thiol group should dissociate with pK above 10.

Three factors enter on the alkaline side of the optimal pH. The decrease of the rate of reduction of cytochrome parallels the transformation of ferricytochrome type III to type IV (pK 9.35 \(^{21}\)). At present it is not possible to eluci-

*Acta Chem. Scand.* 7 (1953) No. 5
diate what structural changes in this transformation would prevent the reduction of type IV by cysteine. Another possibility, although less probable, is that the change in the charge of the substrate molecule due to the ionization of the amino group (pK 10.28) would influence. Third, the dissociation of a proton (pK 8.18) from the reductant in the (cystine/cysteine)-system would decrease the slope of the E'/pH-curve of the system, since no cancelling dissociation occurs in the oxidant. The E'/pH-slope of cytochrome is $-0.06 \text{ mV/pH from pH 7 to 11}^{25a}$. Obviously the distance between $E'_o$ of the two systems must decrease at pH $>8.18$, which also could account for the decrease in reaction velocity.

In the complete system oxygen + cytochrome oxidase + cytochrome c + cysteine the latter is oxidized more rapidly than was believed from previous manometric experiments. The discrepancy between the $Q_{O_2}$-values from manometric and amperometric experiments may be accounted for by the following facts.

1. The substrate concentration in the amperometric experiments was much lower than in the manometric determinations. This must of course be of importance when substrates are employed which inhibit the reaction, e.g. cysteine.

2. The oxygen supply was much better in the amperometric than in the manometric experiments. This is in agreement with the wellknown observation that an increase in the number of oscillations per minute of the manometers will increase the $Q_{O_2}$-value. It must be considered that to oxidize completely to cystine 45 μmoles of cysteine in 3 ml of water at 37° with air as the gas phase, the oxygen, dissolved in the liquid, must be renewed more than 20 times.

In the oxidation of succinate by (heart muscle preparation + cytochrome c) also other catalysts are involved (succinic dehydrogenase and Slater’s factor), which may have been partly damaged during the preparation. Since these catalysts are probably not acting in the oxidation of cysteine a direct comparison is not justified between our amperometric and manometric $Q_{O_2}$-values with cysteine as substrates and those of Keilin and Hartree and Slater with succinate. Slater $^{16}$ found the $Q_{O_2}$ of cytochrome oxidase to be 3 400 (38°, fat free dry weight, ascorbate as substrate).

The pH optimum of the over-all reaction from oxygen to cysteine via(cytochrome oxidase + cytochrome c) was found as 8.0, whereas the optimum of the part-reactions, cytochrome oxidase / cytochrome c and cytochrome c / cysteine, were found to be 6.67 $^{20}$ and 8.8 (present investigation), respectively. Since the three values have been obtained with different techniques a coordination will not be attempted. The position of the rate-determining step in the

*Acta Chem. Scand. 7 (1953) No. 5*
reaction chain will, as is evident from the above, be dependent upon the pH at which the reaction is carried out.

Fromageot has pointed out that the term cysteine oxidase should be considered as no more than a general expression which covers enzymes whose action involves simple elementary transformations. With regard to this and to the fact that in the oxidation of cysteine by (cytochrome oxidase + cytochrome c) oxygen is the ultimate electron acceptor with the formation of water, it is correct to say that (cytochrome oxidase + cytochrome c) functions as a cysteine oxidase. Preliminary experiments have shown that not only glutathion but also numerous other low-molecular thiols can substitute cysteine as a reducer of cytochrome c. The expression cysteine oxidase could therefore just as well be exchanged for thiol oxidase in this case.

 Succinic dehydrogenase contains one or two SH-groups, essential for its activity. It is tempting to think that (cytochrome oxidase + cytochrome c) could oxidize directly thiol groups in enzymes in a way similar to what it does with the simple thiols.

SUMMARY

1. Using an amperometric technique for the determination of cysteine and cystine it has been confirmed that a system containing oxygen + heart muscle preparation + cytochrome c + cysteine oxidizes cysteine only to cystine.

2. The reaction is dependent on the available amount of oxygen. If oxygen is bubbled through the vessel, and low concentrations of cysteine are used, the reaction proceeds more rapidly than found in manometric experiments.

3. The reaction is of order one with respect to the concentration of cysteine. \( \mu \) is around 11 800 cal \( \times \) mole\(^{-1}\).

4. An increase in the concentration of cysteine decreases the rate constant because of the inhibition of the oxidase by cysteine.

5. The maximum efficiency (rate constant per mg of oxidase preparation) is obtained at low concentration of the oxidase preparation. These low concentrations are to be used in any assay of the cytochrome oxidase activity of a preparation when cysteine is the substrate.

6. The addition of cytochrome c increases the rate constant, but not along a hyperbolic function.

7. Autooxidizable cytochrome c oxidizes cysteine at a significantly lower rate than normal cytochrome c does.

8. Only non-autooxidizable cytochrome c is effective in promoting the catalysis of cysteine oxidation by oxidase and oxygen.

*Acta Chem. Scand.* 7 (1953) No. 5
9. Both at low and high concentrations of cysteine the optimal pH for the activity of the system containing oxygen + oxidase + cytochrome c + cysteine lies around pH 8.0.

10. Cysteine reduces ferri cytochrome c with a typical bimolecular kinetics. The reaction has a pH optimum at 8.8.

REFERENCES

5. Schneider, W. C., and Potter, V. R. J. Biol. Chem. 149 (1943) 217.

Received February 5, 1953.

Acta Chem. Scand. 7 (1953) No. 5