CXXXIV. THE CATALYTIC ACTION OF TRACES OF IRON ON THE OXIDATION OF CYSTEINE AND GLUTATHIONE.

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The great acceleration produced in the atmospheric oxidation of cysteine by small quantities of iron was first shown by Mathews and Walker [1909], who observed that the reaction velocity was doubled by the addition of M/100,000 ferric chloride. These investigators also showed that the oxidation is inhibited by cyanides and nitriles, one molecule of hydrogen cyanide sufficing to inhibit the oxidation of one thousand molecules of cysteine. In its behaviour towards various reagents and in its susceptibility to changes in hydrogen ion concentration, the oxidation of cysteine closely resembles the oxidations taking place in the living cell, and has on this account received a considerable amount of attention.

Warburg and Sakuma [1923] put forward the theory that the so-called autooxidation of cysteine is in reality a catalysis by iron. In the oxidation of cysteine hydrogen cyanide inhibits the accelerating action of added iron, and its inhibiting effect on the oxidation of cysteine alone is ascribed to the same anticatalytic action on traces of iron present in the cysteine. It is assumed that the oxidation of cysteine takes place through the formation of an intermediate cysteine iron complex which is autooxidisable, but on addition of cyanide, a complex of cysteine, iron and cyanide is formed which is incapable of oxidation by molecular oxygen. By using quartz apparatus and taking special precautions to eliminate traces of iron, these workers claimed to have obtained samples of cysteine which were oxidised by atmospheric oxygen at an extremely slow rate.

Abderhalden and Wertheimer [1923, 1] attach great importance to the small residual oxidation which Warburg and Sakuma found to occur even in their purest samples of cysteine and attribute the inactivation by cyanide to another cause. The same investigators [1923, 2], working with glass apparatus, claimed to have prepared a specimen of cysteine free from iron, which was autooxidisable and the oxidation of which was still greatly inhibited by small quantities of cyanide. Consequently they assumed that the inactivation of the cysteine was not due to the action of the cyanide on iron. Mauthner [1912] found that cystine can be reduced by an aqueous solution of potassium cyanide, and Abderhalden and Wertheimer [1923, 2] confirmed this, finding that cystine is reduced by dilute potassium cyanide solution, the reduction
being detected by the development of the nitroprusside reaction. Dixon and Tunnicliffe [1923] found that the oxidation of cysteine is catalysed by cystine, probably by the formation of an intermediate cysteine-cystine complex. Abderhalden and Wertheimer ascribe the inactivation by cyanide to its reducing action on cystine, thus preventing the formation of the autocatalytic cysteine-cystine complex.

In view of the importance of information on the mechanism of the oxidation of sulphhydryl compounds for a proper understanding of oxidations taking place in the living cell, it was thought desirable to carry out further work on the subject. In the first part of the present paper the effect of iron and cyanide on the oxidation of cysteine has again been studied and much of Warburg and Sakuma’s work confirmed; while in the second part, the investigation has been extended to glutathione, the dipeptide which Hopkins has shown to play an important part in tissue oxidations.

**Experimental.**

**I. CYSTEINE.**

*Preparation of iron-free cysteine.*

The method used in the preparation of iron-free cysteine was essentially the same as that employed by Sakuma [1923]. The purification was carried out entirely in vessels of fused quartz, since the use of glass is liable to cause considerable contamination with iron, while difficulty was found in obtaining glazed porcelain quite free from iron. The quartz flasks were repeatedly boiled out with pure concentrated hydrochloric acid and were proved to be free from iron by the method employed by Sakuma. Water was distilled from a quartz distilling flask into a quartz receiver cooled with a stream of water, a platinum spiral being used to prevent bumping of the liquid. Pure concentrated hydrochloric acid was then boiled in the distilling flask, the side tube of the latter being placed just above the level of the water in the receiver. The distillation was continued until the acid in the receiver was six times normal. 20 cc. of this acid were evaporated to dryness in a quartz dish in presence of a crystal of pure potassium chlorate, the residue being dissolved in 1 cc. of water. 1 cc. of 10% potassium thiocyanate solution was added together with 1 cc. of the 6N distilled acid, and the solution was viewed in a test-tube above a perfectly white background. By comparing this with a similar tube containing 3 cc. of pure water, any trace of yellow colour indicating the presence of iron could be observed. The final concentration of hydrochloric acid in the test-tube was 2N, at which concentration this test is most sensitive [Lachs and Friedenthal, 1911]. In a control experiment, the presence of 1/10,000 mg. of iron which had been added was readily detected. The distillation of hydrochloric acid was repeated until no trace of the iron reaction could be obtained. The water, ethyl alcohol and ammonia used in this work were freshly distilled in quartz vessels.
The starting product was L-cystine which was prepared from hair and twice recrystallised from 20% hydrochloric acid. The cystine was reduced by heating for six hours on the water-bath with tin and 33% hydrochloric acid in presence of a few drops of dilute platinum tetrachloride solution. During this reduction a slight evolution of hydrogen sulphide was observed. The tin was removed by diluting considerably with water and passing hydrogen sulphide. After filtering, the solution was evaporated to dryness in vacuo.

20 g. of the crude cystine hydrochloride so obtained were dissolved in 100 cc. of water and saturated with pure hydrogen sulphide (prepared from antimony trisulphide and thoroughly washed with water). 2 mols. of finely powdered baryta were added, hydrogen sulphide passed for half an hour and the solution allowed to stand for some hours in a closed vessel. The dark green precipitate which separated was filtered off through an acid-extracted quantitative filter-paper and was found to contain large quantities of iron. Hydrogen sulphide was again passed through the filtrate and after standing, the solution was filtered into a quartz flask, further traces of the dark precipitate being so removed.

The solution was saturated with pure HCl gas, using the quartz distilling flask as a wash bottle, the side tube being placed just above the surface of the liquid and was then placed in a vacuum desiccator to remove hydrogen sulphide and evaporated to dryness on the water-bath in a quartz dish. The powdered residue was dried in vacuo over potassium hydroxide and extracted repeatedly with ethyl alcohol. The solution was evaporated to dryness in vacuo and the residue was recrystallised from alcohol. The yield of pure cystine hydrochloride at this stage is small, but I have found that by the addition of a small quantity of chloroform (distilled in quartz vessels) to the mother-liquor a further quantity of cysteine hydrochloride is obtained which appears to be almost equally free from iron.

As found by Sakuma, the dried cystine hydrochloride when titrated with iodine, required considerably more than the theoretical quantity. Doubtless this is due to the presence of a certain amount of free cystine. A quantity of the cystine precipitated by iodine was dissolved in normal hydrochloric acid and the specific rotation measured in a 2 dm. tube. 0.0623 g. of cystine dissolved in 15.3 cc. gave a rotation of $-1.825^\circ$ which is equivalent to a specific rotation $[\alpha]_D^{20} = -224^\circ$. E. Fischer and Susuki gave $-222^\circ$ as the specific rotation of L-cystine, while Sakuma obtained the value $-229^\circ$.

**Method of measurement of oxidation.**

The rate of oxygen uptake by the reduced sulphydryl compounds was measured by means of the Barcroft micro-respirometer the bottles of which were made of fused quartz, sufficiently thick to withstand evacuation, and with necks ground to fit inside the glass stems, thus avoiding risk of impurities from the stopper grease.

Each bottle had a volume of 35 cc. and was calibrated to hold 3 cc. of
liquid. The apparatus was mechanically shaken in a water-bath maintained at constant temperature, and except where otherwise stated all measurements were made at 20°C ± 0.1°C.

In dealing with slow oxygen uptakes the apparatus was allowed to attain equilibrium by standing in the water-bath for about 7 minutes before taking readings. In measuring the more rapid reactions the method used by Dixon and Tunncliffe [1923] was employed, the apparatus being evacuated, filled with pure nitrogen and allowed to attain equilibrium. It was then evacuated again and filled with air. The rate of oxygen uptake was found to be independent of the rate of shaking within wide limits.

The pure sulphhydryl compounds were dissolved in water and brought to the required hydrogen ion concentrations by the addition of dilute ammonium hydroxide solution added from a quartz burette. The stopcock of the latter was large and well ground to eliminate the necessity for tap grease. The volume of alkali required was determined by a separate titration and was added to the solution in the Barcroft apparatus, together with sufficient water to make a total volume of 3 cc. The pH was checked at the end of each experiment by means of Clark and Lubs' standard indicators.

The results obtained by direct measurement of the oxygen uptake agreed well with those obtained by shaking a larger volume of the solution in a quartz flask, pouring out small quantities at intervals and titrating with N/100 iodine solution from a micro-burette.

**Rate of oxidation of purified cysteine.**

Table I gives the results of a number of experiments with different samples of cysteine hydrochloride at various hydrogen ion concentrations. The rate of oxidation of iron-free cysteine is seen to be more than one hundred times slower than that of a crude sample. As is shown in Fig. 1 the velocity of oxygen uptake of pure cysteine is linear over a considerable period, and consequently can be expressed in the form 

$$ V = \frac{\text{cmm. of oxygen}}{\text{mg. of cysteine, HCl}} \times \text{(minutes)} $$

**Table I.**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>mg. of cysteine, HCl in 3 cc. solution</th>
<th>pH</th>
<th>Oxygen uptake in cmm. per hour</th>
<th>cmm./(mg. × mins.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine (A)</td>
<td>10</td>
<td>7-7</td>
<td>6-22</td>
<td>0-0194</td>
</tr>
<tr>
<td>Cysteine (B)</td>
<td>10</td>
<td>7-7</td>
<td>2-49</td>
<td>0-0041</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7-7</td>
<td>4-21</td>
<td>0-0058</td>
</tr>
<tr>
<td>Cysteine (C)</td>
<td>9</td>
<td>7-3</td>
<td>3-32</td>
<td>0-0061</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7-7</td>
<td>4-60</td>
<td>0-0077</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7-7</td>
<td>2-80</td>
<td>0-0047</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7-8</td>
<td>5-00</td>
<td>0-0083</td>
</tr>
<tr>
<td></td>
<td>13-5</td>
<td>8-0</td>
<td>4-80</td>
<td>0-0059</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8-0</td>
<td>17-5</td>
<td>0-0081</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7-6</td>
<td>2-32</td>
<td>0-0039</td>
</tr>
<tr>
<td>Crude cysteine</td>
<td>10</td>
<td>8-0</td>
<td>380-0</td>
<td>0-63</td>
</tr>
<tr>
<td>Cysteine (C)</td>
<td>10</td>
<td>7-6</td>
<td>1-86</td>
<td>0-0031</td>
</tr>
<tr>
<td>+ M/1000 HCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
obtained are given in the last column of Table I and are of the same order as the average values obtained for pure cysteine by Warburg and Sakuma.

The upper curve in Fig. 1 shows the oxygen uptake of a sample of pure cysteine hydrochloride, while the lower curve shows the effect of the addition of M/1000 hydrogen cyanide. Potassium cyanide solution together with the equivalent amount of hydrochloric acid was added to the acid cysteine solution before adjusting the $p_H$ with ammonia. Measurements were taken over a period of 24 hours and it will be observed that the rate of oxidation of pure cysteine is linear over a considerable part of the curve, showing a slight falling off after 9 hours, while the rate of uptake of cysteine + cyanide is linear throughout the whole 24 hours. This will be referred to later.

Fig. 1. Absorption of oxygen by pure cysteine.
A. 36 mg. pure cysteine hydrochloride.
B. 36 mg. ,, ,, + M/1000 HCN.

$P_H=8.0$. Temp. =20·0°.

Catalytic action of traces of iron on the oxidation of purified cysteine.

The oxidation of pure cysteine is very strongly catalysed by iron, and Warburg and Sakuma found that a marked increase in velocity is produced even by the addition of a few 1/100,000ths of a mg. of iron to the solution. These workers expressed the catalytic activity of the iron in the form,

\[
\text{quotient} = \frac{\text{increase of oxygen uptake in cmm.}}{(\text{mg. of added iron}) \times (\text{time in minutes})} = n_{Fe},
\]

and found the average value of this quotient to be 1700.

In my earlier experiments, using ferric chloride, the values obtained were considerably lower than this and were very variable. It was thought that this might be due to the inactivation of the iron on standing in very dilute solution, possibly owing to some physical change in the ferric hydroxide formed by hydrolysis; and on using a freshly prepared solution of ferric chloride and diluting immediately before each experiment, consistent results were obtained.
Ferrous iron, added as ferrous ammonium sulphate, was found to be equally active.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>mg. of cysteine, HCl</th>
<th>Initial $O_2$ uptake in cmm. per hr.</th>
<th>mg. of iron added</th>
<th>Final $O_2$ uptake in cmm. per hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6.6</td>
<td>0.0001 (ferric)</td>
<td>15-6</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>4.2</td>
<td>0.0002</td>
<td>19-8</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>2.8</td>
<td>0.0001 (ferrous)</td>
<td>12-8</td>
</tr>
<tr>
<td>4</td>
<td>13.5</td>
<td>4.8</td>
<td>0.0004 (haematin)</td>
<td>23-2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>7.2</td>
<td>0.0008</td>
<td>47.7</td>
</tr>
</tbody>
</table>

The first part of Table II shows the catalytic effect of ferric and ferrous ions on cysteine oxidation and the values obtained for $n_{Fe}$ agree well with those found by Warburg and Sakuma.

In the second part of the same table is shown the catalytic effect of the iron in haematin. The haematin was prepared by washing crystals of pure haemin (prepared from ox blood) successively with alcohol, pure concentrated HCl, and distilled water, to remove any traces of free iron, dissolving in two equivalents of $N/2$ ammonium hydroxide and diluting with water. The iron in haematin, while less active as a catalyst than inorganic iron, appears to be less sensitive to the action of cyanide.

Fig. 2 shows the effect of adding increasing amounts of iron (as ferric chloride) on the velocity of oxygen uptake of pure cysteine. The increase in velocity is seen to be directly proportional to the quantity of iron added. The initial velocity of uptake of the pure cysteine was 4.2 cmm. per hour and if this residual uptake were assumed to be due to a trace of iron remaining in the purified cysteine the amount of this remaining iron would be given by the
distance $AO$, the curve being produced to meet the abscissa at $A$. The distance $AO$ would then represent 0-00005 mg. of iron present in 12 mg. of cysteine hydrochloride.

In order to test this assumption, 0-10 g. of the same sample of cysteine hydrochloride was ashed in a quartz crucible which was then washed out with 1 cc. of hot 6N hydrochloric acid. This was added to 2 cc. of 5 % KCNS solution and comparisons made with solutions containing known amounts of iron. In this way the amount of iron was found to be about 0-0001 mg. (certainly less than 0-0002 mg.). This represents only 0-00001 mg. in 12 mg. of cysteine hydrochloride, and consequently, the residual uptake does not appear to be entirely due to the presence of iron.

This is confirmed by the last experiment in Table I which shows that the inhibiting action of $M/1000$ HCN on the oxidation of pure cysteine is small, the value of $V$ being reduced from 0-0039 to 0-0031. If the oxygen uptake of pure cysteine were entirely due to traces of residual iron, it would be expected that $M/1000$ HCN would produce a considerably greater inhibition, assuming Warburg’s theory that iron is inactivated by cyanide. Evidence in favour of the correctness of this assumption will be given later in the paper.

Hence it would appear either that traces of catalysts other than iron are present in the purified cysteine or that a minute extent cysteine is strictly autoxidisable.

II. GLUTATHIONE.

Preparation of iron-free glutathione.

The purification of glutathione involved the use of pure mercuric sulphate reagent and pure barium hydrosulphide solution.

The mercuric sulphate was prepared by heating together 18 g. of mercury with 27 g. of sulphuric acid in a quartz dish. Both the mercury and the sulphuric acid were previously distilled from quartz. 15 g. of the crystalline products were added to 150 cc. of water and pure sulphuric acid was added until the mercuric sulphate was just dissolved.

The solution then contained about 9 % of sulphuric acid.

The barium hydrosulphide was prepared by shaking excess of finely powdered baryta with water and saturating with pure hydrogen sulphide. The solution was poured off from the excess of baryta, allowed to stand overnight and filtered to remove traces of iron.

2 g. of oxidised glutathione prepared from yeast were dissolved in 10 cc. of water. The solution was saturated with pure hydrogen sulphide, a total of 7 g. of finely powdered baryta being meanwhile added at intervals. The solution was allowed to stand overnight in a closed vessel, and a considerable quantity of a dark greenish precipitate was filtered off and washed with a little barium hydrosulphide solution. 3 g. of finely powdered baryta were added to the filtrate and washings and hydrogen sulphide passed for $\frac{1}{2}$ hour.
After standing in a closed vessel for 2 days the solution was filtered into a quartz flask, diluted with 100 cc. of water, made just acid with sulphuric acid and again filtered. A slight excess of mercuric sulphate reagent was added and the precipitate filtered off, washed with water, suspended in 75 cc. of water and decomposed with hydrogen sulphide. The precipitated mercuric sulphide was allowed to settle and the sulphuric acid was removed exactly with barium hydrosulphide.

The solution which was then still distinctly acid to litmus, was filtered into the quartz distilling flask and evaporated down to about 5 cc. in hydrogen at 40 mm. pressure.

![Graph](image)

**Fig. 3.** Comparison of rates of oxygen uptake of pure and impure glutathione. Catalytic effect of a trace of iron on the rate of oxygen uptake of pure glutathione, and the inhibiting effect of hydrogen cyanide.

A. 16 mg. pure glutathione + \( \frac{M}{1000} \) HCN.
B. 16 mg. " "
C. 16 mg. " " + 0.0004 mg. Fe (as FeCl₃).
D. 16 mg. crude " " \( p_H = 8.0 \).
Temp. = 20°.

The product was poured into 80 cc. of absolute alcohol and allowed to stand for 24 hours. The alcohol was then poured off and 80 cc. of fresh alcohol added. The glutathione was allowed to stand under alcohol until it became quite friable. The alcohol was then poured off and the pure glutathione dried in a vacuum desiccator.

By titration with iodine the product was found to contain 66% of the reduced form of glutathione.

The dark green precipitate which formed on passing hydrogen sulphide through the solution of crude glutathione in baryta was found after washing to contain large quantities of iron, the only other metal found being barium.

The precipitate was dissolved in acid and the iron estimated; 0.0178 g. of Fe₂O₃ were obtained from the 2 g. of glutathione. This is equivalent to over 0.6% of Fe in the crude glutathione.
Rate of oxidation of glutathione.

The rate of oxidation of purified glutathione is shown by curve B in Fig. 3. It will be observed that as in the case of iron-free cysteine the velocity of oxygen uptake is linear over a considerable part of the curve. By comparison with curve C it is evident that the oxidation of purified glutathione is strongly catalysed by minute quantities of iron, the rate of uptake of oxygen being increased from 42 cmm. to 57 cmm. in the first hour, by 0.0004 mg. of iron.

Curve D shows the rate of oxidation of an impure specimen of glutathione under the same conditions. The oxygen uptake is extremely rapid, the glutathione being almost completely oxidised in half an hour. Comparing this with curve B, it is seen that by removing iron from glutathione its initial velocity of oxygen uptake is reduced from about 760 cmm. to 42 cmm. per hour.

Curve A shows the effect of the addition of M/1000 HCN to the purified glutathione. The rate of uptake is still further reduced, though it is considerably greater than that of pure cysteine in presence of M/1000 cyanide.

At point P on the curve 0.004 mg. of iron was added which produced an enormous acceleration in the rate of oxygen uptake, the oxidation being complete in a few minutes. The total uptake in this case is somewhat less than theoretical, owing partly to a certain amount of oxidation having taken place on the addition of the iron before the apparatus could be evacuated and brought to equilibrium in nitrogen, and partly to the removal of 0.1 cc. of solution before adding an equal volume of the iron solution, in order to keep the volume of liquid in the apparatus constant.

As was found in the case of pure cysteine + M/1000 cyanide, the rate of oxygen uptake of pure glutathione + M/1000 cyanide is perfectly linear.

The effect of varying concentrations of cyanide on impure glutathione was then tried. Fig. 4 shows that increasing inhibition of the rate of oxidation is brought about by increasing cyanide concentration. Here again the velocity of uptake is seen to be practically linear, and to remain so until the oxidation is almost complete. The significance of these linear curves will be discussed later.

In a similar experiment using a sample of impure glutathione which was known to contain less iron than that used in the experiments shown in Fig. 4, the actual reduction in the rate of oxidation brought about by varying concentrations of cyanide was relatively greater.

Warburg and Sakuma showed that from pH 9.2 to 10.2 the oxidation of cysteine was inhibited by M/10 sodium pyrophosphate to the same extent as by M/1000 hydrogen cyanide. They assume that this is due to the formation of a non-catalytic complex of iron with the pyrophosphate, this complex being more stable at an alkaline pH. I find that the oxidation of glutathione is also inhibited by pyrophosphate and Fig. 5 shows that a considerable inhibition is produced even by M/50 pyrophosphate at pH 7.6, and that the rate of oxygen uptake becomes linear over a considerable period.
It was thought to be of interest to try the effect of the addition of cyanide to the system oxidised glutathione + thermostable tissue described by

Hopkins and Dixon [1922]. The tissue was prepared by boiling finely chopped rat muscle with water, treating with alcohol and drying in vacuo. The finely powdered product when shaken in buffer solution at $p_H 7.6$ shows only a small uptake of oxygen. The tissue is capable of reducing oxidised glutathione,
and on adding glutathione in the oxidised form, the system takes up oxygen rapidly to the extent of about 400 to 500 cmm. per gram of dry tissue. Fig. 6 shows the effect of hydrogen cyanide on this uptake of oxygen. A definite inhibition is evident, though this is much less than that produced on the rate of oxidation of reduced glutathione alone, and it will be seen that in the presence of tissue, the curves obtained with cyanide are no longer linear.

It will be noticed that in presence of cyanide the total uptake of oxygen by the system is somewhat increased. This was found to be the case in a number of experiments. Abderhalden and Wertheimer [1923, 2] have shown that cystine oxidises dilute potassium cyanide solution and it is possible that the increased oxygen uptake may be due to a similar reaction between the cyanide and oxidised glutathione, leading to the formation of more reduced glutathione and consequently to a higher oxygen uptake. It may be mentioned that Ellinger and Landsberger [1922] claim that hydrogen cyanide may be oxidised on the muscle surface.

**DISCUSSION.**

It would appear from the foregoing experiments that in their behaviour during oxidation cysteine and reduced glutathione are essentially analogous. The normal rate of oxidation of both compounds is greatly reduced by careful purification, and both show a marked acceleration in presence of iron and an inhibition in presence of cyanide. On adding iron to a solution of reduced glutathione a deep violet colour is produced similar to that given with iron by cysteine and if the solution be shaken in air, the colour persists until the
glutathione is completely oxidised. It appears probable that, as in the case of cysteine the acceleration in the rate of oxidation of glutathione produced by iron is due to the formation of an autoxidisable complex of glutathione with iron.

The difference between the aerobic oxidation of cysteine and that of glutathione appears to be one of degree rather than kind, glutathione being oxidised more rapidly than cysteine. That this is not a fundamental difference is shown by Dixon and Tunnicliffe who have found that in the anaerobic oxidation of these compounds, using methylene blue instead of oxygen as the hydrogen acceptor, the order is reversed, cysteine being oxidised more rapidly than glutathione.

There appears to be much evidence in favour of the assumption that the inhibiting action of cyanide on oxidations is due to its combining with traces of catalytic iron to form a non-catalytic complex. Warburg and Brefeld [1924] have shown definitely that in the catalytic oxidation of a solution of leucine by iron on the surface of charcoal, the inhibition caused by hydrogen cyanide is due to its inactivating action on the iron, and it seems highly probable that the same is true in the case of the oxidation of cysteine and glutathione. Further evidence for this point of view is afforded by the observation mentioned earlier in the paper that with samples of cysteine and glutathione containing small amounts of iron the relative inhibition by cyanide was less than with those containing more iron.

Meyerhof [1923] has shown that the inhibition by cyanide of the catalytic action of copper on the oxidation of thioglycollic acid is due to the formation of a CuCN complex, and the action of cyanide on iron is believed to be analogous to this.

Definite evidence for the theory of cyanide inactivation of iron is afforded by Warburg's observation that sodium pyrophosphate which, like cyanide, is known to form a complex with iron, also inhibits the oxidation of cysteine.

In Fig. 5 it was shown that sodium pyrophosphate inhibits glutathione in a similar manner, and further that, as in the case of cyanide inhibition, the curve becomes linear.

It will be observed that in oxidations taking place in presence of a considerable amount of ionised iron, the form of curve for oxygen uptake is such as would be expected if the concentration of sulphydryl compound formed the limiting factor in the reaction velocity. The falling off in velocity with decreasing concentration of sulphydryl compound is of course modified by the autocatalytic nature of the reaction.

On the other hand, in those oxidations in which the quantity of ionised iron in solution is very small, owing either to careful purification or to complex formation with cyanide or pyrophosphate, the curves are linear until oxidation is almost complete. The reason for these two different types of curve seems to be as follows.

The sulphydryl compounds are apparently incapable of combining directly
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with molecular oxygen, the latter requiring first to be activated by means of iron. It has not been definitely proved, however, that the very slow rate of oxidation of the purified compounds is not due to their autoxidation, although it may well be due to traces of catalysts which have escaped removal.

Suppose that cyanide forms a complex either with the catalytic iron alone, or more probably with iron together with the sulphhydryl compound. Let us assume that this complex is slightly dissociated and that a very small concentration of ionised iron is thus present in solution. It is evident that with such a small concentration of iron, the relatively large quantity of sulphhydryl compound would be capable of taking up the activated oxygen more quickly than it could be formed. Consequently the rate of activation of oxygen by iron becomes the limiting factor in the reaction, and this rate being constant, the oxygen uptake curve is linear. When the oxidation is almost complete, the concentration of sulphhydryl compound becomes so small that the rate at which it can take up active oxygen becomes the limiting factor, and consequently a progressive falling off in velocity then takes place.

This theory explains the curves in Fig. 4 which show that increasing the concentration of cyanide produces a progressive increase in the inhibition. If the cyanide complex with iron were entirely undissociated, it would be expected that on increasing the concentration of cyanide, the amount of inhibition would reach a maximum when all the free iron was taken up.

On the other hand, if we assume that the complex is slightly dissociated, it is evident that increasing the concentration of cyanide will decrease the amount of this dissociation and so bring about an increased inhibition of oxidation.

In the same way if the concentration of iron in solution be rendered very small by careful purification of the sulphhydryl compound, the rate of activation of oxygen is again the limiting factor and again linear curves are obtained.

On the other hand, when a comparatively large amount of iron is free in solution as in the case of solutions of ordinary samples of cysteine and glutathione, excess of active oxygen is available throughout the whole reaction and the limiting factor becomes the rate of uptake of active oxygen by the sulphhydryl compound. Therefore as the concentration of sulphhydryl compound falls off, a progressive decrease in velocity takes place, though this decrease is modified by the catalytic effect of the oxidised form as shown by Dixon and Tunnlicliffe.

In the case of the system glutathione + tissue, the inhibiting effect of hydrogen cyanide on the rate of oxygen uptake is much less marked than in the case of the oxidation of reduced glutathione in the absence of tissue. It was recorded earlier in the paper that the catalytic effect of iron in haematin is inhibited to a smaller extent by cyanide than that of inorganic iron, and as it is probable that oxidations in the tissue may be catalysed by organic iron compounds allied to haematin, the smaller inhibition by cyanide would be expected.
It is not difficult to understand why the iron in the haematin compound should be active as a catalyst while the cyanide compound with iron is inactive. In cyanide-iron compounds, in presence of oxygen, the ferrocyanide form is known to be more stable than the ferricyanide form, and hence the ferrocyanide compound, when once formed, would show no tendency to take up oxygen. In haematin, however, the ferric compound is the more stable in presence of oxygen, and consequently as soon as this is reduced to the ferrous compound by the sulphphydryl group, it becomes oxidised again by the air, the iron in haematin thus acting as an oxygen carrier.

Further work is being carried out with a view to finding whether there is any connection between the catalysis of the oxidation of sulphydryl compounds by iron and by disulphides.

**Summary.**

1. The purification of cysteine and glutathione has been carried out using quartz vessels and taking special precautions to remove iron. Much of the work of Warburg and Sakuma on cysteine has been confirmed.

2. The rates of atmospheric oxidation of both cysteine and glutathione are found to be very greatly reduced by the removal of traces of iron impurities.

3. It has been shown that the addition of quantities of iron of the order of 1/10,000 mg. produces a marked increase on the rate of oxidation of the purified cysteine and glutathione.

4. Evidence has been given that the inhibition in the oxidation of cysteine and glutathione by hydrogen cyanide is due to its forming a complex with the catalytic iron.

5. The velocities of oxygen uptake in the case of the purified compounds alone, and in the case of the unpurified compounds in the presence of cyanide, have been found to be linear.

6. It has been found that the iron in haematin is also capable of catalysing these oxidations.

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