XXXVII. ON THE CATALYSIS OF THE OXIDATION OF CYSTEINE AND THIOGLYCOLLIC ACID BY IRON AND COPPER.

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MATHEWS and WALKER [1909] studied the apparent autoxidation of cysteine to cystine and showed the acceleration of the velocity of oxidation by very small amounts of iron, copper, and other metals, and the inhibition by small quantities of cyanide. Warburg and Sakuma [1923] further found that metallic catalysis accounted for practically the whole oxidation of cysteine in air, and that cysteine purified from metals was almost, if not entirely, non-autoxidisable in neutral solutions; later, Harrison [1924] confirmed this.

Harris [1922] investigated the complexes formed by cysteine with various metals and proposed a theory for the catalysis in which, in the case of iron say, a ferrous-cysteine complex is first formed, this is oxidised by air to a coloured ferric-cysteine which in turn is rapidly reduced again to ferrous-cysteine at the expense of cysteine, which is thereby oxidised to cystine. Recently Michaelis [1929], from the results of a detailed study of metallic complexes of cysteine, especially a stable cobaltous-cysteine, has put forward a theory of the exact nature of these metallic complexes and their function in the catalytic oxidation.

None of these authors, however, seems to have studied the kinetics of these catalytic oxidations except Mathews and Walker in observing the effect of hydrogen ion concentration. Dixon and Tunnicliffe [1923] noticed the auto-catalytic nature of the aerobic and anaerobic oxidation of glutathione, cysteine, and thioglycollic acid, and showed that the disulphide group accelerates the rate of oxidation. This work appeared just before that of Warburg and Sakuma. Warburg and Sakuma's findings made it seem possible that the acceleration produced by adding —SS— compounds might not be due to the —SS— itself but to catalytic metals in the —SS— preparation. Harrison [1927], however, confirmed Dixon and Tunnicliffe's effect with metal-free preparations of disulphide compounds. This fact has not yet been very satisfactorily accounted for by a theory for the mechanism of the oxidation.

The observations to be reported in this paper show that the aerobic oxidation of —SH groups, in the presence of metallic ions, does not seem to fit in with any simple theory of catalysis.

EXPERIMENTAL.

The oxygen uptake curves were obtained, using the Barcroft differential manometer. The left-hand cups contained 3 cc. of water in all experiments, the right-hand cups containing a total of 3 cc. of the solution of which the oxygen uptake was being studied. All experiments were carried out at room temperature and at an initial p_H of 7.3, except where otherwise stated.

CYSTEINE.

Effect of phosphate buffer.

It was found that phosphate buffer solution has a large inhibiting effect on the velocity of oxygen uptake in presence of iron or copper. In Fig. 1 are shown the results of an experiment to illustrate this. The right-hand cups of four manometers each contained 8 mg. of cysteine hydrochloride at p_H 7.3, two in $M/20$ phosphate buffer and two in water. To one aqueous solution and to one buffer solution, 0.1 cc. of a solution containing 0.05 mg. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc. was added, and to the other two, 0.05 cc. of a fresh solution containing 0.5 mg. of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per cc.

Fig. 1. Effect of phosphate buffer on oxygen uptake of cysteine solutions at p_H 7.3 in the presence of iron and copper.

The extent of the effect of phosphate buffer does not appear to vary much with the concentration between $M/20$ and $M/60$ phosphate.

As cysteine is quite an efficient buffer itself, most of the work was carried out using aqueous solutions of cysteine hydrochloride neutralised with N or $N/10$ sodium hydroxide to p_H 7.3. If the manometer cups are treated with acid and well washed out, there is usually little or no change in p_H at the end of the experiment, though the final liquid is occasionally slightly more alkaline than at first.

Effect of cysteine concentration.

The velocity of oxygen uptake is dependent, not only on the amount of catalyst present, but also upon the amount of cysteine. This was not made clear by Warburg and Sakuma, although Krebs [1929] noticed it in the case of

haematin catalysis but not with pyridine-haematin or nicotine-haematin. That it is true in the case of copper and iron is shown in Figs. 2, 3 *b*, and 10. Fig. 2 records experiments carried out before the effects of phosphate buffer and high concentrations of copper (see below) were known. It was obtained by using a phosphate buffer solution of cysteine at p_H 7.3, to which copper solution was added. Different amounts of this mixture were put in the Barcroft cups and all made up to 3 cc. with buffer solution. The copper present was therefore actually increased proportionately with the cysteine; but in all cases the copper exceeded the minimum amount to give maximum effect. The curves *A*, *B*, and *C* are the results of three experiments, the velocities of uptake obtained being plotted against the amount of cysteine present; points on curve *B* have twice as much, and on curve *C* four times as much copper present as the corresponding points on curve *A*. Thus it is seen that the velocity of uptake is here dependent on the amount of cysteine present (and not on the amount of copper).

Fig. 2. Effect of cysteine concentration. Copper present in all in concentration more than sufficient to give maximum effect. Points on *B* have twice as much, points on *C* four times as much Cu as corresponding points on *A*.

The shape of the uptake curve.

There is also the curious fact that when the conditions are such that the velocity of uptake of 3 cc. is over about 400 mm.³ O₂ per hour, the uptake curve is almost invariably a straight line. That is, the velocity does not fall off as the unoxidised cysteine remaining in solution decreases, until it is completely oxidised, though the angle of the curve depends on the amount of cysteine originally present. Thus, in rapidly oxidising solutions, the velocity of oxygen uptake is dependent on the initial concentration of cysteine, but not on the concentration at any instant. At lower velocities there is generally a continuous falling off in rate of oxygen usage as the oxidation proceeds. Figs. 6 *a*, 7 *a*, and 11 show these effects.

The uptake curve is not autocatalytic in shape, and Dixon and Tunnicliffe [1923] considered this due to the insolubility of the disulphide form, cystine, which in separating out can have no effect on the course of the reaction. However, it has been noticed that at the end of the majority of the experiments, very little or no cystine had precipitated, and the precipitate developed slowly.

It was thought possible that the proportionality of reaction velocity to the initial concentration of cysteine might be explained by presuming that the cysteine hydrochloride used might contain a second substance which acted in a complementary way towards the metal as catalyst. But this could not be proved, though the following experiments were carried out to test it.

Various pure specimens of cysteine hydrochloride, including Kahlbaum's "Warburg" cysteine hydrochloride, were tried and found to give the same results. A sample of cysteine was purified by precipitation as the cuprous compound in the manner described by Hopkins [1929] for the separation of glutathione. 60 mg. of cysteine hydrochloride, thus purified and recrystallised from pure HCl solution, were made up to 15 cc. at p_H 7.3 with water and $N/10$ NaOH. Two Barcroft cups received 1.5 cc. of this (*i.e.* 6 mg. cysteine hydrochloride) and 1.5 cc. water, and two received 3 cc. of the solution. To one of each was added 0.033 cc. of a fresh solution containing 0.5 mg. $FeSO_4 \cdot 7H_2O$ per cc., and to each of the others 0.1 cc. of a solution containing 1 mg. per cc. The initial velocities of O_2 uptake obtained with these are plotted in Fig. 3 *a* against the amount of iron present, and in Fig. 3 *b* against the cysteine-HCl content.

Effects of changing iron and cysteine concentrations on the velocity of O_2 uptake.

It is seen that this behaved in the usual way, the velocity of O_2 uptake being proportional both to the cysteine and the iron present. The other samples of cysteine were all tested in this manner, similar curves being obtained if small amounts of copper were used instead of iron. A sample of optically inactive cysteine hydrochloride (obtained by boiling cystine in acid for a long time, and

then reducing), kindly given me by Messrs N. W. Pirie and A. Leese, gave the same results. As the addition of sodium chloride had no effect on the course of the reaction, the chloride and sodium ions present in neutralised cysteine hydrochloride solutions evidently have no effect. An attempt was made to concentrate the possible catalytic complement in the following way. 0.25 g. of cysteine hydrochloride was made up to 20 cc. at p_H 7.3 in water. A small amount of copper (0.1 cc. of a solution containing 0.5 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc.) was added and the liquid aerated until completely oxidised, and then concentrated by distillation *in vacuo* to 4 cc. and filtered. (A separate bulk experiment with ten times the quantities but the same proportions showed that only about one-third of the copper remained in the filtrate, the rest being in the cystine precipitate.) Portions of this extract were added to solutions of cysteine containing copper or iron, but no acceleration of the oxygen usage was observed.

Fig. 4 a. Effect of adding extra cysteine during oxidation in presence of iron.

Fig. 4 b. Effect of adding extra cysteine during oxidation in presence of copper.

The effect of cysteine concentration was further studied by adding extra cysteine during the course of the oxidation, and when the oxidation was complete. The right-hand cups of two manometers contained respectively 2 cc. and 2.5 cc. of water, and each received 0.5 cc. of an aqueous solution containing 6 mg. of cysteine-HCl at p_H 7.4. In the first an extra 0.5 cc. of this solution was contained in a small tube suspended from the potash tube in the middle of the cup in the manner described by Keilin [1929]. To each cup was added 0.05 cc. of a fresh solution containing 0.2 mg. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per cc. The oxygen uptakes obtained with these are shown in Fig. 4 a. At the point marked with an arrow, the one manometer was tapped so that the extra cysteine in the "Keilin tube" was added to the solution. Fig. 4 b was obtained in the

same way, but the 0.5 cc. cysteine solution contained 8 mg. cysteine-HCl, and 0.1 cc. of a solution containing 0.5 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ replaced the iron solution.

It is seen that on adding fresh cysteine solution to a solution in which cysteine has been partly oxidised in the presence of iron or copper, the oxygen uptake velocity increases, the new velocity of uptake being about what would be expected from the sum of the two amounts of cysteine.

Next, a manometer was set up having in the right-hand cup 1 cc. of an aqueous solution containing 4 mg. cysteine-HCl at p_{H} 7.3, and 0.05 cc. of a solution of 0.5 mg. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per cc. It was shaken until the oxygen uptake had ceased. Then a further 1 cc. of the cysteine solution was added and the O_2 uptake observed. A second manometer contained in its right-hand cup, 2 cc. H_2O , 1 cc. of the cysteine solution and the same amount of iron as before, and its O_2 uptake was compared with the first. The uptake curves obtained are shown in Fig. 5 *a*, and it is seen that the presence of oxidised cysteine in the first manometer has practically no effect on the O_2 uptake.

Fig. 5 *a*. Effect of the presence of previously oxidised cysteine on the oxidation of fresh cysteine. Iron catalyst.

Fig. 5 *b*. Effect of the presence of previously oxidised cysteine on the oxidation of fresh cysteine. Copper catalyst.

Fig. 5 *b* shows the result of a similar experiment to the above, except that the iron solution was replaced by 0.1 cc. of a solution containing 1 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc. Here it is seen that in the presence of oxidised cysteine, the rate of O_2 uptake is about what would be expected if the whole of the cysteine had been added at once.

Thus in the presence of iron and completely oxidised cysteine, addition of fresh cysteine causes oxygen uptake to recommence at a velocity to be expected from the fresh cysteine alone, but with copper, the velocity is again what would be expected from the sum of the oxidised and fresh cysteine. This result has been repeatedly obtained.

Concentration of catalyst.

Harrison [1924] showed that the velocity of oxygen uptake of cysteine in the presence of iron is proportional to the amount of iron present up to a concentration of 1.6×10^{-3} mg. of iron in 3 cc. The following experiment was made in order to see whether there was a limit to this proportionality. The right-hand cups of four manometers each contained 8 mg. cysteine-HCl in 3 cc. water at

p_H 7.3, and they received respectively the following amounts of iron: 0.1 cc. of a solution containing 0.05 mg. $FeSO_4 \cdot 7H_2O$ per cc.; 0.03 cc. of 0.5 mg. per cc.; 0.1 cc. of 1 mg. per cc.; and 0.5 cc. of 5 mg. per cc. The uptake curves obtained with these increasing amounts of iron are shown in Fig. 6 *a*, and in Fig. 6 *b* the velocities of O_2 uptake are plotted against the amounts of iron.

It is thus seen that the velocity of oxygen uptake is proportional to the amount of iron at least up to a concentration of iron of 0.05 mg. per cc.

With copper the case is very different. Up to a certain small concentration, the uptake velocity is proportional to the copper, but at higher copper concentrations the velocity/copper curve falls off sharply, becoming horizontal, and any further increase in copper concentration causes no further increase in reaction velocity. In unbuffered solutions a very large amount of copper added actually causes the velocity to decrease again slightly. This decrease is, however, explained by the fact that on adding a fair amount of copper sulphate, an insoluble Cu-cysteine precipitate is formed, leaving H_2SO_4 in solution so that the p_H is lowered. Smaller additions of copper sulphate cause no appreciable change in p_H .

Fig. 6 *a*. Oxygen uptake curves given by 8 mg. cysteine hydrochloride in water at p_H 7.3 in presence of:

(A) 0.0011 mg. Fe, (B) 0.0033 mg. Fe,
(C) 0.022 mg. Fe, (D) 0.054 mg. Fe.

Fig. 6 *b*¹. Effect of increasing iron on the velocity of oxidation of 8 mg. cysteine hydrochloride at p_H 7.3.

The following is a typical experiment illustrating this effect. The right-hand cups of eight manometers, each containing 8 mg. of cysteine hydrochloride in 3 cc. water at p_H 7.3, received amounts of copper increasing from 0.05 cc. of a solution containing 0.05 mg. $CuSO_4 \cdot 5H_2O$ per cc., to 0.2 cc. of a solution containing 1 mg. $CuSO_4 \cdot 5H_2O$ per cc., and a ninth received no added metal. The uptake curves given by these are shown in Fig. 7 *a*, and Fig. 7 *b* is obtained by plotting initial velocities against copper present.

The failure of the velocity of O_2 uptake to increase with copper concentration cannot be explained by oxygen lack in the liquid, as with iron, and in

The upper points on this curve should actually be higher, as the rate of shaking during the experiment was afterwards found not to be sufficiently great to eliminate all diffusion effect at these high velocities.

other experiments shown, much higher velocities were obtained under the same conditions of temperature and rate of shaking.

This minimal concentration of copper for maximal effect is not greatly affected by temperature, change in cysteine concentration or oxygen tension, the presence or absence of phosphate buffer, by increasing the liquid-glass surface by the introduction of glass beads, or by change of hydrogen ion concentration between p_H 6 and 8.

Fig. 7a. Oxygen uptake curves given by 8 mg. cysteine hydrochloride in water at p_H 7.3 in presence of:

—○— 0.00064 mg. Cu.	—x— 0.00128 mg. Cu.
—△— 0.0042 mg. Cu.	—□— 0.017 mg. Cu.
—○— 0.0038 mg. Cu.	—x— 0.0128 mg. Cu.
—▲— 0.0256 mg. Cu.	—□— 0.051 mg. Cu.
—●— No added metal.	

Fig. 7b. Effect of increasing amounts of copper on the velocity of oxidation of 8 mg. cysteine hydrochloride at p_H 7.3.

In Fig. 8 is given a series of curves obtained under various conditions, which show the effect of increasing copper concentration.

A converse effect with copper occurs in high concentrations of cysteine with low copper. If increasing amounts of cysteine are put up with a constant very small amount of copper, the oxygen uptake velocities are at first proportional to the amount of cysteine, and then a further increase in cysteine concentration causes scarcely any increase in uptake velocity. If now the

copper is increased, the proportionality to cysteine continues to a higher level before ceasing. Fig. 9 illustrates these observations, and it was obtained in the following manner. A solution of 250 mg. cysteine-HCl in 15 cc. water at p_H 7.3 was made up, and the right-hand cups of four manometers received respectively 3 cc., 2.2 cc., 1.2 cc., and 0.4 cc. of this. The volume in each was made up to 3 cc. with water, and to each was added 0.05 cc. of a solution containing 0.05 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc. The initial velocities of O_2 uptake obtained from these are shown in the lower curve in Fig. 9, plotted against the amount of cysteine-HCl present. The upper curve is the result of an exactly similar experiment, using just double the amount of copper solution.

Fig. 8. Effect of increasing amounts of copper on the velocity of oxidation of cysteine under various conditions.

Fig. 9. Effect of high concentration of cysteine on the velocity of oxygen uptake in presence of very small amounts of copper.

Fig. 10. Effect of high cysteine concentration on the velocity of oxygen uptake in presence of a small amount of iron.

In the case of iron catalysis, in the presence of a very small amount of iron, the uptake velocity with large amounts of cysteine remains proportional to the cysteine as seen in Fig. 10, which was obtained in the same way as Fig. 9, the copper solution being replaced by 0.1 cc. of a solution containing 0.05 mg. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per cc.

Oxygen tension.

The right-hand cups of four manometers, *A*, *B*, *C*, and *D*; each contained 10 mg. cysteine-HCl in 2.5 cc. water at p_{H} 7.3. "Keilin tubes" were suspended in all, those in *A* and *C* each holding 0.05 cc. of a solution containing 0.5 mg. $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per cc., plus 0.5 cc. water, and those in *B* and *D* having 0.1 cc. of a solution containing 0.05 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc., plus 0.5 cc. water. *C* and *D* were evacuated and filled with oxygen at atmospheric pressure. The oxygen uptake in all was noted, and after 21 minutes all were tapped so that the iron and copper solutions became mixed with the cysteine, and the new uptakes were observed. The results of this experiment, which are seen in Fig. 11, show that increasing the oxygen tension causes a very considerable increase in the rate of cysteine oxidation in the presence of either iron or copper, though the velocity does not appear to be proportional to the partial pressure of the oxygen in the gas. Similar results were obtained by Krebs [1929] with haematin or nicotine-haematin as catalyst.

Fig. 11. Effect of increased oxygen tension on the velocity of oxygen uptake of cysteine in presence of iron and copper. Cu or Fe solutions added 22 minutes from start.

THIOGLYCOLLIC ACID.

The acid used for these experiments was distilled *in vacuo*, using vessels that had been previously well boiled out with pure hydrochloric acid solution.

Phosphate buffer.

Phosphate buffer has a considerable inhibiting effect on the velocity of oxidation, which does not vary much between phosphate concentrations of $M/20$ and $M/60$. But, since thioglycollic acid has no buffering properties, it was necessary to use the buffer solution, as without it the solutions became acid on oxidation.

Oxygen uptake curve.

With thioglycollic acid the autocatalytic acceleration, noted by Dixon and Tunnicliffe, is obtained, but, as with cysteine at high velocities of uptake, the velocity does not fall off until oxidation is complete (see Fig. 12). In the case of copper catalysis the initial velocity of uptake depends under the usual conditions on the amount of thioglycollic acid present. With iron this is possibly masked by another phenomenon described in the next section.

Fig. 12. Oxygen uptake curves given by 3.5 mg. thioglycollic acid in $M/60$ phosphate buffer at p_H 7.3 in presence of:

- (A) 0.0054 mg. Fe. (B) 0.011 mg. Fe. (C) 0.022 mg. Fe.
(D) 0.044 mg. Fe. (E) No added metal.

Concentration of catalyst.

When iron is the catalyst, the behaviour of thioglycollic acid is very different from that of cysteine. In this case we have a type of auto-inhibition, the catalyst is largely inactivated by excess of thioglycollic acid. The following experiments show this. 280 mg. thioglycollic acid were made up to 80 cc. at p_H 7.3 with $M/20$ phosphate buffer solution. The right-hand cups of four manometers each received 1 cc. of this and 2 cc. of water, and the following different amounts of iron were added: 0.05 cc. of a solution containing 0.5 mg. $FeSO_4 \cdot 7H_2O$ per cc.; 0.1 cc. of the same; 0.1 cc. of a solution of double that strength; and 0.2 cc. of the second solution. A control was also run containing no added metal. Fig. 12 gives the uptake curves thus obtained.

The upper curve in Fig. 13 was obtained by plotting the velocities of O_2 uptake, given by the above experiment, against the iron concentration. The

two lower curves in Fig. 13 were obtained in a similar way, the one being the result of using 3 cc. of the same thioglycollic solution (and no water), and the other using 3 cc. of a thioglycollic acid solution of the same strength but only one-third the concentration of phosphate buffer.

It is seen that the first additions of iron cause very little increase in velocity, but after a certain concentration of iron is reached, the velocity increases rapidly, and the increase is proportional to the extra iron added. Where the thioglycollic acid is present in three times the strength, it is seen that not only is more iron required before its effect is appreciable, but also the velocity never increases, with increasing iron, at such a rate as in the case of the weaker solution. Velocity/iron curves of the same type, for the two different concentrations of thioglycollic acid, were obtained by using neutral water solutions without buffer; the velocities obtained were all considerably higher but the shapes of the curves were exactly the same as in the presence of phosphate buffer.

Fig. 13. Effect of increasing amounts of iron on the velocity of oxygen uptake of thioglycollic acid solutions at p_H 7.3.

It was thought that this inhibition might possibly be due to the presence of H_2S in the distilled thioglycollic acid, but it was actually found that with thioglycollic acid and iron, some acceleration of the reaction was produced by adding small quantities of Na_2S solution (though the p_H was not affected). Krebs [1929] has found the same thing with cysteine oxidation in presence of iron or nicotine-haematin.

With thioglycollic acid, similar curves of copper concentration against velocity of O_2 uptake are obtained as with cysteine; *i.e.* a concentration of copper is reached when further addition of copper gives no increase in velocity. The minimum copper for maximum effect is just about the same as for cysteine. This is seen in Fig. 14, which was given by the following experiment. 280 mg. of thioglycollic acid were made up to 80 cc. at p_H 7.3 with $M/60$ phosphate

buffer solution. The right-hand cups of four manometers each received 1 cc. of this and 2 cc. of *M*/60 buffer. To the cups were added the following amounts of copper: 0.1 cc. of a solution containing 0.05 mg. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per cc.; 0.2 cc. of the same; 0.1 cc. of a solution containing 0.25 mg. per cc.; and 0.2 cc. of a solution containing 0.5 mg. per cc. The lower curve in Fig. 14 shows the velocities of O_2 uptake obtained, plotted against the amount of copper present. The upper curve is the result of a similar experiment in which 3 cc. of the thioglycollic acid solution were used.

It will be noticed that in the curve obtained for the higher concentration of thioglycollic acid, the velocity corresponding to a very low copper concentration falls very low. This low point could be repeatedly obtained and corresponds with either the low oxygen uptake of excess of cysteine in presence of low copper (Fig. 9), or the auto-inhibition of thioglycollic acid on the oxidation with iron as catalyst.

Fig. 14. Effect of increasing amounts of copper on the velocity of oxygen uptake of thioglycollic acid solutions at p_{H} 7.3.

Complexes of thioglycollic acid with iron and copper.

Harris [1922] described the complexes formed between cysteine and iron, copper, and other metals, and Hopkins [1929] described the cuprous compound of glutathione. Thioglycollic acid forms a similar series of complexes. The cuprous compound formed with cuprous oxide is white and resembles those of glutathione and cysteine in being readily soluble in strong HCl or in alkaline solution but insoluble in neutral or H_2SO_4 solution. On adding cupric sulphate

to a neutral thioglycollic acid solution, a brown precipitate is momentarily formed and disappears immediately; this is probably a cupric thioglycollic acid compound, which is rapidly reduced to the cuprous compound, and corresponds to the blue-black momentary precipitate noticed in the case of cysteine.

On adding an iron salt to neutral thioglycollic acid solutions, a purple colour appears which is presumably the ferric complex of thioglycollic acid, as it fades on standing and reappears on aerating, thus corresponding to the violet colour obtained with cysteine. But here there is a noteworthy deviation from correspondence with cysteine. Whereas, as Harris showed, the rate of disappearance of the violet ferric cysteine colour varies directly with the concentration of cysteine, the rate of disappearance of the purple ferric thioglycollic compound is retarded very greatly by increasing the concentration of thioglycollic acid. This fits in with the observation mentioned above, that the aerobic oxidation of thioglycollic acid in the presence of iron is retarded by increasing thioglycollic acid concentration.

DISCUSSION.

The results reported in this paper show that the oxidation of sulphydryl compounds in presence of metals is not to be accounted for on any classical theory of catalysis. It has been found possible to make a fairly simple hypothesis, involving only principal valencies, which will fit the facts more or less satisfactorily; but considering Michaelis's [1929] view of the complex nature of the metallo-cysteine compounds, it would be unwise to suggest more than one or two of the main ideas on which the hypothesis is based.

It is somewhat difficult to decide what kinetic relations Michaelis's series of reactions would imply, but they do not seem to suggest the facts mentioned in this paper; namely, that the velocity of oxygen uptake of a given volume of solution should be proportional to the amount of metal present, and to the initial concentration of cysteine at high velocities, and also dependent on the oxygen tension.

It is tentatively suggested that the active catalyst may be considered to be not merely the metallic ion, but a reversible monomolecular combination between the metal and cysteine or cystine. This complex is then alternately oxidised by O_2 to the ferric state and reduced by cysteine back to the ferrous state as Harris [1922] showed. If the reduction process at p_H 7.3 is very rapid compared with the oxidation process, then the oxidation process would limit the reaction, and the velocity of oxygen uptake should be dependent on the O_2 tension, and on the amount of catalytic complex, which in turn, as it is reversibly formed, is proportional to both the iron and [cysteine + cystine] concentrations. As oxidation proceeds and cysteine is replaced by cystine, the amount of catalyst remains constant and the velocity does not fall until nearly all the cysteine has disappeared.

acid with iron could possibly be explained by the formation of an inactive metallic complex containing more molecules of thioglycollic acid than the active catalyst.

The hypothesis readily explains the catalytic effect of added disulphide compounds; the autocatalytic effect, which has been observed by other authors, is not noticeable in cysteine oxidation at p_H 7.3, but appears to some extent in thioglycollic acid oxidation; it can only be explained on this hypothesis by the assumption that the catalytic metallic complex is more readily formed with a disulphide acid than with a sulphhydryl acid.

Since this work was done, a paper has appeared by Cannan and Richardson [1929] on other aspects of the same subject. They have also put forward certain hypotheses for the mechanism of sulphhydryl oxidation; but it is unfortunate that these have been generalised from a study of the complexes of thioglycollic acid with iron, since it has been shown above (see Fig. 13 and the section on metallic complexes) that thioglycollic acid and iron behave in an anomalous way.

SUMMARY.

1. The rate of oxidation of cysteine at p_H 7.3 is proportional to the amount of iron present up to the highest concentration tried.
2. It is proportional to the amount of copper present up to a certain small concentration. Further increase of copper, provided it does not affect the p_H , causes no effect. This minimal concentration of copper, giving maximal effect, does not appear to be greatly affected by change in temperature, oxygen tension, p_H , cysteine concentration, or by the presence of phosphate buffer or increased liquid-glass interface.
3. The velocity of oxygen uptake is also proportional to the amount of cysteine originally present.
4. The oxygen uptake curve is linear when the velocity of uptake is fairly rapid; it tends to fall off when the reaction is not so rapid.
5. In presence of very low concentrations of copper, a concentration of cysteine can be reached beyond which further additions of cysteine cause only slight increase in velocity.
6. Addition of an extra amount of cysteine during the course of the oxidation of cysteine, causes the velocity to increase to the rate that would be expected if both amounts of cysteine had been added at first, in the case of both iron and copper catalysis. If, however, the extra cysteine is added when the oxidation of the first lot is complete, the O_2 uptake recommences, when copper is the catalyst, at a rate to be expected from the total cysteine, while, in the case of iron, the new velocity is only what would be expected from the fresh cysteine.
7. The velocity of oxidation in presence of iron or copper is increased by increasing the oxygen tension.
8. The velocity of oxygen uptake in presence of iron or copper is decreased by the presence of phosphate buffer.

9. Thioglycollic acid in solution at p_H 7.3 shows similar behaviour to the above, except that:

(a) the velocity of O_2 uptake in the presence of iron is very low up to a certain concentration of iron, and thereafter increases rapidly in proportion to the amount of extra iron added;

(b) higher concentration of thioglycollic acid decreases the rate of oxygen uptake in the presence of iron.

10. A *résumé* of the behaviour of complexes of iron and copper with thioglycollic acid is given.

11. The above phenomena are discussed in relation to the type of hypothesis that may be necessary to correlate them.

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