L. FACTORS AFFECTING THE CATALYTIC ACTION OF COPPER IN THE OXIDATION OF CYSTEINE.

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THE catalytic effect of salts of copper and other metals on the oxidation of cysteine was first demonstrated by Mathews and Walker [1909]. The greatest activity was obtained in a neutral solution of cysteine and the least in an acid solution. In spite of this work the reaction was considered for many years to be one of autoxidation in which the cysteine, when under favourable conditions, is oxidised directly by atmospheric oxygen. However, the so-called autoxidation was shown by Warburg and Sakuma [1923] to be due entirely to the presence of heavy metals, and to be to a large extent inhibited by KCN, the effect of which they ascribed to the formation of an inactive compound with the heavy metals present.

Toda [1926] showed that the addition of iron accelerated the reduction of methylene blue by cysteine and that this reaction was inhibited by KCN. Harrison [1927] found that copper was also active in stimulating the anaerobic oxidation of cysteine and thioglycollic acid, and that cyanide was equally effective in the inhibition of the reaction under these conditions as in aerobic oxidation. These observations undoubtedly prove that the oxidation of cysteine in any appreciable amount takes place only in the presence of certain heavy metals.

The catalytic effect of heavy metals on the oxidation of cysteine has been used by Warburg [1927] for the quantitative determination of these elements when present in minute traces. He found that pyrophosphate inhibited the activity of iron and manganese completely, but did not affect the action of copper in the oxidation of cysteine. He could therefore determine quantitatively small amounts of copper in the presence of iron and manganese by following the rate of oxygen uptake with cysteine in a pyrophosphate buffer solution under fixed conditions.

Apart from these studies very little work has been done on the various factors influencing the action of copper in the oxidation of cysteine. Dixon and Tunnicliffe [1923], before the importance of the presence of heavy metals

was realised, made a very complete study of the factors affecting the oxidation of cysteine, as well as of glutathione and thioglycollic acid. It is difficult to say, however, whether the oxidation obtained in the case of these sulphhydryl compounds was due to the presence of iron, copper, or even other metals. A study of the factors operative when copper alone is responsible for the oxidation is, therefore, important.

The fact that copper may interfere when cysteine is used for the study of oxidase activity was recently demonstrated by Keilin [1930], who used the rate of cysteine oxidation to determine the activity of an oxidase-cytochrome system. When he added $M/100$ pyrophosphate to the mixture of cysteine, cytochrome, and oxidase, in an orthophosphate buffer at p_H 7.3, to determine if any of the activity was due to free iron, which pyrophosphate would inhibit, he found that there was a great acceleration in the oxygen uptake rather than an inhibition. Keilin suggests that this acceleration is due to the action of pyrophosphate on the copper which may be present in the heart muscle preparation used as the source of oxidase. A quantitative determination of copper shows that there is a sufficient amount of this element present to account for the increased oxygen uptake observed.

Dixon and Elliott [1929] found that the addition of small concentrations of pyrophosphate caused a slight acceleration in the respiration of liver in buffer solutions. This may also be due to the action of pyrophosphate on the copper in the liver, because no acceleration was observed in the case of muscle which is much lower in copper. Of course in the direct measurements on tissues the action of pyrophosphate is complicated by the presence of iron.

The following experiments are recorded to explain this phenomenon as well as to demonstrate other factors which affect the catalytic properties of copper.

EXPERIMENTAL.

All the measurements were made aerobically and the rate of oxygen uptake was measured in Barcroft differential manometers. The right hand flask of the apparatus contained in most cases 3 cc. of buffer solution including the cysteine and copper additions. Equivalent amounts of buffer solution were always placed in the left hand flask together with similar amounts of muscle tissue, when measurements were made with muscle as a source of copper. 0.3 cc. of 10 % NaOH was placed in a small tube fused to the bottom of each flask. The apparatus was shaken in a water-bath at either 20° or 37°. Three to five minutes were allowed for equilibration, then the stopcocks closed, and readings taken at definite intervals. Extreme care was taken to free the flasks from any traces of copper by washing them with cleaning solution, tap water, HCl, tap water, and finally rinsing them several times with redistilled water.

All solutions and reagents were especially prepared to eliminate traces of copper. The water used was redistilled from an all glass apparatus.

Cysteine hydrochloride. Pure cysteine hydrochloride was prepared by

recrystallising twice from concentrated redistilled HCl¹. The crude cysteine hydrochloride was dissolved in the least amount of hot HCl necessary for complete solution, the solution cooled on ice, and the crystals filtered. After this process had been twice repeated, the crystals were dried in a vacuum desiccator over calcium chloride and sodium hydroxide. The purity of this preparation is demonstrated by the exceedingly low oxygen uptake recorded when it is subjected to optimum conditions of oxidation. A considerable loss of cysteine is encountered in this method, but the quality of the product justifies the loss. The cysteine hydrochloride was brought to the proper p_H with pure NaOH before being added to the mixture.

Pyrophosphate. A concentrated solution of sodium pyrophosphate was made, filtered, adjusted to approximately p_H 8.0, at which p_H copper pyrophosphate is soluble, and cooled with ice to allow the salt to crystallise. The salt was filtered off and dried at 100°. $M/5$ and $M/10$ solutions of sodium pyrophosphate were made by dissolving the theoretical amount in water and adjusting the p_H , in most cases to 7.6, with N HCl.

Sodium hydroxide. Sodium hydroxide free from iron and copper was prepared by first allowing a 40 % solution to stand for several days and decanting the clear solution from any precipitate formed. A 0.2 M solution was then made and treated in the same manner.

Phosphate buffer. This was made according to Clark and Lubs from acid potassium phosphate and sodium hydroxide. The 0.2 M solution of acid phosphate was allowed to stand several days to precipitate any iron or copper. After adding the proper amount of NaOH to give the desired p_H , the solution was diluted to $M/15$ or $M/30$ concentration.

Borate buffer. This was made from $M/20$ borax and $M/5$ boric acid according to the table of Palitzsch. These salts were used directly without further purification, but blank determinations showed that they contained very little copper.

Copper solution. Made by diluting a standard solution of copper sulphate containing 0.1 mg. Cu per 1 cc. to give one which contained 1×10^{-4} mg. Cu per 0.1 cc.

Blank determinations were made first in order to determine the purity of the cysteine hydrochloride and the buffer solutions used. The oxygen uptake was measured with cysteine in $M/15$ orthophosphate buffer p_H 7.6, and also with cysteine in the phosphate buffer containing $M/100$ pyrophosphate. Similar measurements were made with Kahlbaum's cysteine (nach Prof. Warburg) to compare the degree of purity of the two samples. The results in Table I show that the cysteine prepared by recrystallisation contained less metal than the Kahlbaum preparation. The addition of pyrophosphate increased the oxygen uptake in both cases very slightly. This increase was not due entirely to any possible metals in the pyrophosphate but to a large extent to the accelerating action of the pyrophosphate on the slight

¹ I am indebted to Dr M. Dixon for suggesting this method of purification.

amount of copper in the solutions used. When larger amounts of pyrophosphate were used, the oxygen uptake was no higher. One of the manometers in each series was generally used for a blank run and the oxygen uptake due to cysteine alone was always deducted in calculations involving an absolute amount of copper, but not in the results where only relative values were required. Whenever a relatively large oxygen uptake was observed in the blank, the contaminated solution was replaced by a new one. It is very difficult to prevent contamination when dealing with such minute quantities, therefore the solutions used for each series were never pipetted from the stock solution but from aliquots.

Table I. *Oxygen consumption by pure cysteine.*

Cysteine No. 1 Kahlbaum	mm. ³ oxygen absorbed per hour	
	<i>M</i> /15 buffer	Buffer plus <i>M</i> /100 Na ₄ P ₂ O ₇
	1.2 5.4	5.0 12.5

Effect of pyrophosphate.

Results demonstrating the accelerating action of pyrophosphate on a heart muscle preparation of oxidase are given in Fig. 1. The concentrated heart muscle suspension was prepared by Dr Keilin according to his usual procedure [Keilin, 1929]. 10 cc. of this strong suspension was diluted to 50 cc. with *M*/15 phosphate buffer p_H 7.6 and 1.5 cc. of the diluted mixture used in the Barcroft flask, or an amount equivalent to 0.3 cc. of the strong mixture.

Fig. 1. Effect of pyrophosphate on the activity of copper in muscle tissue. 8 mg. cysteine in 3 cc. phosphate buffer p_H 7.6. A, pyrophosphate alone. B, muscle alone. C, muscle + pyrophosphate. D, 2×10^{-4} mg. copper + pyrophosphate.

Fig. 2. Effect of pyrophosphate on the activity of copper solutions. 8 mg. cysteine in 3 cc. phosphate buffer p_H 7.6. A, copper alone 4×10^{-4} mg. Cu. B, same amount of copper + pyrophosphate.

Each flask contained a final volume of 3 cc. of orthophosphate buffer together with the various additions. A, contained the buffer plus *M*/100 pyrophosphate; B, the buffer plus muscle alone; C, the buffer plus muscle and pyrophosphate; D, the buffer plus 2×10^{-4} mg. Cu with pyrophosphate. The rate of oxidation with the same amount of muscle is twice as rapid in the presence of pyrophosphate as in the buffer solution alone. The rate obtained with 0.3 cc. strong muscle suspension with pyrophosphate is about the same as that with 2×10^{-4} mg. Cu under the same conditions. Therefore, 0.3 cc.

contains about 2×10^{-4} mg. Cu. A determination of Cu in 75 cc. of the muscle suspension by the method of Elvehjem and Lindow [1929] gave a copper content of 0.0006 mg. Cu per 0.3 cc. Only about one-third of the copper present is therefore available for catalytic action. This is not surprising because undoubtedly a large proportion of the copper present comes from the sand used during the preparation (the muscle pulp contains considerably more copper than that attributed to the heart muscle itself), and all the copper is made soluble only by ignition and extraction with strong HCl. Several other determinations gave results of similar order.

Pyrophosphate has the same accelerating action whether the source of copper is muscle tissue suspension or soluble copper sulphate. In the presence of 4×10^{-4} mg. Cu the rate of oxidation is almost three times as rapid when pyrophosphate is added as when the phosphate buffer is used alone. Curves demonstrating the differences in the rate of oxidation are given in Fig. 2.

Fig. 3. Action of H_2S in presence and absence of pyrophosphate. 8 mg. cysteine and 4×10^{-4} mg. copper in 3 cc. phosphate buffer p_H 7.6. A, copper alone. B, copper + H_2S . C, copper, H_2S and pyrophosphate. D, copper and pyrophosphate.

Fig. 4. Effect of phosphate buffer concentration on the activity of copper. 8 mg. cysteine and 4×10^{-4} mg. copper in 3 cc. phosphate buffer. A, $M/15$ buffer. B, $M/20$. C, $M/30$. D, $M/60$.

This difference in activity is more readily explained by the inhibitory effect of the phosphate buffer than by the accelerating action of the pyrophosphate. The actual inhibitory effect of $M/15$ orthophosphate buffer p_H 7.6 was noticed when the influence of H_2S on the catalysis was studied. The addition of sulphide decreased the activity of the copper in the presence of pyrophosphate, but a decided increase was observed in the case of the buffer solution alone. The oxygen uptake of the different solutions is given in Fig. 3. The inhibitory action of sulphides agrees with the earlier results obtained with H_2S and is probably due to the formation of copper sulphide, but the accelerating effect must be explained in another way. Since the H_2S was added as an aqueous solution of Na_2S (0.3 cc. $M/100$ Na_2S per 3 cc.), the increased action is probably due to the dilution of the buffer solution. The copper becomes more soluble or more available in the more dilute solution. Fig. 4 shows that dilution actually hastens the rate of oxygen consumption. Decided increases in the rate are noted as the buffer is diluted from $M/15$ to $M/20$ and $M/30$, but further dilution causes very little change in the reaction.

Effect of hydrogen ion concentration.

These facts suggest that the solubility of the copper in the buffer solution is the limiting factor. If a weak solution of copper sulphate is added to the phosphate buffer at p_H 7.6 a precipitate of copper phosphate is formed immediately, but if the same amount of copper is added to a pyrophosphate solution at the same p_H no precipitate is formed. Variations in the p_H undoubtedly change the solubility of the copper and thus should bring about certain differences in the rate of oxygen consumption. Results of the measurements made at different H ion concentrations for $M/15$ and $M/30$ phosphate, and for $M/15$ phosphate plus pyrophosphate are given in Fig. 5. In the case of $M/15$ phosphate variations in the p_H have no significant effect on the oxidation rate, because at this concentration the added copper is changed to insoluble copper phosphate at all degrees of acidity. However, when the lower concentration of phosphate was used a maximum activity was noted at p_H 7.6.

Fig. 5. The effect of p_H on the activity of copper in different buffer solutions. Oxygen uptake at the end of 30 minutes. 8 mg. cysteine and 4×10^{-4} mg. copper. A, $M/15$ phosphate buffer. B, $M/30$ phosphate buffer. C, $M/15$ phosphate buffer + pyrophosphate.

Fig. 6. The solubility of copper in different buffer solutions. 25 cc. buffer solution with 0.1 mg. copper. A, $M/15$ phosphate buffer. B, borate buffer. C, $M/30$ phosphate buffer. D, $M/15$ phosphate buffer + pyrophosphate.

The copper is most soluble at this H ion concentration and decreases in solubility as the solution becomes more alkaline. The presence of pyrophosphate gives some increase in activity over $M/30$ buffer at p_H 7.6, but a maximum is obtained at p_H 8.0 with a decrease as the p_H is further increased. Since copper pyrophosphate is soluble in more alkaline solutions than copper phosphate, it is possible for the maximum activity to occur at a higher p_H . Borate buffer solutions were used for p_H values above 8.0. The oxygen uptake with pyrophosphate in phosphate buffer and in borate buffer at p_H 8.0 was measured under the same conditions. Identical results were obtained showing that the type of buffer in which the pyrophosphate acts has no influence on the rate of reaction.

In order to demonstrate that the suggested changes in solubility occur, the solubility of a definite amount of copper at the various H ion concentrations was determined. 0.1 mg. of Cu was added to 25 cc. of the buffer solution adjusted to the proper p_H , allowed to stand 2 hours, filtered, and the amount of copper remaining in the filtrate determined. 3 cc. of $M/10$ $Na_4P_2O_7$ were added to the solution containing the pyrophosphate. The percentage of soluble copper is plotted against p_H in Fig. 6.

Although it is difficult to compare the solubility curves with those obtained by oxygen consumption, because the former were measured when 0.1 mg. Cu was used with 25 cc. of buffer, and the latter when 0.0004 mg. was used with 3 cc. of buffer, it is readily seen that the curves are similar for the phosphate buffer on the alkaline side of p_H 7.6 and for the pyrophosphate buffer on the alkaline side of p_H 8.0. The retarding action of increased alkalinity in either of the buffers is due to the formation of insoluble copper salts. The slope of the oxygen uptake curves on the acid side of p_H 7.6 and 8.0 cannot be explained by the solubility of the copper in relation to the buffer, because the solubility increases with the increased acidity in all cases.

Fig. 7. Comparison of copper activity with and without buffer. 8 mg. cysteine and 2×10^{-4} mg. copper. A, cysteine in buffer p_H 6.8. B, cysteine alone p_H 6.8. C, cysteine in buffer p_H 7.6. D, cysteine alone p_H 7.6.

Fig. 8. Effect of copper concentration on the rate of oxidation. 8 mg. cysteine in $M/15$ phosphate buffer p_H 8.0 with $M/100$ pyrophosphate. A, 0.5×10^{-4} mg. Cu. B, 1×10^{-4} mg. Cu. C, 2×10^{-4} mg. Cu.

Because the solubility determinations were made on the buffer solutions alone without the addition of cysteine, the decreasing activity with increased acidity may be attributed to the action of cysteine. The cysteine is undoubtedly the active factor because the slopes of the curves are identical irrespective of the buffer used. The same oxygen consumption is obtained whether the oxidation is carried out in buffer solution, or in water solution where the cysteine itself is used as the buffer. The results of these experiments are given in Fig. 7. If a line be drawn in Fig. 6 between p_H 6.8 and 8.0 which has the same slope as the curve for oxygen uptake in Fig. 5, it will intersect the solubility curves at p_H 7.2 for $M/15$ phosphate, at p_H 7.6 for $M/30$ phosphate, and at p_H 8.0 for pyrophosphate. The optimum oxygen uptake for the different solutions is actually observed at these p_H .

The retarding effect of cysteine in more acid solutions cannot be explained by the insolubility of a copper-cysteine complex, for, although copper forms

a precipitate with cysteine at p_H 6.0 and below, no precipitate is formed between p_H 6.0 and 8.0 even though 1 to 2 mg. Cu are added to 10 mg. cysteine. However, an inactive copper-cysteine complex may be more readily formed in acid solutions than in neutral or alkaline solutions. Further reference to this complex will be made when the results obtained at 37° are presented.

Since the rate of oxidation of cysteine is greatest with pyrophosphate in buffer solution at p_H 8.0, the buffer and the cysteine are exerting the least retarding effect, and, therefore, this H ion concentration should give the proper conditions for the quantitative determination of copper. The curves in Fig. 8 show that the rate of oxidation of 8 mg. cysteine in 3 cc. $M/15$ phosphate or borate buffer at p_H 8.0 and containing $M/100$ pyrophosphate is directly proportional to the copper present for amounts below 4×10^{-4} mg. Cu. This procedure is quite similar to that used by Warburg [1927] for the determination of copper except that a low concentration of pyrophosphate in a rather dilute buffer is used in place of a strong pyrophosphate buffer alone. This method allows the use of more dilute buffer solutions because the orthophosphate forms a more efficient buffer than pyrophosphate.

Interfering action of iron.

The above results were obtained when copper was the only heavy metal present, but the interfering effect of other metals must also be considered. Addition of iron in amounts equivalent to the copper, or to 2 to 3 times the amount of copper, does not affect the oxygen uptake materially as is shown in Table II. The iron was added as ferric chloride which was prepared free from traces of copper. The failure of small amounts of iron to interfere is, of course, in agreement with Warburg's findings. However, if larger amounts of iron (0.02 mg., approximately equivalent to the amount present in 0.5 cc. whole blood) are used, a very large inhibition is observed. The amount of inhibition decreases as the amount of pyrophosphate used is increased but the normal rate is not reached even if $M/5$ pyrophosphate, suggested by

Table II. *Effect of iron on the catalytic action of copper in the presence of pyrophosphate.*

Conditions of cysteine oxidation					mm. ³ oxygen per ½ hour		Recovery %
Phosphate buffer	Pyro- phosphate	Copper mg. $\times 10^{-4}$	Iron mg. $\times 10^{-4}$	Temp. ° C.	Copper	Copper + iron	
$M/15$ p_H 8.0	$M/100$	4	4	20	142.0	136.0	96.0
" "	"	2	400	"	86.7	23.0	26.0
" "	$M/30$	1	"	"	50.7	30.1	59.0
" p_H 7.6	$M/100$	1	"	"	35.4	16.0	45.0
" —	$M/5$ p_H 8.0	1	200	"	58.3	48.0	83.0
$M/15$ p_H 7.8	$M/10$	1	"	"	46.7	34.8	75.0
" p_H 7.6	$M/10$	1	"	37	63.3	54.3	86.0
" "	$M/8$	1	"	"	72.9	70.8	97.0

Warburg, is added. Warburg's figures show the same effect; when he added 0.02 mg. Fe together with 2×10^{-4} mg. Cu to $M/5$ pyrophosphate at p_H 7.6 only an 80 % recovery was obtained.

Figures given in Table II show that a complete recovery of copper is never obtained, although the procedure is modified as to p_H , concentration of pyrophosphate, etc., when the reaction is carried out at 20°. When the oxidation takes place at 37° with rather high concentrations of pyrophosphate, the rate in the presence of relatively large amounts of iron is practically identical with that obtained with copper alone. The retarding effect of iron in the presence of pyrophosphate is most probably due to the formation of insoluble ferric pyrophosphate which inactivates some of the copper. There are two reasons for this view. Firstly, it is known that iron pyrophosphate is soluble in alkaline solutions only in the presence of excess pyrophosphate, and the addition of more pyrophosphate increases the recovery of copper; secondly, ferric pyrophosphate is more soluble at 37° than at 20° and the recovery of copper is better at 37°. The latter fact can be demonstrated by taking two solutions of pyrophosphate at p_H 7.6 and adding a few drops of dilute iron solution to one at 37° and the other at 20°, the precipitate formed, upon the addition of iron, disappears most rapidly in the first solution.

The complete recovery of copper in the presence of iron at 37° is further demonstrated when the copper content of a solution from the ash of liver is determined by this method and by the method of Elvehjem and Lindow [1929]. 140 mg. Cu was found by the first method and 125 mg. Cu by the latter. These results agree exceedingly well when one considers that the copper content of the sample analysed was of the order of 1×10^{-4} mg. in one case and 0.1 mg. in the other. They further show that there are no other elements in the ash of liver which accelerate the oxidation of the cysteine in the presence of pyrophosphate.

Effect of temperature.

It is interesting to note that the optimum p_H at the higher temperature (37°) is no longer at 8.0 but nearer 7.0. This is not merely a temperature effect, because in a number of carefully controlled experiments the oxygen consumed per 30 minutes in the presence of 1×10^{-4} mg. Cu at p_H 7.6 was found to be 32–36 mm.³ at 20° and 50–54 at 37°, but 80–85 for p_H 7.0 at the latter temperature. The relation of p_H to the oxygen uptake at 37° in the presence of pyrophosphate is shown in Fig. 9. An optimum is observed at p_H 7.0 with a rapid falling off as p_H 6.6 is reached. The pyrophosphate curve for 20° is replotted in this figure to show the relationship of the two.

The fact that the inhibiting action of cysteine is decreased in acid solution at 37° allows one to make several other observations on the activity of copper. Firstly, curve *C* in Fig. 9 shows that even in the less alkaline solution pyrophosphate is still needed for the optimum activity of the copper. The uptake, however, is higher with phosphate alone at 37° than at 20° and the p_H curve

is similar to the solubility curve, which further proves that the retarding effect of the more acid solution is due to the inactive copper-cysteine complex formed.

Greater activity of the copper is not obtained by carrying out the reaction in water, with cysteine instead of phosphate as a buffer. Undoubtedly in the absence of phosphate the copper is precipitated as hydroxide instead of copper phosphate, because it can be shown that a precipitate is formed when a dilute solution of copper sulphate is brought to p_H 7.0. Fig. 10 gives the results obtained when the oxygen consumption was measured in a watery solution, phosphate solution, or a phosphate plus pyrophosphate solution. The activity without the pyrophosphate is similar whether in watery or phosphate solution, but considerably lower than when pyrophosphate is added. Results of the same order were obtained when heart muscle preparation was used as the source of copper.

Fig. 9. The effect of p_H on the activity of copper at 37° . Oxygen uptake at the end of 30 minutes with 8 mg. cysteine and 4×10^{-4} mg. copper. A, with pyrophosphate. B, same conditions at 20° . C, without pyrophosphate.

Fig. 10. The influence of buffer on the activity of copper at 37° . 8 mg. cysteine and 1×10^{-4} mg. copper at p_H 7.0. A, without buffer. B, phosphate buffer. C, buffer + pyrophosphate.

Fig. 11. The effects of H_2S on the activity of copper. 8 mg. cysteine and 1×10^{-4} mg. copper in $M/15$ phosphate p_H 7.0 with pyrophosphate. A, copper + $M/1000 H_2S$. B, copper alone.

Secondly, by working at the higher temperature a high rate of oxygen uptake is obtained over a relatively wide p_H range, which allows a study of the effect of p_H on inhibition by H_2S . A typical result obtained by the addition of H_2S at p_H 7.0 is given in Fig. 11. The percentage inhibition at this p_H is 60, but the amount decreases at higher p_H values and 40 to 50 % is obtained at p_H 7.6. At p_H 8.0 very little inhibition is observed and in some cases an actual increase results by the addition of H_2S , possibly due to the oxidation of the sulphide.

Krebs [1929] found that copper as well as other heavy metals catalyses the oxidation of sulphides. It is difficult, however, to compare his results with these experiments because he worked with very high concentrations of sulphide (0.5 *M*) and used the sulphide without buffer. The solution was undoubtedly quite alkaline, because when similar reactions were carried out in *N* HCl the rate of oxidation was very slow.

Further observations on the activity of copper, which need merely be recorded without including the complete experimental results because they only confirm previous work, are the following. The activity is entirely unaffected by the presence of carbon monoxide but is inhibited to at least 95 % of the total by *M*/1000 KCN. *M*/100 sodium pyrophosphate is the optimum concentration for the activity of copper in absence of iron, but *M*/5 may be used without any interference. However, when the concentration is decreased to *M*/1000 a definite falling off in the activity is observed and considerably less than 50 % of the total activity is obtained with *M*/3000 pyrophosphate.

DISCUSSION.

Although the work presented in this paper deals entirely with the quantitative study of the catalytic action of copper in the oxidation of cysteine, the results have undoubtedly a broader significance. It would be entirely erroneous to say that the facts observed in this particular system can be applied directly to the action of copper on other compounds, but the tremendous activity of this element in the oxidation of cysteine under optimum conditions may explain how the presence or absence of minute traces of it can bring about profound changes in plant and animal phenomena.

The optimum conditions observed in the case of cysteine may be totally different from those for the optimum oxidation of other compounds, even such as thioglycollic acid and glutathione which closely resemble cysteine. Independent studies need to be made for each of the materials activated by copper.

All the factors shown to be necessary for the optimum activity of copper are ordinarily present in most plants and animals. Copper is universally distributed in small amounts in all plants and animals thus far analysed [Lindow, Elvehjem, and Peterson, 1929]. The optimum p_H at 37° is between 7.0 and 7.6 which is the hydrogen ion concentration associated with a large portion of living matter. Copper is more active in the presence of pyrophosphate and Lohmann [1928] has found pyrophosphate in the muscle of certain animals and in yeast, while Eggleton and Eggleton [1929] have reported its presence in blood.

SUMMARY.

1. The oxygen uptake of a system of cysteine and heart muscle preparation in orthophosphate buffer p_H 7.6 is increased two or threefold by the addition of pyrophosphate.

2. The same acceleration is obtained when the muscle preparation is replaced by a minute amount of copper salts. The increased rate of oxidation in the presence of pyrophosphate is due to the formation of copper pyrophosphate, which is more soluble at p_H 7.6 than copper phosphate and therefore more catalytically active.

3. Curves are presented to show the rate of cysteine oxidation by copper in phosphate buffers of different concentrations, and in a phosphate buffer plus pyrophosphate at varying hydrogen ion concentrations.

4. A study of the solubility of copper in different buffer solutions shows the decreased activity with increased p_H to be due to the formation of insoluble copper phosphates. The decrease of activity, as the p_H becomes more acid, can only be explained by the formation of a soluble inactive copper-cysteine complex.

5. The optimum activity of copper in phosphate buffers containing pyrophosphate occurs at p_H 8.0 at 20°. At this hydrogen ion concentration the rate of oxidation is directly proportional to the copper present, providing the copper is not in excess of 4×10^{-4} mg. Cu, and thus provides a method for the quantitative estimation of minute amounts of copper.

6. Relatively large amounts of iron inhibit the activity of copper in the presence of pyrophosphate. This retarding effect can be eliminated by using larger concentrations of pyrophosphate and by carrying out the reaction at 37°. At 37° the optimum activity is observed at p_H 7.0, and this is undoubtedly due to the fact that the copper-cysteine complex is less readily formed at the higher temperature.

7. The inhibiting action of cysteine at p_H 7.0 disappears at 37°, but the copper is sufficiently insoluble in buffer or watery solutions even at this p_H to prevent its complete activity in the absence of pyrophosphate.

8. $M/1000$ H_2S inhibits the activity of copper in phosphate buffers containing pyrophosphate to the extent of 60 to 70 % at p_H 7.0, but the degree of inhibition is much less at higher p_H values.

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