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THE ACTION OF HEAVY METALS ON CYSTEINE AND ON SULPHYDRYL GROUPS OF PROTEINS

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I. BREAKDOWN OF THE CYSTEINE MOLECULE BY COPPER

The oxidation of cysteine by molecular oxygen in the presence of iron has been carefully studied in recent years and the factors influencing this reaction are well known. It has been shown by Warburg and Sakuma (1) that the oxidation of cysteine is dependent on the presence of a heavy metal catalyst, and that under the influence of iron, cysteine in neutral or slightly alkaline solution is oxidized to cystine. The reaction does not go further, and cystine in aqueous solutions is quite stable in the presence of iron. Warburg and Negelein (2) showed that aqueous suspensions of blood charcoal (containing iron, nitrogen, and carbon) had the property of oxidizing cysteine, cystine, and other amino acids, so that the final products were the end products of oxidations in the animal body—carbon dioxide, ammonia, and sulphuric acid.

It was shown by Voegtlin, Rosenthal, and Johnson (3) that copper can oxidize reduced crystalline glutathione solutions to the disulphide compound, while iron, manganese, and certain other heavy metals are without such an effect.

It is generally assumed that copper behaves similarly to iron in the oxidation of cysteine, and that in aqueous or buffer solutions the reaction proceeds only to the formation of cystine. We have found, however, that small amounts of copper can cause a breakdown of the cysteine molecule so that carbon dioxide, ammonia, and sulphuric acid can be recovered as end products.

METHODS AND MATERIAL

The cysteine hydrochloride was prepared by recrystallization from a commercial sample, and purification by Warburg's method (7) to render it free from heavy metals.[1] The water employed for making

[1] This was done by Dr. J. M. Johnson of the National Institute of Health.

the solutions and for rinsing the glassware was triple distilled, the last two times in glass. The copper, iron, and manganese salts were commercial samples of high purity, analyzed by Doctor Johnson for their copper content.

The oxidations were followed by determining the oxygen uptake with the Barcroft-Warburg microrespiration apparatus. The manometer and vessels were shaken at a rate of 90 oscillations per minute. The temperature employed was 37.6° C., unless mentioned differently. Unless otherwise stated, the gas employed was air and, except for estimations of carbon dioxide formation, the carbon dioxide was absorbed by alkali in the central container of the respiration vessel. Where carbon dioxide production was measured, determinations were done in an atmosphere of oxygen; and the technique employed was that which has been summarized by Richardson (5).

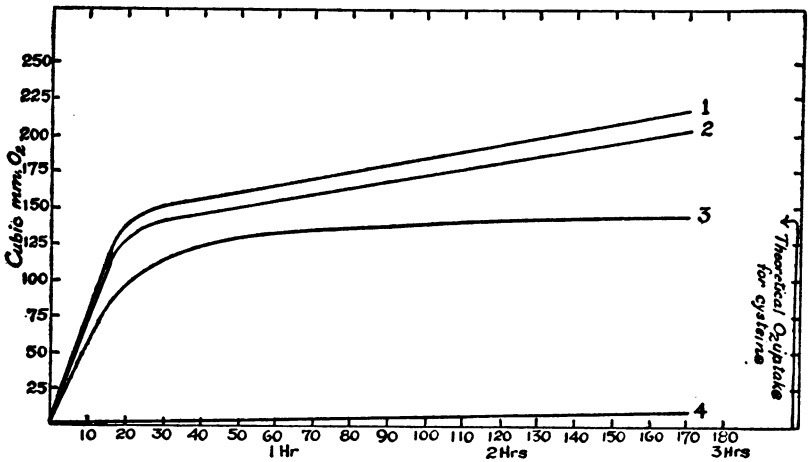

CHART 1.—The ability of copper to oxidize cysteine beyond the disulphide stage. Curve 1, 4 mg cysteine hydrochloride +0.01 mg Cu (as cupric ammonium sulphate). Curve 2, cysteine +0.01 mg of Cu +0.01 mg Fe (as ferric ammonium sulphate). Curve 3, cysteine +0.01 mg Fe. (Iron brings about an oxidation only to the S–S stage.) Curve 4, cysteine alone. All experiments done in phosphate buffer at pH 7.3; temperature 37.6° C.; atmospheric air. Total volume 2.5 cc. Theoretical O_2 uptake refers to that required to oxidize cysteine to cystine

The action of copper upon cysteine.—The rate of the oxidative breakdown of cysteine by copper depends upon the quantity of copper, the hydrogen ion concentration, temperature, oxygen tension, and nature of the solution used.

The addition of 0.01 mg of copper (as cupric ammonium sulphate) to 4 mg of cysteine hydrochloride in phosphate buffer at pH 7.3 and at 37.6° C. caused an oxygen consumption in five hours of 230 cu mm, or 1.6 times the amount that is required to oxidize the SH to the S–S group. (Chart 1.) The oxygen uptake had not ceased at the end of five hours.

When the amount of copper is increased from 0.01 mg to 0.1 mg, the other conditions remaining the same, the oxygen consumed in

five hours increased from 1.6 to 3.0 times that required for the S–S compound. (Chart 2.)

When 0.1 mg of copper was used and the hydrogen-ion concentration of the phosphate buffer solution varied, the oxygen uptake in five hours was as follows:

pH 6.6—1.22 times the oxygen required for cystine.
pH 7.15—1.9 times the oxygen required for cystine.
pH 7.3—3.0 times the oxygen required for cystine.

In all of the above experiments the oxygen consumption had not ceased after five hours, and so these values do not represent the end results of the oxidation.

In order to determine whether the phosphate was essential to this reaction, an experiment was done in water. Alkali was added to an aqueous solution of cysteine to bring about the desired pH. The

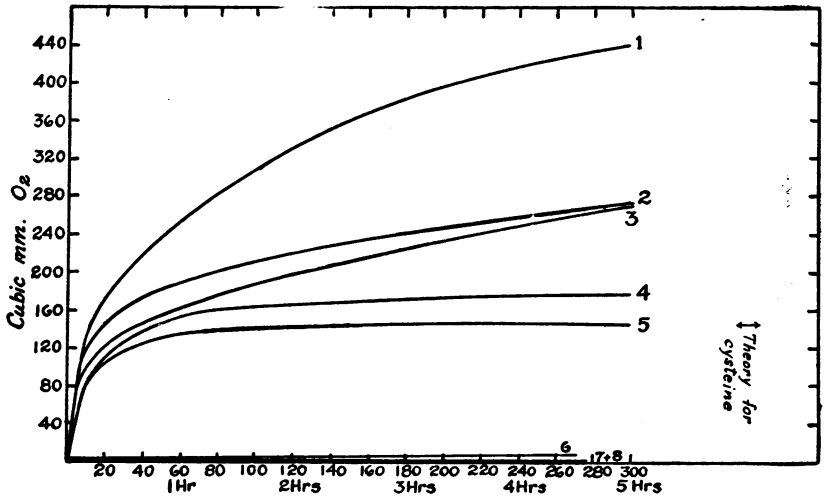

CHART 2.—The oxidative breakdown of the cysteine molecule by copper at varying hydrogen ion concentrations. The lack of effect of copper or iron upon cystine solutions. Curves 1, 2, and 4, 4 mg cysteine HCl +0.1 mg Cu (as cupric ammonium sulphate) in phosphate buffer at pH 7.3, 7.15, and 6.6, respectively. Curve 3, in water at pH 7.3. Curve 5, 4 mg cysteine HCl+0.1 mg Fe (as ferric ammonium sulphate) in phosphate buffer, pH 7.3 Curve 6, 20 mg cystine +0.1 mg Fe in phosphate buffer at pH 6.6. Curves 7 and 8, 20 mg cystine +0.1 mg Cu in phosphate buffer at pH 6.6 and 7.3. Total volume of solutions 2.5 cc.

cysteine has sufficient buffer capacity to stabilize the hydrogen-ion concentration of the solution, which was pH 7.3 at the end of the experiment. With 0.1 mg of copper, the oxygen uptake in five hours (oxidation incomplete at the end of that time) was 1.87 times the calculated amount for S–S, as compared to 3.0 times when phosphate buffer of the same pH was employed. (Chart 2.)

Warburg (4) has employed the catalytic action of copper on cysteine in pyrophosphate buffer as a means of estimating small amounts of copper. The method is based upon his observation that iron and manganese are inactivated by the pyrophosphate. In those experiments which he carried to completion in pyrophosphate and

in borate buffer the oxygen uptake corresponded very closely to the amount needed for oxidizing cysteine to cystine. We have repeated this experiment in pyrophosphate buffer and obtained similar results. The ability of copper to cause the oxidative breakdown of the cysteine molecule is abolished in pyrophosphate buffer, while its ability to oxidize cysteine to the disulphide compound is retained. (Chart 3.) Indeed, Elvehjem (6) has shown that the oxidation of cysteine to cystine by copper is enhanced in pyrophosphate as compared with that obtained in phosphate buffer solutions.

Experiments were done next to show the production of carbon dioxide during the oxidation of cysteine by copper in phosphate buffer. An experiment was run under the following conditions: 4 mg cysteine hydrochloride in phosphate buffer of pH 7.5, tem-

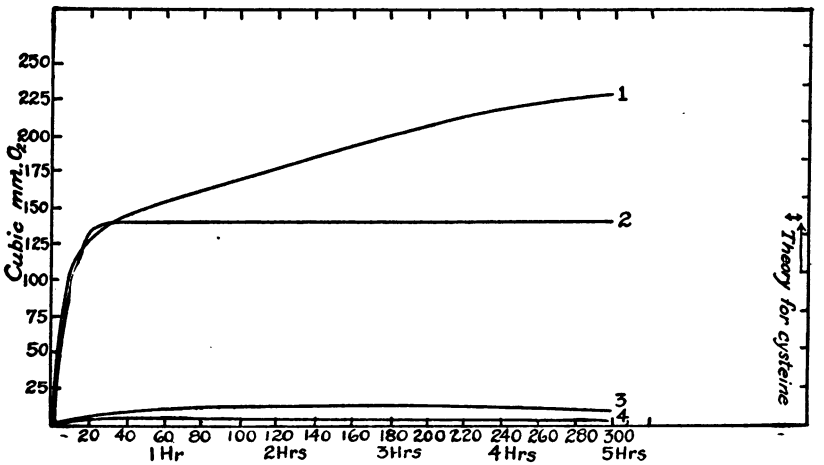

CHART 3.—The ability of pyrophosphate solutions to prevent the oxidation of cysteine by copper beyond the S-S stage. The stability of cystine toward Cu and Fe is not affected by the addition of a small amount of cysteine. Curve 1, 4 mg cysteine +0.01 mg Cu in phosphate buffer, pH 7.3. Curve 2, in pyrophosphate buffer at pH 7.6. Curve 3, 10 mg cystine +0.1 mg cysteine +0.1 mg Cu, phosphate buffer pH 7.3. Curve 4, 10 mg cystine +0.1 mg cysteine +0.1 mg Fe, phosphate buffer pH 7.3. Volume of solutions 2.5 cc.

perature 41° C., 0.1 mg copper, and an atmosphere of oxygen. At the end of 6 hours 746 cu mm, or 5.2 times the amount of oxygen necessary for the formation of the S-S compound, had been consumed and 385 cu mm of carbon dioxide produced. (Chart 4.) The oxidation had not been completed at the end of that time. For the complete oxidation of 4 mg of cysteine hydrochloride to CO_2, ammonia, and sulphuric acid, 2,559 cu mm of oxygen would be required and 1,714.5 cu mm of CO_2 would be produced. In this experiment, therefore, at the end of six hours there was 29 per cent of the oxygen consumed and 22 per cent of the CO_2 produced that would be required for the complete oxidation of the cysteine. These values are the same as those obtained by Warburg and Negelein (2) for the oxidation of cysteine on blood charcoal under conditions

of temperature, oxygen tension, etc., comparable to those of our experiment.

Other observations dealing with the products of oxidation of cysteine by copper may be summarized as follows:

(A) To 5 grams of cysteine hydrochloride in 25 c c water brought to pH 7.3, were added 50 mg of copper. The solution in an Ehrlenmeyer flask of 300 c c capacity was placed in an incubator at 37.5° C. A mechanism in the incubator permitted the flask to be slowly tipped from side to side. After 3 days the precipitate was tested by Doctor Johnson for cysteic acid, after the method of Friedmann (7), with negative results.

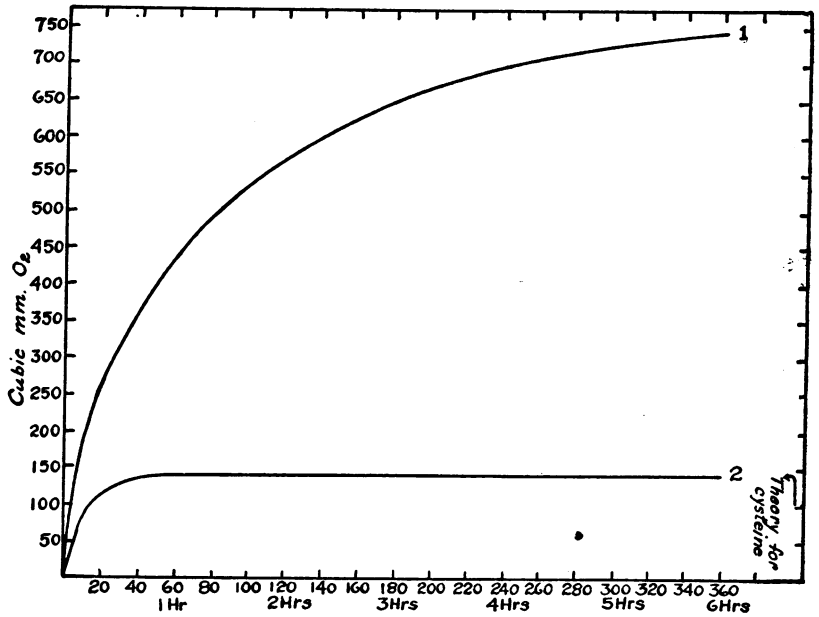

CHART 4.—The effects of copper and of manganese upon 4 mg of cysteine HCl in phosphate buffer, pH 7.5 at 41° C. and in an atmosphere of oxygen. Curve 1, cysteine +0.1 mg Cu (as cupric ammonium sulphate). Separate determinations showed that 385 cu mm of CO_2 were produced in the 6-hour period. Curve 2 cysteine +0.1 mg manganese (as $MnSO_4$). No CO_2 was produced

(B) 250 mg of cysteine hydrochloride were dissolved in water, adjusted to pH 7.5 and made up to 500 c c. The solution was equally divided into two half-liter flasks. To one was added 3.1 mg of copper (as cupric chloride) and to the other 3.1 mg of iron (as ferric chloride). Oxygen was run into the stoppered flasks, they were placed in a water bath at 41° C. and shaken for nine hours. Several replacements of oxygen were made during this time. At the end of this period, determinations of ammonia were run by Folin's method and of sulphates by the barium sulphate method.

Ten c c of the solution oxidized by copper contained 0.1 mg of ammonia. This represents 19 per cent of the amount that would be

found if the cysteine were completely broken down into carbon dioxide, ammonia, and sulphuric acid. The solution oxidized by iron contained no ammonia.

Two hundred and forty c c of the solution oxidized by copper contained 0.19 mg of sulphur which could be precipitated as barium sulphate after acidification of the solution with hydrochloric acid. This represents, therefore, a recovery of 0.8 per cent of the total sulphur as sulphuric acid. In the cysteine solution oxidized by iron no sulphuric acid could be detected.

(C) When copper is allowed to oxidize cysteine in phosphate buffer or water, the solution turns dark brown, suggesting an oxidation of the amino group. This does not occur with iron or manganese.

(D) When cysteine is slowly oxidized by iron, crystals characteristic of cystine are obtained. When copper is employed there are present several kinds of crystals. Those predominating are small thick irregular needles arranged in thick clumps or bundles. A few characteristic cystine crystals are usually present. The nitroprusside test in the presence of cyanide is strongly positive, revealing the presence of a disulphide compound.

Action of copper upon cystine.—We were first of the opinion that the action of copper was to oxidize cysteine to cystine and that the further oxidation which occurred was due to a breakdown of the cystine. Such a mechanism would require that cystine under the conditions of these experiments be oxidized by copper. This is not the case. When 20 mg of cystine were added to phosphate buffer at pH 6.6, or pH 7.3, no uptake of oxygen results from the addition of 0.1 mg of copper or iron. (Chart 2.)

The possibility remained that the cysteine-copper combination formed a catalytic system capable of oxidizing cystine. To test this hypothesis, respiration vessels were set up containing 10 mg of cystine and 0.1 mg of copper in phosphate buffer at pH 7.3. After readings were begun, 0.1 mg of cysteine hydrochloride was added from the side arm. The oxygen consumption after five hours was 11 cu mm or three times the amount required to oxidize 0.1 mg of cysteine to cystine. (Chart 3.) This oxygen uptake can therefore be accounted for by the action of copper on the cysteine present. There is no evidence that any of the cystine originally present in the solution was oxidized by the copper.

The negative results of these experiments suggest that the breakdown of cysteine by copper involves not only an oxidation of the SH radical but also an action upon another part of the cysteine molecule. In such a process cystine would not necessarily be an intermediate step in the reaction.

Action of iron and manganese upon cysteine.—In accordance with the results of previous workers, we have found that iron, when added

to cysteine in neutral or slightly alkaline solutions, brings about an oxidation to the cystine stage at which point the oxidation ceases. This is true in water, in phosphate buffer, and when iron is added to 10 mg of cystine containing 0.1 mg of cysteine. (Charts 1, 2, 3.) The presence of iron does not appreciably alter the action of copper upon cysteine in phosphate buffer. (Chart 1.)

Warburg (4) has shown that manganese is an active catalyst of the oxidation of cysteine in borate buffer solutions, but the total oxygen uptake was not reported. However, we have found that manganese is not a very active catalyst in water or phosphate buffer at hydrogen ion concentrations near neutrality, at 37.6° C. and in an atmosphere of air. When 0.1 mg of manganese (as manganous sulphate) was added to 4 mg of cysteine in phosphate buffer at pH 6.6 and 7.1, or to 4 mg of cysteine in water at pH 7.1, the oxidation to cystine was not completed after 11 hours of shaking (Chart 5), and the nitroprusside

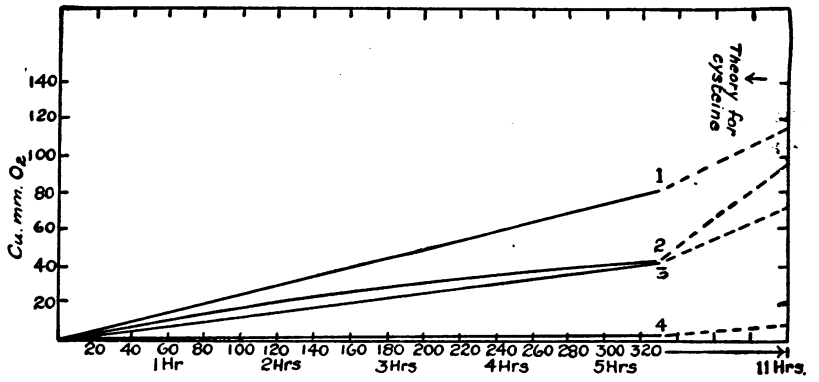

CHART 5.—The feeble catalytic activity of manganese upon cysteine in water or phosphate buffer. Curve 1, 4 mg cysteine +0.1 mg Mn in water at pH 7.1. Curve 2, in phosphate buffer at pH 7.3. Curve 3, in phosphate buffer at pH 6.6. Curve 4, cysteine alone in water at pH 7.1

test on the solutions were still positive after this time. However, in an experiment run at 41° C. in an atmosphere of oxygen, and in phosphate buffer solution at pH 7.5, it was found that the oxygen consumption ended with the amount required for the formation of cystine. (Chart 4.)

II. THE ACTION OF HEAVY METALS UPON THE FIXED SULPHYDRYL GROUPS OF PROTEINS

For the purpose of studying the oxidation of the SH groups of proteins, twice recrystallized egg albumin was employed. The ammonium sulphate was removed by dialysis in a stream of distilled water for two days at 3° C. This was accomplished by preparing a glass jacket that encased the collodion sacs with a few millimeters' clearance so that when the sac was in place 10 to 20 c c of water would fill

the jacket to overflowing. The water was conducted to the bottom of the jacket through a fine glass tube placed in the space between the collodion sac and the jacket. About 20 liters of water were used in 24 hours. The rate of dialysis was greatly accelerated by a motor-driven stirring rod placed in the albumin solution.

Tests were made for ammonium sulphate by precipitating the albumin solution with trichloracetic acid and by adding a drop of Nessler's solution to a drop of the filtrate. In determining the nitrogen content, the trichloracetic acid precipitate was washed upon filter paper with trichloracetic acid until the filtrate gave no color with Nessler's reagent.

To bring out the sulphydryl groups in the dialyzed albumin, sodium chloride was added to make 0.8 per cent, and the solution was coagulated by immersion in boiling water with stirring for three to five minutes. It was then rapidly cooled and pipetted into the respiration vessels.

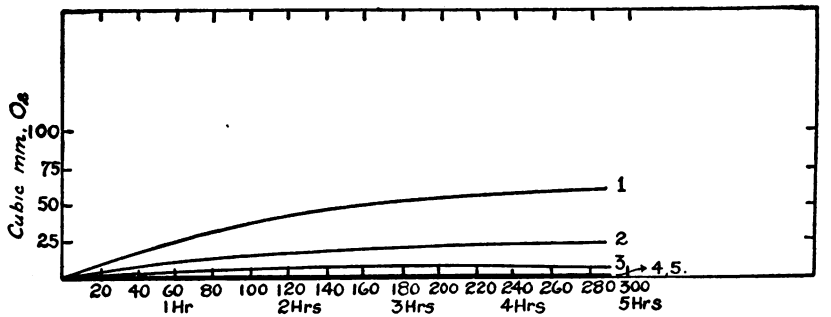

CHART 6.—The oxygen uptake of coagulated crystalline egg albumin brought about by copper and the lack of effect of iron. Curve 1, 60 mg coagulated albumin +0.2 mg copper (as citrate). Curve 2, albumin +0.02 mg Cu. Curve 3, albumin +0.002 mg Cu. Curve 4, albumin +0.5 mg Fe (as citrate). Curve 5, albumin alone. Experiments run in aqueous solutions at pH 7.8 Total volume of fluid 2.5 c c.

Effects of iron and copper on egg albumin.—For the purpose of this study cupric and ferric citrate, cupric and ferric ammonium sulphate, cupric and ferric sulphate, and cupric and ferric chloride were employed. The ferric salts were analyzed by Dr. J. M. Johnson for the presence of copper and found to contain amounts of no significance for this work.

The sulphydryl groups of egg albumin are more resistant to oxidation than those of glutathione and cysteine. This is shown by the fact that in slightly alkaline solution (pH 7 to 8) the coagulated albumin may be kept at room temperature, exposed to air, for one to two weeks before the nitroprusside test disappears. It is also manifested by the larger amounts of copper required to bring about their oxidation.

We have found that among the heavy metals only copper and manganese can bring about an oxidation when added to coagulated

egg albumin. With copper the oxidation involves a breakdown of the protein molecule with the liberation of carbon dioxide, while with manganese less oxygen is consumed and no carbon dioxide formed.

The ability of copper to bring about the oxidation of the coagulated egg albumin, and the ineffectiveness of iron, is shown in Chart 6. From 0.002 to 0.2 mg of copper (as citrate) and 0.5 mg of iron were employed. In this experiment, made at pH 7.8, at 37.6° C. in an atmosphere of air, the maximum oxygen uptake from 60 mg of protein plus 0.2 mg of copper was 46 cu mm in four hours.

The effect of 0.5 mg of copper (as citrate) upon 80 mg of albumin is shown in Chart 7. It is also seen that no uptake of oxygen results when copper is added to the native egg albumin (containing no free sulphydryl groups). This absence of effect of copper upon native albumin suggests that there is no breakdown of the molecule unless the protein contains free sulphydryl groups.

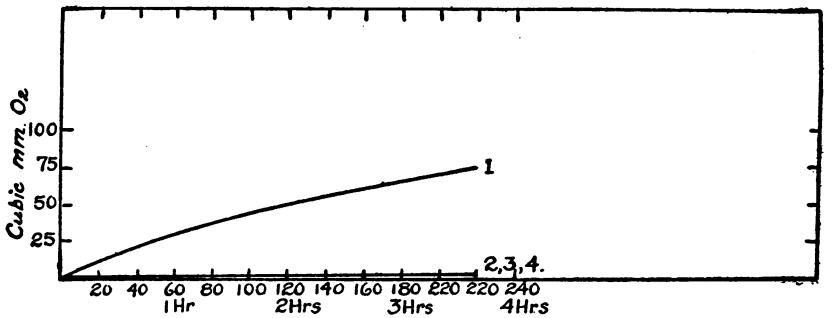

CHART 7.—The inability of copper to bring about an oxidation of egg albumin when in the native state. Curve 1, 84 mg coagulated ovalbumin +0.5 mg Cu (as citrate). Curve 2, 84 mg native albumin +0.5 mg Cu. Curve 3, coagulated albumin +0.5 mg Fe (as citrate). Curve 4, coagulated albumin alone. Experiments in water at pH 7.5. Total volume of fluid 4.5 c c.

In the above experiments the oxidations did not proceed to completion, and so a further experiment was done in which the rate of oxidation was increased by carrying out the oxidation in an atmosphere of oxygen and at a higher temperature (41° C.). (Chart 8.) Seventy-six mg of coagulated protein to which was added 0.3 mg of copper (as chloride) consumed in five hours 138 cu mm of oxygen, or 1.82 cu mm O_2 per milligram of protein.

Although it is possible to estimate approximately the sulphydryl content of proteins, satisfactory methods for exact quantitative estimation are at present not available. If the cystine sulphur of egg albumin can be taken to represent the maximum amount of sulphur that could be converted into sulphydryl sulphur by denaturation of this protein, then the actual oxygen uptake of the coagulated egg albumin in the presence of copper is greater than that required for the oxidation of SH to S–S groups. Thus, Sullivan (8) found 1.2

per cent of cystine in egg albumin. If this sulphur is expressed as SH (and it is unlikely that all of the cystine sulphur is converted into SH sulphur in the coagulated protein) then the oxygen required to convert it to the S–S state would be 0.432 cu mm per milligram of protein, whereas in this experiment 1.82 cu mm were consumed. Since the analysis of crystalline egg albumin (Calvary) shows that only one-eighth of the total sulphur is present as cystine sulphur, it is also possible that some of this noncystine sulphur may give rise to SH groups upon denaturation of the protein. However, we have obtained proof that the oxidation of the coagulated albumin by copper proceeds further than the oxidation of SH to S–S compounds, in the production of carbon dioxide during the process. An experiment was run upon 76 mg of protein in 4 c c of water at pH 7.4; temperature of 41° C. and in an atmosphere of oxygen; 0.3 mg of copper was added before readings were begun so that the measure-

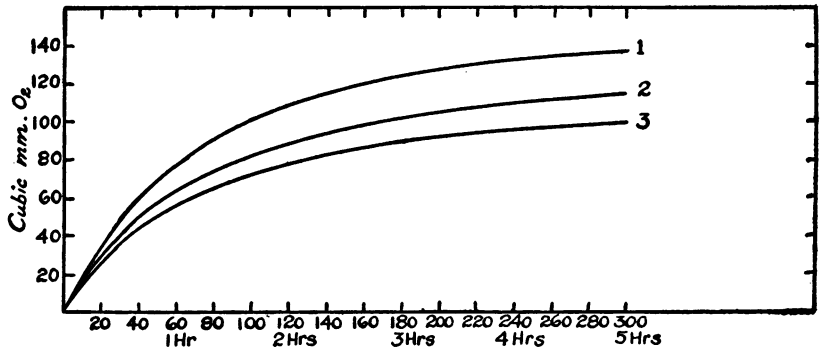

CHART 8.—The oxygen consumption and CO_2 production of 76 mg coagulated egg albumin +0.3 mg Cu (as $CuCl_2$) in an atmosphere of oxygen and at 41° C. Curve 1, total O_2 consumption in 5 hours. Curves 2 and 3, oxygen consumption during a 5-hour period, with and without alkali for absorption of CO_2. The corrected values for this period were 116 cu mm O_2 taken up and 18 cu mm CO_2 produced. Aqueous solutions, pH 7.4; volume of fluid 4.2 to 4.6 c c.

ments do not represent total values. In five hours 116 cu mm of oxygen were consumed and 18 cu mm of carbon dioxide were liberated. (Chart 8.) The ratio of carbon dioxide to oxygen is thus 0.155. This is less CO_2 than was produced from the oxidation of cysteine by copper, where the ratio was 0.561.

Further proof that the oxidation of the albumin by copper does not proceed to the same extent as with cysteine is shown in that no sulphuric acid or appreciable quantities of ammonia could be detected as end products. For this purpose 200 c c of egg white (diluted with equal parts of 0.8 per cent salt solution; protein content = 6 per cent) were placed in each of two 500-c c flasks. The protein was coagulated by immersion into boiling water with stirring, for nine minutes. After cooling the solutions, to one flask was added 33 mg of copper (as chloride) in 50 c c of water, and to the other 33 mg of iron (as chloride) in 50 c c of water. After nine hours'

shaking at 41° C. in an atmosphere of oxygen, only a trace of ammonia could be detected in two 10-c c samples of the solution oxidized with copper, using the iron-containing solution as a control. The remaining 230 c c in each flask were precipitated with equal parts of 20 per cent redistilled trichloracetic acid and the filtrates were tested for sulphuric acid by the addition of hydrochloric acid and barium chloride. Negative results were obtained upon both solutions.

Experiments were done to determine whether the nitroprusside test would disappear before the completion of the oxidation by copper. These tests were done upon some of the coagulated albumin solution in a separate respiration vessel reserved for this purpose. In harmony with the other results it was found that the nitroprusside test became negative early in the course of the oxidation. This is also illustrated

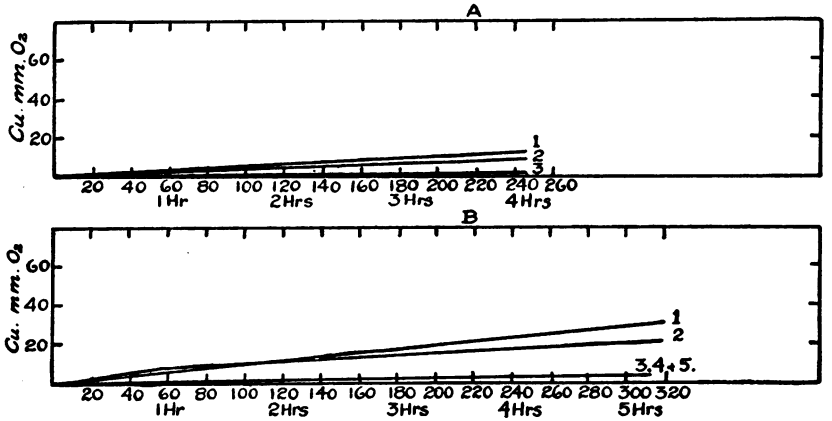

CHART 9.—The ability of manganese to bring about an oxidation of the coagulated albumin, and lack of effect of cobalt, zinc, and tin. A, curve 1, 50 mg albumin +0.3 mg Cu (as cupric ammonium sulphate), pH 6.8. Curve 2, albumin +0.3 mg Mn (as $MnSO_4$), pH 7.0. Curve 3, albumin +0.3 mg Co (as $CoCl_2$), pH 7.8. Experiments run in water; total volume 4.6 c c. B, curve 1, 60 mg albumin +0.2 mg Cu, pH 6.9. Curve 2, +0.2 mg Mn, pH 7.4. Curve 3, +0.2 mg Co, pH 7.2. Curve 4, +0.2 mg Zn (as $ZnSO_4$), pH 6.9. Curve 5, +0.1 mg Sn (as $SnCl_2$), pH 7.1. Experiments run in water; total volume 5.4 c c. The nitroprusside tests were positive at the end of all of the above experiments, with the exception of copper.

in Chart 7, where the oxidation was still proceeding rapidly at the end of the experiment, although the nitroprusside test was negative.

Effects of cobalt, zinc, and tin salts on protein sulphydryl groups.—Cobalt has been shown by Michaelis and Barron (9) to form a complex with cysteine, the cobaltous cysteine complex being susceptible of oxidation. Similarly, Voegtlin, Johnson, and Rosenthal (3) found that cobalt and glutathione in the presence of oxygen form a complex which is susceptible of oxidation. However, in three experiments, employing from 0.2 to 0.5 mg of cobalt, no appeciable oxidation could be demonstrated with the coagulated egg albumin. (Chart 9.) With the 0.5 mg addition of cobalt the nitroprusside test at the end of the experiment was less strongly positive than the control, and so it is possible that to some extent a stable cobalt protein complex was formed.

Zinc was found by Voegtlin, Johnson, and Rosenthal (3) to have an inhibitory action on the oxidation of glutathione, while tin in small concentrations was without effect. Large amounts of stannous chloride, under the conditions of these experiments, of themselves take up oxygen due to oxidation to the stannic state. However, when 0.1 mg of stannous chloride was added to 60 mg of coagulated albumin at pH 7.1, no oxygen uptake occurred in five hours, at which time the nitroprusside test on the solution was strongly positive. (Chart 9.)

Zinc was without effect on the coagulated protein. (Chart 9.) Since the protein solution itself does not take up any oxygen, these experiments do not reveal any inhibiting effect that might be shown by tin or zinc upon the oxidation of coagulated albumin.

Effect of manganese on coagulated egg albumin.—It was found by Warburg (4) that manganese could bring about the oxidation of

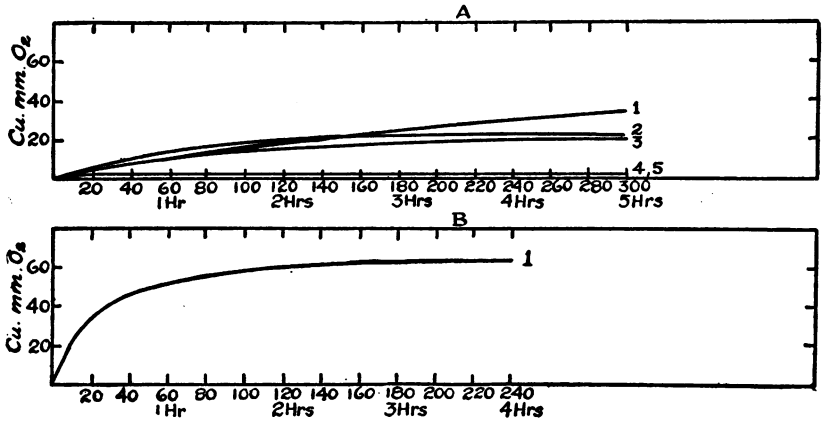

CHART 10.—Effects of manganese ($MnSO_4$) on native and coagulated albumin. A, Curve 1, 52 mg coagulated albumin +0.2 mg Mn, pH 7.1. Curve 2, 47 mg coagulated albumin +0.5 mg Mn, pH 7.0. Curve 3, 47 mg coagulated albumin +0.2 mg Mn, pH 6.8. Curve 4, 47 mg native albumin +0.5 mg Mn, pH 6.9. Curve 5, coagulated albumin alone. Experiments in water, total volume 4.6 c c; Temperature 37.6°C. Atmosphere air. The nitroprusside tests were faintly positive at termination of experiments with curves 1, 2, and 3; B, Curve 1, 76 mg coagulated albumin +0.3 mg of Mn in an atmosphere of oxygen, temperature 41° C., pH 7.6. Aqueous solution, volume 4.6 c c. Nitroprusside test practically negative at end of experiment. Separate determinations revealed no CO_2 formation

cysteine. In the first half of this paper it was shown that in phosphate buffer at pH 7.5, this oxidation proceeds only to the disulphide state. Voegtlin, Johnson, and Rosenthal (3) found manganese without effect upon the oxidation of glutathione.

Manganese added to native egg albumin at pH 7 to pH 8.4 brought about no oxygen consumption. (Chart 10.) Such experiments also serve as a control to show that the manganous sulphate is not oxidized to manganic compounds under the condition of these experiments.[2] No dark color could be detected in any of the solution at the termination of the experiments.

[2] Such oxidation does occur at pH 10 or above.

Experiments with coagulated albumin showed that manganese could bring about a considerable oxygen uptake. As with copper, this oxidation proceeds more rapidly on the alkaline side of neutrality, but differs in several respects from the oxidation by copper.

In five experiments 0.2 to 0.5 mg of manganese were added to coagulated albumin in aqueous solution, at pH 6.8 to pH 7.4, in an atmosphere of air, and at 37.6° C. The oxygen uptake in five hours was slightly less than with similar amounts of copper; the oxidations had not ceased at the end of this time and the nitroprusside tests were faintly positive at the completion of the experiments. (Charts 9, 10A.)

An experiment was run at 41° C., at pH 7.6, and in an atmosphere of oxygen. Under these conditions 0.3 mg of manganese added to 76 mg of albumin caused an oxygen uptake which had reached completion after 2½ hours. (Chart 10B.) The total oxygen uptake amounted to 63 cu mm of oxygen, or 0.83 cu mm per milligram of protein. This is less than half the total oxidation caused by copper under the same conditions. Further studies showed that, under these conditions, the oxygen uptake in the presence of manganese was not accompanied by carbon dioxide production.

A third difference between the action of manganese and that of copper is that in the case of manganese the nitroprusside test persists throughout the duration of the oxygen uptake.

The evidence at hand suggests, therefore, that the oxidation by manganese involves only an oxidation of the SH groups. This can not be established with certainty until the actual sulphydryl content of the coagulated albumin and its oxygen requirements can be quantitatively determined.

Oxidation of dialyzed tissues by copper and iron.—It was previously observed (10) that when tissues were dialyzed for two or three days in running water, the glutathione was all washed out, while the residue gave a positive nitroprusside test, as evidence of the presence of protein sulphydryl groups. The technique of the dialysis was similar to that for egg albumin. The rat testis was principally used for the present study. Such a residue at pH 6 to 8 shows a very low consumption of oxygen at 37.6°. When iron was added to the dialyzed testis there was a large uptake of oxygen. Ferric ammonium sulphate was more than seven times as effective as ferric citrate. The oxidation with iron proceeded much more rapidly at an acid reaction. (Charts 11, 12.)

Copper was less effective than iron in causing the oxygen consumption with the dialyzed testis, although an appreciable oxygen uptake occurred. (Chart 11.)

Heating the solution to 100° C. did not diminish the effect of iron and so the possibility seemed remote that the activation of an enzyme was concerned in the oxidation.

The depressant effect of hydroxyl ions suggested the oxidation of fats. A further experiment proved this to be the case. Some of the dialyzed tissue was precipitated with trichloracetic acid and the residue washed with water until free from acid. The residue was then repeatedly extracted with alcohol and ether. The extracts were then combined and the alcohol and ether removed *in vacuo*. The residue of the alcohol-ether extract was taken up in absolute alcohol

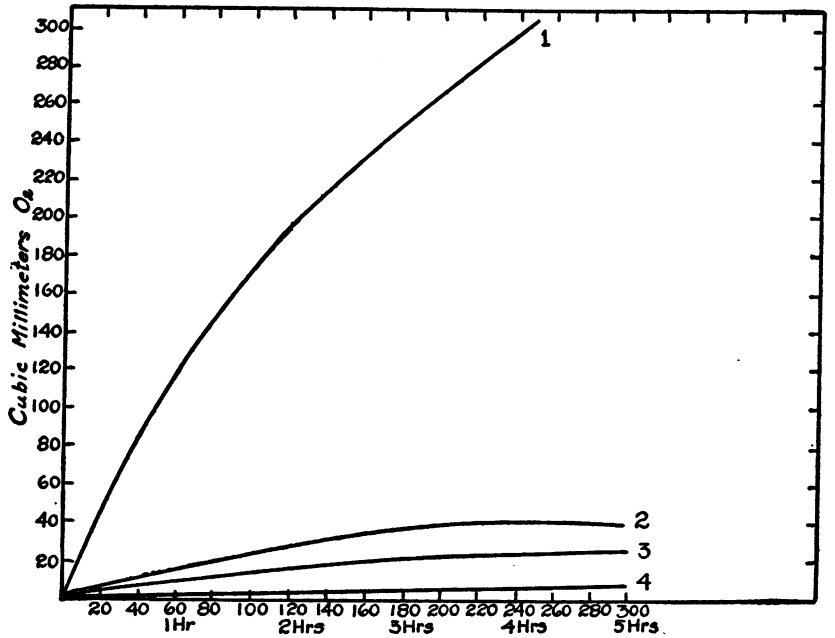

CHART 11.—Oxygen uptake of rat testes dialyzed for 2 days in distilled water at 3° C. Effects of iron and copper. Curve 1, testes +0.1 mg Fe (ferric ammonium sulphate), pH 6.8. Curve 2, testes +0.1 mg Cu (cupric ammonium sulphate), pH 7.0. Curve 3, testes +0.05 mg Cu, pH 7.0. Curve 4, testes alone, pH 7.2. Fluid volume 2.5 c c; protein content 36.7 mg

and filtered, and the filtrate was evaporated *in vacuo*. An emulsion of the lipoid extract was then made in 0.8 per cent salt solution.

It was found that the emulsion of the alcohol-ether extract took up oxygen upon the addition of iron or copper at pH 6.6 to practically the same extent as the original dialyzed testis, while the testicular residue that was extracted showed no oxygen uptake upon the addition of iron or copper. It can be concluded, therefore, that we are chiefly dealing with an oxidation of the lipoids of the tissue residue by these metals. The disappearance of the nitroprusside reaction, particularly in the case of copper, indicates that a simultaneous oxidation of the protein sulphydryl groups occurs.

DISCUSSION

The ability of copper to oxidize cysteine solutions to carbon dioxide, ammonia, and water is apparently specific for this metal. The fact that copper is without action upon cystine solutions is of particular interest from the point of view of the mechanism of the oxidation, for it demonstrates that a reaction between copper and sulphydryl radical is essential to the process and further suggests that the oxidation does not pass through the cystine stage. In accordance with this view we have also found that taurine in aqueous solution is stable in the presence of copper, while Friedmann (7) has prepared stable copper salts of cysteic acid.

The fact, shown by Voegtlin, Johnson, and Rosenthal (3), that copper can oxidize reduced glutathione only to the disulphide state

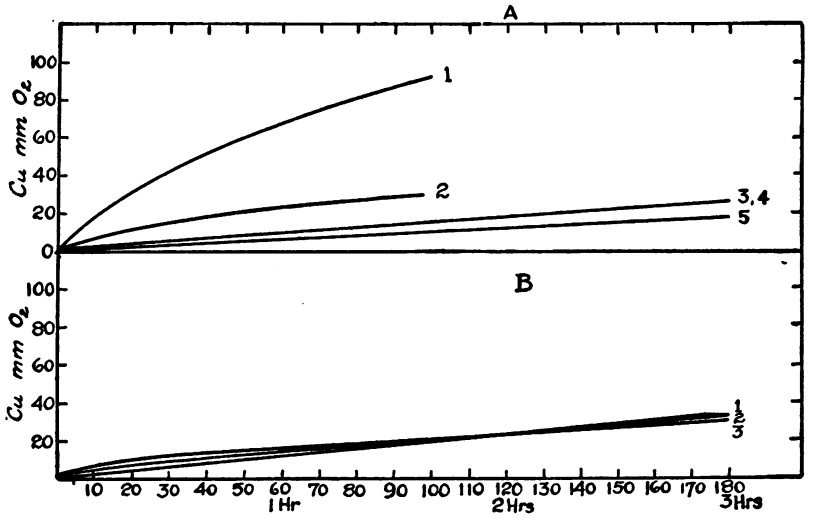

CHART 12.—Effects of iron and copper salts on dialyzed (2 days) rat testes at various hydrogen ion concentrations. A, Curves 1 and 2, testes +0.1 mg Fe (ferric ammonium sulphate) at pH 6.7 and 7.6, respectively. Curves 3, 4, and 5, testes +0.1 mg Fe (ferric citrate) at pH 6.6, 7.0, and 7.6, respectively. B, Curves 1, 2, and 3, testes +0.1 mg Cu (cupric citrate) at pH 6.6, 7.0, and 7.6. Volume of fluid 2.5 c c. protein content 29 mg

is also of physiological interest, for it places this oxidation among the reversible reactions, while the breakdown of the cysteine molecule by copper is irreversible. These observations may be of significance in explaining the fact that cysteine has not been recovered from normal tissues in any appreciable quantities.

The ability of pyrophosphate solutions at pH 7.6 to prevent the oxidation of cysteine by copper beyond the disulphide stage is of interest in that pyrophosphate has been shown by Elvehjem (6) to augment the catalytic action of copper in the oxidation of cysteine to cystine.

The behavior of the coagulated crystalline egg albumin toward heavy metals presents some interesting comparisons. The oxidation

of this protein by copper resembles that of cysteine in that a breakdown of the molecule occurs. This is evidenced by a large excess of oxygen uptake and by the formation of carbon dioxide during the oxidation. The resemblance to cysteine is also shown in that this oxidation does not occur if no free sulphydryl groups are present (as in native albumin). The behavior toward manganese also resembles that of cysteine in that an oxidation is effected (reduced glutathione is not oxidized by manganese) which, from the smaller amount of oxygen consumed, the persistence of the nitroprusside test throughout the oxidation, and the absence of carbon dioxide formation, seems to stop at the disulphide stage. On the other hand, the behavior of the coagulated egg albumin containing SH groups towards iron differs

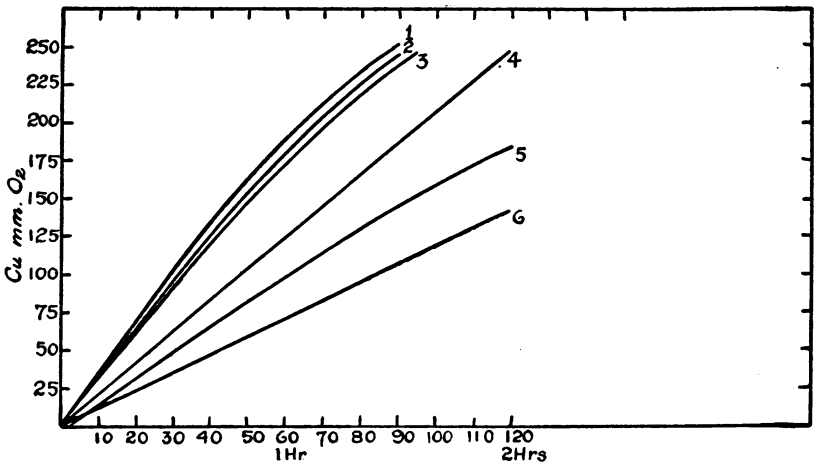

CHART 13.—No acceleration of oxygen uptake from the addition of Cu (citrate) to living rat tissues *in vitro*. Curve 1, 0.2 gm liver alone. Curves 2 and 3, liver +0.00318 mg Cu (m/50,000) and +0.0318 mg Cu, respectively. Curve 4 represents four curves showing 0.2 gm testes alone, testes +0.0159 mg Cu (m/10,000), testes +0.00318 mg Cu, and testes +0.00159 mg Cu. Curve 5, 0.2 gm testes +0.0795 mg Cu (m/2,000). Curve 6 represents four curves showing 0.15 gm Jensen rat sarcoma alone, sarcoma +0.0795 mg Cu, sarcoma +0.0159 mg Cu, and sarcoma +0.00318 mg Cu. Tissues in Locke's solution with 0.3 per cent bicarbonate and 0.2 per cent glucose. Total volume 2.5 c c. (Molarity refers to final concentrations)

from cystine and resembles that of glutathione in that no appreciable oxidation is brought about during the course of the experiments.

The oxidations effected by copper and manganese may also involve other portions of the protein molecule as well as the sulphydryl-containing radical, but the absence of effect in the absence of free sulphydryl groups is good evidence that the SH group is essential to the oxidation.

The ability of copper to bring about oxidations in the coagulated egg albumin and of iron and copper to oxidize the fats in dialyzed tissue residues suggests that these metals might stimulate oxidations when added to living cells. However, Rosenthal and Voegtlin (12) were unable to show any increase in oxygen consumption of rat tissues, *in vitro*, or of yeast cells following the addition of various iron

salts in low concentrations, while high concentrations caused a depression of oxygen uptake. We have found similar results with cupric citrate upon rat liver, rat testis, and Jensen rat sarcoma. (Chart 13.) It is possible that iron and copper do bring about such oxidations in living cells, but that these effects are obscured by a simultaneous inhibition of oxygen consumption because of a depression of other phases of cell respiration. These negative results may also be due to lack of penetration of these salts into the interior of the intact cells.

SUMMARY

The addition of copper salts to cysteine in aqueous solution or in phosphate buffer causes an oxidative breakdown of the molecule. This is shown by a consumption of oxygen which can exceed five times the amount necessary for the formation of cystine, by the production of carbon dioxide, ammonia, and sulphuric acid, and by a darkening of the solution during the oxidation. Cysteic acid could not be recovered as an end product.

The addition of copper salts to cystine or taurine solutions is without effect.

The oxidative breakdown of cysteine by copper is completely inhibited in pyrophosphate buffer solutions, the oxidation proceeding only to the cystine stage.

Iron salts and manganese (manganous sulphate) in aqueous solutions or phosphate buffer oxidize cysteine only to cystine.

Copper, iron, or manganese added to solutions of crystalline native egg albumin cause no uptake of oxygen.

When the egg albumin is subjected to heat coagulation to bring out the protein sulphydryl groups, the addition of copper causes an oxidation which also involves an oxidative breakdown in the molecule. This is shown by an oxygen uptake of more than four times the theoretical maximum oxygen consumption attributed to sulphydryl groups, by the production of carbon dioxide, and by the disappearance of the nitroprusside test long before the completion of the oxygen uptake.

The addition of manganese to coagulated egg albumin causes a maximum oxygen consumption of approximately one half that of copper, and is not attended by the formation of carbon dioxide. The nitroprusside test persists throughout the oxidation.

The addition of iron, cobalt, tin, or zinc to coagulated egg albumin causes no appreciable uptake of oxygen.

The addition of iron or copper salts to dialyzed tissues brings about an oxygen consumption which is largely concerned with the oxidation of fats.

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As has been shown with iron salts, the addition of cupric citrate to rat tissues and Jensen rat sarcoma in low concentrations does not alter the oxygen consumption. Higher concentrations cause some inhibition of oxygen uptake. Under these circumstances stimulation of oxidations may be obscured by depression of other phases of cell respiration.

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COURT DECISION RELATING TO PUBLIC HEALTH

County required to pay specified monthly sum for maintenance in State narcotic hospital of addict who is resident of such county.—(California District Court of Appeal, First Dist., Div. 2; Riley *v.* Stack et al., 18 P. (2d) 110; decided Dec. 29, 1932.) The narcotic rehabilitation act authorized the superior court to commit drug addicts to the State narcotic hospital and provided, among other things, that the county of which an addict was a bona fide resident should pay the State at the rate of $25 per month for the time such committed addict remained an inmate of the institution. By order of the superior court sitting in the city and county of San Francisco, a certain addict was duly and regularly committed to the State narcotic hospital. The court found the addict to be a resident of San Mateo County and ordered that county to make payments to the State for the support of the addict pursuant to the statute. In a mandamus proceeding to require the auditor and treasurer of San Mateo County to comply with the act, the respondents defended upon the ground that San Mateo County was not a party to the proceeding leading to the commitment and had no opportunity to contest the issue of the residence of the addict. They argued that the due process clauses of the Federal and State Constitutions, guaranteeing that no *person* should be deprived of his property without due process of law, required that the county or its taxpayers

should be heard before an obligation to pay was placed upon them. The appellate court rejected this view and granted the writ prayed for. It concluded its opinion with the following language:

Our conclusion is that the act does not offend the due process clause in so far as the county is concerned, because the county is not a "person" within the meaning of either the Federal or the State Constitution but is a mere subdivision of the State, and, in so far as the individual taxpayer of the county is concerned, his property is not taken without due process, because when the legislature itself fixes the taxing district (i. e., the county) it is presumed to have taken such evidence upon the question of benefits to the local taxpayer as may be necessary and its determination of that matter is conclusive. [Cases cited.] The right which the taxpayer then has is not a right to question the public necessity for the tax which he is to pay. Id. This right is preserved in the general tax laws, but it is not necessary to make specific references to these provisions, because no taxpayer of San Mateo County is proceeding under them.

DEATHS DURING WEEK ENDED MARCH 18, 1933

[From the Weekly Health Index issued by the Bureau of the Census, Department of Commerce]

	Week ended Mar. 18, 1933	Corresponding week, 1932
Data from 85 large cities of the United States:		
Total deaths	8,609	9,769
Deaths per 1,000 population, annual basis	12.1	13.9
Deaths under 1 year of age	641	729
Deaths under 1 year of age per 1,000 estimated live births [1]	55	60
Deaths per 1,000 population, annual basis, first 11 weeks of year	12.4	12.5
Data from industrial insurance companies:		
Policies in force	68,819,116	73,791,756
Number of death claims	13,721	16,289
Death claims per 1,000 policies in force, annual rate	10.4	11.5
Death claims per 1,000 policies, first 11 weeks of year, annual rate	11.2	10.2

[1] 1933, 81 cities; 1932, 80 cities.

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Reports for Weeks Ended March 25, 1933, and March 26, 1932

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended March 25, 1933, and March 26, 1932

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932
New England States:								
Maine	1	3	2	16	----	286	0	0
New Hampshire	----	----	1	----	----	8	0	0
Vermont	----	2	----	----	44	70	0	0
Massachusetts	17	42	5	16	375	514	1	4
Rhode Island	2	11	1	----	----	254	0	0
Connecticut	6	7	19	38	240	154	1	1
Middle Atlantic States:								
New York	76	116	[1] 36	[1] 97	3,903	2,255	3	5
New Jersey	22	32	9	164	1,716	339	1	0
Pennsylvania	73	84	----	----	1,176	1,681	6	3
East North Central States:								
Ohio	40	40	10	94	639	618	0	6
Indiana	24	24	90	186	112	72	10	12
Illinois	48	82	32	145	398	365	29	3
Michigan	18	27	12	71	823	906	3	6
Wisconsin	7	13	64	505	390	570	0	1
West North Central States:								
Minnesota	27	6	2	5	1,326	20	2	3
Iowa	11	8	----	----	5	4	0	1
Missouri	30	23	22	55	250	45	1	1
North Dakota	9	1	----	----	21	55	4	1
South Dakota	4	2	1	----	3	9	0	0
Nebraska	13	6	2	----	27	8	0	1
Kansas	5	15	3	22	309	128	1	1
South Atlantic States:								
Delaware	1	8	----	----	7	3	0	0
Maryland [2]	8	12	24	348	12	25	0	1
District of Columbia	3	7	1	11	5	2	1	2
Virginia	13	----	----	----	480	----	2	----
West Virginia	14	17	12	284	276	438	0	1
North Carolina	17	17	64	169	509	670	0	2
South Carolina	7	6	751	1,909	171	114	0	0
Georgia [3]	8	15	319	125	64	25	1	1
Florida [3]	5	6	10	----	57	3	0	0
East South Central States:								
Kentucky	6	25	53	790	130	118	0	0
Tennessee	9	3	105	1,137	53	174	2	1
Alabama [3]	14	10	121	123	15	12	2	1
Mississippi	5	7	----	----	----	----	4	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended March 25, 1933, and March 26, 1932—Continued

Division and State	Diphtheria		Influenza		Measles		Meningococcus meningitis	
	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932
West South Central States:								
Arkansas	9	3	48	308	152	5	3	0
Louisiana	17	17	33	60	31	219	1	0
Oklahoma [4]	9	24	56	534	77	21	2	1
Texas [3]	132	49	147	33	1,180	35	1	0
Mountain States:								
Montana				44	57	113	1	1
Idaho		1	5	2	32		0	0
Wyoming	4				4	4	0	0
Colorado	1		31		11	183	0	0
New Mexico	14	10		2	10	92	2	0
Arizona	3	4	1	24	33	1	0	0
Utah [2]	3	1	3		2	1	0	1
Pacific States:								
Washington	9	1	3	9	37	649	1	1
Oregon		2	42	170	64	219	0	0
California [3]	55	64	50	113	1,378	431	7	4
Total	799	853	2,190	7,609	16,604	11,918	92	66

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932
New England States:								
Maine	0	0	8	33	0	0	1	0
New Hampshire	0	0	25	46	0	0	0	0
Vermont	1	0	26	10	0	4	0	0
Massachusetts	0	0	456	586	0	0	4	2
Rhode Island	0	0	31	78	0	0	0	2
Connecticut	0	0	147	91	0	2	0	0
Middle Atlantic States:								
New York	3	2	1,110	1,789	0	3	11	7
New Jersey	0	1	354	345	0	0	6	4
Pennsylvania	1	1	1,069	524	0	0	8	10
East North Central States:								
Ohio	0	0	635	302	23	21	3	1
Indiana	0	0	175	151	6	10	2	0
Illinois	1	1	535	433	16	13	2	14
Michigan	0	0	603	459	2	6	5	2
Wisconsin	1	0	154	95	2	0	1	1
West North Central States:								
Minnesota	0	1	109	103	0	1	0	0
Iowa	0	0	54	56	42	26	0	1
Missouri	1	0	78	72	21	4	5	2
North Dakota	0	0	15	13	0	6	1	0
South Dakota	0	2	19	4	0	8	1	1
Nebraska	0	0	42	37	3	14	0	0
Kansas	0	0	65	56	1	4	4	6
South Atlantic States:								
Delaware	0	0	12	26	0	0	1	0
Maryland [2]	0	0	110	136	0	0	1	0
District of Columbia	0	0	15	20	0	0	0	0
Virginia	0	1	63		0		5	
West Virginia	1	0	31	26	1	17	8	7
North Carolina	0	0	51	63	1	2	2	8
South Carolina	0	3	3	9	0	1	5	19
Georgia [3]	0	0	7	5	3	0	3	1
Florida [3]	0	0	7	5	0	0	18	23
East South Central States:								
Kentucky	0	0	64	82	0	8	6	6
Tennessee	1	0	41	18	2	17	8	10
Alabama [3]	1	0	13	18	14	5	2	3
Mississippi	0	0	3	18	0	8	10	0

See footnotes at end of table.

Cases of certain communicable diseases reported by telegraph by State health officers for weeks ended March 25, 1933, and March 26, 1932—Continued

Division and State	Poliomyelitis		Scarlet fever		Smallpox		Typhoid fever	
	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932	Week ended Mar. 25, 1933	Week ended Mar. 26, 1932
West South Central States:								
Arkansas	0	0	8	12	15	8	3	1
Louisiana	0	0	11	6	0	5	7	12
Oklahoma [4]	0	0	15	35	7	16	1	0
Texas [2]	1	0	37	36	8	32	12	4
Mountain States:								
Montana	0	1	10	37	0	0	5	1
Idaho	0	0	7	0	6	0	1	4
Wyoming	0	0	8	8	0	0	1	2
Colorado	0	0	11	35	0	1	1	1
New Mexico	1	0	17	9	0	0	1	0
Arizona	0	0	23	6	0	0	0	3
Utah [2]	0	0	6	6	0	0	0	1
Pacific States:								
Washington	0	0	61	34	8	29	0	2
Oregon	0	0	29	12	2	23	3	2
California [3]	3	0	176	135	48	23	5	8
Total	16	13	6,519	6,080	231	317	163	171

[1] New York City only.
[2] Week ended Friday.
[3] Typhus fever, week ended Mar. 25, 1933, 7 cases: 1 case in Georgia, 2 cases in Florida, 2 cases in Alabama, 1 case in Texas, and 1 case in California.
[4] Figures for 1933 are exclusive of Oklahoma City and Tulsa and for 1932 are exclusive of Tulsa only.

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of cases reported monthly by States is published weekly and covers only those States from which reports are received during the current week.

State	Meningococcus meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
January, 1933										
Hawaii Territory		9	142				1		0	8
Washington	2	44	206		14		1	142	53	15
February, 1933										
California	10	213	759	1	1,873		4	879	145	29
Georgia	4	44	2,111	203	116	17	0	47	0	17
Illinois	66	197	363	1	883	1	3	1,742	33	21
Iowa	6	45			20		0	175	160	2
Maryland	2	45	513		24	1	0	394	0	12
Minnesota	10	24	8		4,197		1	385	1	10
North Carolina	11	76	1,104		1,578	75	1	144	4	15
Ohio	3	168	499	1	2,453		0	2,580	29	16
Oregon		14	383		401		0	88	9	5
Rhode Island		13	63		4		0	149	0	0
South Carolina		134	7,671	438	318	139	1	14	0	4
South Dakota	2	16	42		51		0	67	7	3
West Virginia	3	61	843		1,696		4	124	0	21

January, 1933

	Cases
Chicken pox:	
Hawaii Territory	28
Washington	561
Conjunctivitis, epidemic:	
Hawaii Territory	5
Conjunctivitis, follicular:	
Hawaii Territory	24
Dysentery:	
Washington	1
German measles:	
Washington	9
Hookworm disease:	
Hawaii Territory	63
Impetigo contagiosa:	
Hawaii Territory	1
Washington	1
Leprosy:	
Hawaii Territory	6
Lethargic encephalitis:	
Washington	1
Mumps:	
Hawaii Territory	4
Washington	92
Plague:	
Hawaii Territory	1
Rabies in animals:	
Washington	1
Scabies:	
Washington	9
Septic sore throat:	
Washington	1
Tetanus:	
Hawaii Territory	2
Trachoma:	
Hawaii Territory	1
Undulant fever:	
Washington	2
Whooping cough:	
Hawaii Territory	28
Washington	51

February, 1933

	Cases
Actinomycosis:	
California	1
Illinois	1
Botulism:	
California	2
Chicken pox:	
California	2,687
Georgia	154
Illinois	1,938
Iowa	161
Maryland	485
Minnesota	452
North Carolina	547
Ohio	2,282
Oregon	199
Rhode Island	49
South Carolina	155
South Dakota	68
West Virginia	225
Conjunctivitis:	
Illinois	1
Dengue:	
South Carolina	3
Diarrhea:	
South Carolina	315
Diarrhea and enteritis:	
Ohio (under 2 years)	17
Dysentery:	
California (amebic)	5
California (bacillary)	14
Georgia	17
Illinois (amebic)	1
Maryland	3
Minnesota (amebic)	3
Ohio	1
Food poisoning:	
California	28
Ohio	16
German measles:	
California	36
Illinois	41
Iowa	5
Maryland	15
North Carolina	11
Ohio	34
Rhode Island	1
South Carolina	5
Granuloma, coccidioidal:	
California	3
Hookworm disease:	
South Carolina	124
Impetigo contagiosa:	
Iowa	2
Maryland	16
Oregon	43
Lead poisoning:	
Illinois	12
Ohio	9
Lethargic encephalitis:	
California	3
Georgia	1
Illinois	2
Iowa	2
Minnesota	1
Ohio	1
Oregon	1
South Carolina	6
Mumps:	
California	873
Georgia	105
Illinois	236
Iowa	190
Maryland	404
Ohio	227
Oregon	10
Rhode Island	16
South Carolina	35
South Dakota	22
West Virginia	12
Opthalmia neonatorum:	
Illinois	7
Minnesota	1
North Carolina	1
Ohio	86
South Carolina	15
South Dakota	1
Paratyphoid fever:	
California	3
Ohio	1
Puerperal septicemia:	
Illinois	6
South Dakota	1
Rabies in animals:	
California	40
Illinois	27
Maryland	2
South Carolina	22
Rabies in man:	
Illinois	1
Rat-bite fever:	
Maryland	1
Scabies:	
Maryland	6
Oregon	58
Septic sore throat:	
California	11
Georgia	23
Illinois	10
Maryland	5
North Carolina	9
Ohio	316
Oregon	8
Rhode Island	1
South Dakota	3
Tetanus:	
California	4
Illinois	1
Maryland	2
Ohio	3
South Carolina	1
Trachoma:	
California	15
Illinois	1
Ohio	3
Trichinosis:	
California	1
Illinois	1
Tularaemia:	
California	1
Georgia	4
Illinois	7
Maryland	1
North Carolina	8
Ohio	2
South Carolina	1
Typhus fever:	
California	1
Georgia	19
Illinois	1
Undulant fever:	
California	8
Georgia	1
Illinois	10
Iowa	6
Maryland	2
Minnesota	1
North Carolina	3
Ohio	2
Oregon	1
Rhode Island	1
South Dakota	1
Vincent's angina:	
Illinois	44
Iowa	2
Maryland	8
Oregon	7
Whooping cough:	
California	1,121
Georgia	159
Illinois	278
Iowa	42
Maryland	119
Minnesota	383
North Carolina	601
Ohio	502
Oregon	53
Rhode Island	54
South Carolina	124
South Dakota	4
West Virginia	118

WEEKLY REPORTS FROM CITIES

City reports for week ended March 18, 1933

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Maine:											
Portland	0	-------	1	2	3	2	0	0	0	13	28
New Hampshire:											
Concord	0	-------	0	0	1	0	0	0	0	0	11
Manchester	0	-------	0	0	2	8	0	0	0	0	7
Nashua	0	-------	0	0	0	0	0	0	0	0	-------
Vermont:											
Barre	0	-------	0	0	0	0	0	0	0	5	1
Burlington	1	-------	0	0	0	3	0	0	0	0	5
Massachusetts:											
Boston	3	1	0	70	27	99	0	9	0	71	223
Fall River	1	-------	0	0	5	9	0	1	0	9	41
Springfield	0	-------	0	0	2	10	0	0	0	31	30
Worcester	0	-------	0	3	5	33	0	2	0	5	0
Rhode Island:											
Pawtucket	0	-------	0	0	0	0	0	0	0	0	8
Providence	2	2	2	0	10	20	0	2	0	14	70
Connecticut:											
Bridgeport	0	3	0	18	0	11	0	2	0	3	35
Hartford	0	-------	0	9	8	10	0	2	0	3	60
New Haven	0	-------	0	0	4	8	0	0	0	16	51
New York:											
Buffalo	7	-------	7	12	28	74	0	11	0	23	175
New York	36	21	9	2,108	192	402	0	96	6	145	1,565
Rochester	0	-------	0	1	2	24	0	2	0	22	73
Syracuse	0	-------	0	1	2	47	0	0	0	10	46
New Jersey:											
Camden	0	-------	0	0	1	13	0	0	0	0	28
Newark	0	6	0	589	14	32	0	6	1	23	112
Trenton	4	-------	1	16	5	27	0	2	0	0	38
Pennsylvania:											
Philadelphia	5	8	4	97	61	142	0	39	2	5	542
Pittsburgh	10	4	3	4	16	76	0	9	1	18	153
Reading	2	-------	0	77	6	11	0	0	0	7	28
Ohio:											
Cincinnati	1	-------	4	1	16	36	0	7	0	1	128
Cleveland	10	78	2	1	15	206	0	15	0	40	217
Columbus	1	2	2	68	2	10	0	5	0	0	99
Toledo	2	2	1	208	3	84	0	5	0	5	57
Indiana:											
Fort Wayne	4	-------	0	0	2	3	0	0	0	0	15
Indianapolis	3	-------	1	110	12	32	0	3	0	17	-------
South Bend	0	-------	0	0	1	0	0	1	0	2	22
Terre Haute	1	-------	0	0	2	10	0	1	0	0	20
Illinois:											
Chicago	2	13	10	320	80	283	1	42	0	23	750
Springfield	0	1	0	2	3	4	0	0	0	0	23
Michigan:											
Detroit	16	6	1	654	24	233	0	16	1	101	255
Flint	1	5	0	82	7	4	0	3	0	7	39
Grand Rapids	0	-------	2	2	5	5	0	2	0	35	39
Wisconsin:											
Kenosha	0	-------	0	0	0	7	0	0	0	11	3
Madison	1	-------	-------	125	-------	7	0	-------	0	1	-------
Milwaukee	0	-------	0	4	4	43	0	6	0	52	94
Racine	1	-------	0	0	0	9	0	1	0	2	16
Superior	0	-------	0	0	1	0	0	0	0	19	18
Minnesota:											
Duluth	0	-------	0	5	0	1	0	2	0	44	20
Minneapolis	1	-------	1	567	8	31	0	0	0	17	107
St. Paul	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------	-------
Iowa:											
Des Moines	4	-------	-------	0	-------	5	0	-------	0	0	35
Sioux City	0	-------	-------	2	-------	4	0	-------	0	0	-------
Waterloo	1	-------	-------	0	-------	4	2	-------	0	0	-------
Missouri:											
Kansas City	2	-------	1	182	15	40	0	4	0	0	131
St. Joseph	0	-------	0	33	3	2	0	0	0	6	17
St. Louis	15	-------	-------	17	6	20	0	11	1	1	226
North Dakota:											
Fargo	0	-------	0	0	1	1	0	0	0	0	2
Grand Forks	0	-------	0	0	0	0	0	0	0	0	-------
South Dakota:											
Aberdeen	0	-------	0	0	0	2	0	0	0	0	-------

City reports for week ended March 18, 1933—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Nebraska:											
Omaha	6	-------	0	3	9	8	0	0	0	0	64
Kansas:											
Topeka	0	-------	1	131	3	0	0	1	0	0	17
Wichita	0	-------	0	0	3	1	0	1	1	3	36
Delaware:											
Wilmington	2	-------	0	3	12	5	0	0	0	2	38
Maryland:											
Baltimore	5	10	6	2	39	70	0	15	0	18	244
Cumberland	0	1	0	0	0	1	0	0	0	0	13
Frederick	0	-------	0	0	0	0	0	0	0	0	4
Dist. of Columbia:											
Washington	3	3	1	3	14	28	0	13	1	6	148
Virginia:											
Lynchburg	0	-------	0	1	0	2	0	0	0	1	16
Norfolk	0	-------	0	2	5	4	0	1	1	0	31
Richmond	0	-------	4	1	5	4	0	3	0	4	48
Roanoke	0	-------	0	160	0	3	0	0	0	0	14
West Virginia:											
Charleston	0	-------	0	0	0	3	0	1	1	1	4
Huntington	0	-------	0	9	0	1	0	0	0	0	-------
Wheeling	0	-------	0	16	4	1	0	1	0	3	25
North Carolina:											
Raleigh	0	-------	0	0	2	0	0	0	0	0	17
Wilmington	0	-------	0	157	0	1	0	1	0	0	15
Winston-Salem	0	2	1	4	3	7	0	1	0	5	22
South Carolina:											
Charleston	0	14	2	1	2	1	0	3	0	3	29
Columbia	0	-------	0	0	2	0	0	0	0	0	8
Georgia:											
Atlanta	0	52	2	2	6	2	0	3	0	17	83
Brunswick	0	-------	0	0	0	0	0	0	0	0	2
Savannah	1	168	1	0	2	0	0	0	0	0	20
Florida:											
Miami	1	2	0	0	2	0	0	0	1	35	24
Tampa	0	2	2	0	2	1	0	2	4	1	18
Kentucky:											
Ashland	0	-------	0	7	0	0	0	0	1	0	-------
Lexington	0	5	0	5	3	0	0	-------	0	0	15
Tennessee:											
Memphis	4	-------	3	8	8	7	0	3	1	10	74
Nashville	0	-------	1	0	1	1	0	2	1	0	41
Alabama:											
Birmingham	1	7	4	3	12	2	0	5	2	17	87
Mobile	0	-------	0	1	4	1	0	1	0	0	16
Montgomery	1	3	-------	0	-------	0	0	-------	0	0	-------
Arkansas:											
Fort Smith	0	-------	-------	1	-------	0	0	-------	0	0	-------
Little Rock	0	-------	1	3	5	0	0	1	0	0	8
Louisiana:											
New Orleans	6	3	3	3	14	7	0	9	2	4	148
Shreveport	0	-------	0	0	4	0	0	2	0	0	21
Oklahoma											
Tulsa	1	-------	-------	22	-------	2	1	-------	0	1	-------
Texas:											
Dallas	9	2	2	-------	5	0	0	6	1	0	58
Fort Worth	1	-------	0	330	9	4	0	2	0	0	40
Galveston	1	-------	0	3	4	0	0	1	1	0	12
Houston	11	-------	0	29	10	2	0	2	0	0	79
San Antonio	2	-------	4	24	7	2	0	7	0	0	77
Montana:											
Billings	0	-------	0	0	0	0	0	0	0	0	4
Great Falls	0	-------	0	1	0	0	0	1	0	0	6
Helena	1	-------	0	0	0	0	0	0	0	0	5
Missoula	0	-------	0	2	1	0	0	1	0	0	3
Idaho:											
Boise	0	-------	0	29	0	0	1	0	0	1	4
Colorado:											
Denver	2	43	3	4	13	24	0	1	0	2	70
Pueblo	0	-------	1	0	0	4	0	1	0	3	9
New Mexico:											
Albuquerque	0	-------	0	0	2	0	0	3	0	2	9
Arizona:											
Phoenix	0	-------	0	4	0	4	3	3	0	0	-------

City reports for week ended March 18, 1933—Continued

State and city	Diphtheria cases	Influenza		Measles cases	Pneumonia deaths	Scarlet fever cases	Smallpox cases	Tuberculosis deaths	Typhoid fever cases	Whooping cough cases	Deaths, all causes
		Cases	Deaths								
Utah:											
Salt Lake City	0	-------	0	1	1	7	0	1	0	6	31
Nevada:											
Reno	0	-------	0	0	1	0	0	0	0	0	2
Washington:											
Seattle	0	-------	-------	3	-------	7	0	-------	0	1	-------
Spokane	0	-------	-------	0	-------	1	0	-------	0	0	-------
Tacoma	0	-------	1	0	3	1	0	-------	0	0	24
Oregon:											
Portland	0	-------	1	3	4	4	2	2	0	3	68
Salem	1	2	-------	22	-------	0	0	-------	0	0	-------
California:											
Los Angeles	31	26	0	508	14	55	13	20	3	26	277
Sacramento	0	3	0	1	5	0	0	5	0	18	32
San Francisco	2	4	0	6	6	8	0	9	2	78	134

State and city	Meningococcus meningitis		Poliomyelitis cases	State and city	Meningococcus meningitis		Poliomyelitis cases
	Cases	Deaths			Cases	Deaths	
New York:				West Virginia:			
New York	1	1	1	Wheeling	0	0	1
Pennsylvania:				Tennessee:			
Philadelphia	2	1	1	Memphis	1	0	0
Pittsburgh	2	1	1				
Indiana:				Louisiana:			
Indianapolis	7	4	0	New Orleans	5	0	0
Illinois:				Colorado:			
Chicago	20	4	0	Denver	0	1	0
Springfield	0	1	0				
Michigan:				California:			
Detroit	2	0	0	Los Angeles	0	1	0
Grand Rapids	1	0	0				
Missouri:							
Kansas City	3	0	0				
St. Joseph	7	0	0				
St. Louis	2	0	0				

Lethargic encephalitis.—Cases: New York, 3.
Pellagra.—Cases: Winston-Salem, 1; Savannah, 3; Miami, 2; Dallas, 2; Los Angeles, 1.
Typhus fever.—Cases: Charleston, S. C., 1; Tampa, 2; Houston, 1.

FOREIGN AND INSULAR

CANADA

Quebec Province—Communicable diseases—Four weeks ended February 25, 1933.—The Bureau of Health of the Province of Quebec, Canada, reports cases of certain communicable diseases for the four weeks ended February 25, 1933, as follows:

Disease	Weeks ended		Two weeks ended Feb. 25
	Feb. 4	Feb. 11	
Cerebrospinal meningitis	1		
Chicken pox	169	132	354
Diphtheria	22	33	39
Erysipelas	8	5	11
German measles	4	4	8
Influenza	12	2	6
Measles	100	90	303
Poliomyelitis	1	2	5
Puerperal septicemia	1		3
Scarlet fever	99	65	191
Tuberculosis	96	90	159
Typhoid fever	24	24	20
Whooping cough	139	188	294

CUBA

Provinces—Communicable diseases—Four weeks ended February 4, 1933.—During the four weeks ended February 4, 1933, cases of certain communicable diseases were reported in the Provinces of Cuba as follows:

Disease	Pinar del Rio	Habana	Matanzas	Santa Clara	Camaguey	Oriente	Total
Chicken pox	2	2				2	6
Diphtheria	1	20			2		23
Leprosy		2		3			5
Malaria	2	20	67	158	68	439	754
Measles	1	3	6	64	1	13	88
Rabies		1					1
Scarlet fever	1						1
Tetanus, infantile			1			1	2
Tuberculosis	2	22	5	5	4	5	43
Typhoid fever		11	1	11	1	5	29

GREAT BRITAIN

England and Wales—Vital statistics—October–December, 1932.—During the fourth quarter of the year 1932, 140,350 births and 116,458 deaths were registered in England and Wales. The following statistics are taken from the Quarterly Return of Births, Deaths, and Marriages, issued by the Registrar-General of England and Wales. The figures are provisional.

Birth and death rates in England and Wales, October to December, 1932

Annual rates per 1,000 population:	
Live births	13.9
Stillbirths	.61
Deaths, all causes	11.5
Deaths from—	
Diphtheria	.06
Influenza	.18
Measles	.03
Annual rates per 1,000 population—Cont'd.	
Deaths from—Continued	
Scarlet fever	0.01
Typhoid and paratyphoid fever	.01
Violence	.53
Whooping cough	.04
Deaths per 1,000 live births:	
Diarrhea and enteritis (under 2 years)	7.5
Total deaths under 1 year	64.0

England and Wales—Infectious diseases—Thirteen weeks ended December 31, 1932.—During the 13 weeks ended December 31, 1932, cases of certain infectious diseases were reported in England and Wales as follows:

Disease	Cases	Disease	Cases
Diphtheria	12,807	Puerperal pyrexia	1,298
Ophthalmia neonatorum	1,004	Scarlet fever	28,496
Pneumonia	14,379	Smallpox	271
Puerperal fever	481	Typhoid fever	906

PUERTO RICO

Mortality from communicable diseases—Years 1931 and 1932.—The following table shows the number of deaths and death rates per 100,000 population from communicable diseases in Puerto Rico during the years 1931 and 1932.

Cause of death	1931		1932	
	Number of deaths	Death rate per 100,000 population	Number of deaths	Death rate per 100,000 population
All transmissible causes	9,293	590.2	9,580	599.1
Diphtheria	61	3.9	47	2.9
Dysentery	117	7.4	138	8.6
Influenza	246	15.6	449	28.1
Malaria	3,208	203.7	2,797	174.9
Measles	6	.4	51	3.2
Syphilis	341	21.6	413	25.8
Tetanus	426	27.1	447	28.0
Tuberculosis	4,338	275.5	4,753	297.3
Typhoid and paratyphoid fever	104	6.6	82	5.1
Whooping cough	181	11.5	131	8.2
All other transmissible causes	265	16.8	272	17.0

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER

(NOTE.—A table giving current information of the world prevalence of quarantinable diseases appeared in the PUBLIC HEALTH REPORTS for March 31, 1933, pp. 334-345. A similar cumulative table will appear in the PUBLIC HEALTH REPORTS to be issued April 28, 1933, and thereafter, at least for the time being, in the issue published on the last Friday of each month.)

Cholera

Philippine Islands.—During the week ended March 25, 1933, one fatal case of cholera was reported at Ormoc, Leyte Province, Philippine Islands.

Plague

Java—Batavia.—During the week ended March 18, 1933, an imported case of plague was reported at Batavia, Java.

Yellow Fever

Senegal.—On March 17, 1933, a fatal case of yellow fever was reported at Dagana, Senegal, and on March 20 a case was reported at Podor, Senegal.

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