CXIII. THE ACTION OF CARBON MONOXIDE ON THE AUTOXIDATION OF SULPHYDRYL COMPOUNDS.

BY MALCOLM DIXON.

From the Biochemical Laboratory, Cambridge.

(Received June 28th, 1928.)

THE work of Warburg [1928] and others has shown that the greater part of the oxygen uptake by living cells is due primarily to catalytic systems which are poisoned by small amounts of cyanide. According to Warburg's view of cell respiration the oxygen must react in the first place with some iron-containing cell catalyst which activates it, and he believes that the effect of cyanide is due to the formation of an inactive addition compound with this iron. It is well known that carbon monoxide also combines with many iron compounds, and Warburg [1927] has recently shown that cell respiration is also inhibited by carbon monoxide, and he has brought forward good evidence to show that its action is due, like that of the cyanide, to the inactivation of the catalytic iron compounds in the cell.

Warburg supposed that the cell respiration as a whole was poisoned by carbon monoxide, but his experimental data do not exclude the possibility that a part of the oxygen absorbed may be utilised by systems on which CO has no action. Such systems are in fact known to be present in tissues [Dixon, 1927].

Now it has been shown by Hopkins [1921] that part of the oxygen absorption by the tissues is due to the autoxidation of the —SH group of the glutathione present. This autoxidation is dependent on the presence of iron (or similar catalytic metals), and is prevented by the presence of small amounts of cyanide [Warburg and Sakuma, 1923; Harrison, 1924]; and it resembles in this respect the main part of the cell respiration.

In view of these facts it was thought to be desirable that a study of the effect of CO upon this autoxidation should be made, especially as in this system we have a case of a biologically important iron-catalysed oxidation which can be studied *in vitro* apart from other cell constituents. Moreover, by investigating the effect of carbon monoxide on the catalytic action of iron added to the system in various forms, one may expect to obtain information of value in interpreting results got with living tissues.

In the present paper experiments are given on the effect of CO on the catalysis by free iron and copper salts and by haematin of the autoxidation of cysteine and reduced glutathione.

The experiments were carried out by means of the Barcroft apparatus, using the technique already described [Dixon, 1927] for filling the apparatus with the gas mixtures. As light is known to diminish the inhibiting action of CO, the water-bath was equipped with a framework so arranged that a black cloth could be drawn over the whole in such a way as to exclude all light without interfering with the shaking or the reading of the manometers. Unless otherwise stated both flasks of each apparatus contained the same solutions, except that the sulphhydryl compound was present in the right-hand flasks only. The preparations of cysteine and reduced glutathione used, though pure specimens, were not free from traces of catalytic metals; but it was not necessary to use iron-free preparations in this work, since the procedure adopted in all the experiments was to compare the rates of oxygen uptake of identical

Fig. 1. Effect of CO on rate of autoxidation of cysteine. Curves A and B show the oxygen uptake of 20 mg. cysteine, curves C and D that of 10 mg. cysteine. In A and C the composition of the gas mixture was O₂ 5 %, CO 74 %, N₂ 21 %; in B and D O₂ 5 %, N₂ 95 %. Temperature 16°.

solutions in the presence and absence of carbon monoxide. The preparations also contained a small amount of their respective —SS— forms, owing to slight autoxidation having already taken place, and the total oxygen uptake was therefore rather below the theoretical; but this is of course quite immaterial. The experiments were carried out in all cases in phosphate buffer at p_H 7.3, and the solutions of cysteine and glutathione were carefully adjusted to this p_H before use. The volume of liquid in each flask was always made up to 3 cc. with buffer solution.

Fig. 1 shows the autoxidation of cysteine in the presence and absence of CO, with two different concentrations of cysteine. No catalyst was added, and the autoxidation is due to the traces of catalytic metals already present in the preparation. It will be seen that the carbon monoxide produced no inhibition. Some similar results for glutathione are given in Fig. 4 (curves C and D).

Fig. 2 shows the effect of CO on the reaction when catalysed by iron salts. The complete curves are given, but the essential thing is of course the velocity during the linear part of the oxygen uptake. The concentration of the cysteine

Fig. 2. *Effect of CO on catalysis of cysteine autoxidation by inorganic iron.* The solutions contained 20 mg. cysteine + 0.04 mg. Fe (added as FeSO₄). Apparatus A contained O₂ 5 %, CO 74 %, N₂ 21 %; apparatus B contained O₂ 5 %, N₂ 95 %. Temperature 17°.

Fig. 3. *Effect of CO on catalysis of cysteine autoxidation by copper.* The solutions contained 8 mg. cysteine + a very small trace of copper sulphate. Gas mixtures as in Fig. 2. The curves in the presence and absence of CO coincided throughout. The experiment was carried out in duplicate (in different Barcroft apparatus), and the figure represents four completely coincident curves: two with, and two without CO. Temperature 18°.

was the same as in curves A and B of Fig. 1, but the addition of a small trace of ferrous sulphate had produced a threefold increase in the velocity of the oxidation. The curves show that although 70 % of the oxygen uptake was

due to catalysis by the iron added, the carbon monoxide still produced no inhibition.

This result is interesting in view of the fact that Cremer [1928], in a paper which appeared while these experiments were being carried out, has shown that under such conditions the cysteine-iron complex combines at once with carbon monoxide to form an addition compound containing two molecules of CO for every atom of iron. These facts taken together show that the formation of a CO addition compound does not necessarily inactivate iron-containing catalysts, since, while Cremer's experiments make it certain that the iron is entirely or almost entirely converted into the addition compound, the curves show that there is no change in its catalytic activity.

The slight difference between the two curves, though not much greater than the experimental error, is probably due to the absorption of a small amount of CO in addition to the oxygen, since it corresponds roughly with the amount of CO which would be taken up to combine with the amount of iron present in forming Cremer's addition compound.

In Fig. 3 is shown a similar experiment in which copper was added instead of iron. Copper is a much more efficient catalyst than iron. Here only 0.1 cc. of an extremely dilute solution of copper sulphate was added, and it was found that under the conditions of this experiment the velocity of oxidation was thereby approximately doubled. At least half the oxygen uptake shown in Fig. 3 is therefore due to the catalytic action of copper, and the curves show that again carbon monoxide has no effect.

It was shown by Harrison [1924] that not only inorganic iron but also iron in the form of haematin could catalyse the autoxidation of sulphydryl compounds, although the catalytic power of this form of iron was only about half that of iron salts. In order to investigate the effect of carbon monoxide upon this catalysis, solutions of haematin were prepared as follows. Pure crystalline haemin¹ was washed first with pure concentrated hydrochloric acid to remove any traces of free iron and then with water. Solutions containing 1 mg. per cc. were then made up by the careful addition of the minimum amount of dilute NaOH necessary to dissolve the haemin. It was found that the addition of these solutions in the amounts used in the experiments produced no detectable change in the p_H of the buffer solution used.

Some results showing the effect of CO on the catalysis by freshly dissolved haematin are given in Figs. 4 and 5. Fig. 4 shows experiments on glutathione, and Fig. 5, in curves *A* and *B*, gives results on cysteine. These curves are representative of a number of quite similar experiments.

In Fig. 4 the lower pair of curves gives the oxygen uptake by the glutathione alone in the presence and absence of CO; the upper pair gives the uptake in the presence of 0.5 mg. haematin, again in the presence and absence of CO. The difference between the upper and lower pairs of curves therefore represents

¹ I am indebted to Dr Keilin and also to Mr R. Hill for the specimens of haemin used in these experiments.

the oxygen uptake due to the catalytic action of the haematin. In this experiment the haematin was added to both flasks of each apparatus, and the sulphhydryl compound to the right-hand flasks only. In the experiments given

Fig. 4. *Effect of CO on autoxidation of glutathione, alone and after the addition of haematin.* Curves *C* and *D* give the oxygen uptake of 10 mg. glutathione; curves *A* and *B* of 10 mg. glutathione + 0.5 mg. haematin. *A* and *C* in the presence of CO, *B* and *D* in its absence. Gas mixtures as before. Temperature 18°. This experiment was also done in duplicate, and the duplicate curves all coincided completely, so that eight curves are represented in the figure.

Fig. 5. *Effect of CO on catalysis of cysteine autoxidation by freshly dissolved haematin and by haematin previously allowed to stand in solution.* The curves give the oxygen uptake of 10 mg. cysteine + 0.5 mg. haematin, compensated for the spontaneous uptake of the cysteine alone. In curves *A* and *B* the haematin was freshly dissolved, in curves *C* and *D* the solution was several days old. *A* and *C* were in presence, and *B* and *D* in absence, of CO. Temperature 14°. In another experiment curve *D* lay between curves *A* and *B*.

in Fig. 5, however, the method was slightly modified. Here the haematin was added to the right-hand flasks only, and the cysteine was added to both flasks. By this means the oxygen uptake due to the catalytic metals already present in the cysteine was balanced out, and the reading of the manometers indicated

directly the oxygen uptake due to the haematin catalysis. This does not apply of course to the later phases of the experiment, when the rate of oxygen absorption in the right-hand flasks begins to fall off, but since we are only concerned with the rates of autoxidation during the first (linear) part of the curves this is no disadvantage. The figures may be taken as representing the actual oxygen uptake due to the haematin catalysis throughout the period covered by the curves given here.

These experiments make it quite clear that carbon monoxide produces no inhibition of the oxidation induced by haematin. They therefore stand in direct opposition to the recent results of Krebs [1928]¹, who, using similar methods, found that CO produced a 70 % inhibition of the catalysis of the autoxidation of cysteine by haematin.

The discrepancy between my results and those of Krebs seems to be explained by the following observations. It is known that haematin in solution is unstable and breaks down fairly rapidly, giving a mixture of various less complex iron compounds. On allowing a solution of haematin to stand for several days it is usually found that a large part of the haematin has broken down. Such a solution is found to have precisely the same power to catalyse the oxygen uptake of cysteine as it had when freshly made up. That is to say, the mixture of iron compounds formed by the decomposition has exactly the same catalytic power as the original haematin. The behaviour towards CO is however very different. Whereas CO has no effect on the oxygen uptake induced by a freshly made up solution of haematin, it markedly inhibits that induced by the same solution after it has been allowed to stand for a few days. This is shown in Fig. 5, which is again representative of a number of experiments. Curves *A* and *B*, as already mentioned, represent the oxygen uptake due to freshly dissolved haematin in the presence and absence of CO. Curves *C* and *D* show the uptake produced by the same quantity of haematin which had been allowed to stand in solution for one week. Both haematin solutions were made up from the same sample of haemin, and it may be mentioned that the solution used for curves *C* and *D* was the identical solution which had been used, when freshly made up, for the experiment given in Fig. 4. In that experiment CO had no effect upon its catalytic action, whereas here a 50 % inhibition is produced.

It seems possible that Krebs may have used a stock solution of haematin, or haemin which had become partially decomposed, and that this may have been responsible for the inhibition he observed. At any rate, whether this is correct or not, I have never been able to detect any inhibition by CO when the haematin has been freshly made up, and have always found a marked inhibition after it has been allowed to stand for a few days before use; and this effect would seem to provide an adequate explanation of the discrepancy.

The catalytic action of the haematin decomposition products, like that of haematin itself, seems to be practically insensitive to the action of cyanide,

¹ This paper had not appeared at the time when these experiments were first carried out.

at any rate up to a concentration of $M/300$ [see also Harrison, 1924]. It is clear then that cyanide and carbon monoxide are by no means always equivalent in their action as inhibitors of oxidation-catalysis by various forms of iron, since in the —SH system cyanide inhibits iron salts but not the haematin products, while carbon monoxide inhibits the haematin products but not iron salts. Other differences have been pointed out [Dixon, 1927]. These facts must be borne in mind when considering the interpretation of Warburg's results and the chemical nature of his iron-containing "Atmungsferment."

SUMMARY.

1. Carbon monoxide has no effect on the rate of the autoxidation of cysteine or reduced glutathione induced by the addition of iron or copper salts or by freshly dissolved haematin.
2. When a solution of haematin is allowed to stand for several days the haematin partly breaks down into a mixture of simpler iron compounds. This mixture has the same catalytic power as the original haematin, but the catalysis is now markedly inhibited by carbon monoxide.

I should like to express my sincere thanks to Sir F. G. Hopkins for his interest in this work and to Dr Keilin for his kindness in allowing me to use his apparatus and for supplying me with pure carbon monoxide.

REFERENCES.

- Cremer (1928). *Biochem. Z.* **194**, 221.
Dixon (1927). *Biochem. J.* **21**, 1211.
Harrison (1924). *Biochem. J.* **18**, 1009.
Hopkins (1921). *Biochem. J.* **15**, 286.
Krebs (1928). *Biochem. Z.* **193**, 347.
Warburg (1927). *Biochem. Z.* **189**, 354.
—— (1928). Über die katalytischen Wirkungen der lebendigen Substanz (Berlin).
Warburg and Sakuma (1923). *Pflüger's Arch.* **200**, 203.