CLXI. THE ACTION OF CARBON MONOXIDE ON CERTAIN OXIDISING ENZYMES.

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WARBURG [1926] has shown that the respiration of yeast is inhibited by carbon monoxide. Since this inhibition is prevented by light, and the combination of carbon monoxide with many complex iron compounds is known to be suppressed by light, he believes that the inhibition is due to the combination of the CO with the iron-containing "oxygen-transporting component of the respiratory ferment," which results in its inactivation. The action of carbon monoxide is thus analogous to that of cyanides.

Haldane [1927] has shown that carbon monoxide has a similar inhibitory effect on the activity of moths and cress plants, and has made it clear that this is very probably due to the poisoning of an oxidation-catalyst present in the cells.

Keilin [1927] has studied the action of CO on certain oxidising enzymes. He finds that the indophenol oxidases of both yeast and mammalian muscle are strongly inhibited by carbon monoxide and by cyanide, as is also the oxidation of catechol by the oxygenase of the potato.

In general then it would seem that carbon monoxide and cyanide act in a similar way; although the action of cyanide is usually the more marked, and fairly large amounts of carbon monoxide are necessary to produce a large inhibition (*e.g.* Warburg obtained a 60 % inhibition with 600 mm. pressure of CO).

The enzymes of the aldehyde-oxidase type (the dehydrases) have not so far been tested with carbon monoxide; and since the aerobic oxidation by certain of these systems is inhibited by cyanide, while that of others is completely unaffected, it will be interesting to determine whether carbon monoxide will show a similar distinction in behaviour towards these systems.

As convenient examples of these two classes of enzymes the aldehyde (and xanthine) oxidase of milk and the succinoxidase of muscle were chosen for investigation, since these enzymes can readily be obtained in solution free from other interfering enzymes by the methods of Dixon and Kodama [1926] and Ohlsson [1921] respectively. The oxygen uptake of the milk oxidase system has been shown by Dixon and Thurlow [1925] and by Dixon [1927] to be unaffected by cyanide; while Fleisch [1924] found that $M/1000$ cyanide

was sufficient to prevent almost completely the uptake of oxygen by the succinoxidase system, leaving its power to reduce methylene blue unimpaired.

The experiments were carried out by means of the Barcroft differential apparatus at room temperature. The carbon monoxide was prepared from formate and sulphuric acid in the usual way, and was purified by passing it through strong NaOH, then through sulphuric acid, and finally through two long U-tubes filled with adsorbent charcoal. The Barcroft apparatus was filled with the appropriate gas mixture in the following manner. After the necessary solutions had been introduced into the apparatus it was placed in position in the thermostat with the taps open, and the open ends of the tubes were connected by means of rubber pressure-tubing and a glass T-piece with a tube communicating with a graduated mercury pressure gauge. This tube was also in communication, through taps, with a vacuum pump and with reservoirs of carbon monoxide and nitrogen. The apparatus could thus be exhausted until the pressure of air remaining, as shown on the gauge, corresponded with the desired pressure of oxygen. The tap connected with the vacuum pump was then turned off, and CO or nitrogen admitted into the apparatus. The final pressure was slightly above that of the atmosphere, as the gases were stored under slight pressure, and after shutting off the supply of gas the excess pressure in the apparatus was relieved by momentarily opening a fourth tap communicating with the atmosphere. (The outlet of this tap was kept just dipping into water to act as a trap.) The taps of the Barcroft apparatus were then immediately closed, the rubber tubes detached, and after shaking for five minutes in the water-bath readings were commenced. During the whole of the filling operation, which was usually complete in under one minute, the apparatus was gently shaken in the water-bath. The connecting tubes were of fairly narrow bore, so that their volume was small in comparison with that of the Barcroft apparatus; hence dead space effects were negligible, and the actual final pressures of the various gases inside the apparatus were equal (within 1 mm.) to those given by the gauge readings. Strong NaOH solution was placed in the small receptacles provided in the apparatus to absorb any traces of CO₂ which might be given off by the solutions. The same solutions were always added to both flasks of each apparatus, except that the substance to be oxidised (hypoxanthine or succinate) was added to the right-hand flask only. In the case of aldehyde, however, it was necessary to add this to both flasks, on account of its vapour pressure, so that in this case the enzyme solution was added to the right-hand flask only. The volume of liquid in each flask was in all cases made up to 3 cc. with buffer solution.

In Figs. 1 and 2 are given graphically the results of experiments on the aerobic oxidation of aldehyde and hypoxanthine by the milk oxidase. The curves show that carbon monoxide has no inhibitory effect in either case. Several experiments were carried out both with milk and with the oxidase preparation of Dixon and Kodama [1926] and the curves given are quite typical of the results obtained.

Fig. 1. Effect of CO on the Schardinger enzyme. Each apparatus contained 1 cc. fresh milk + 1 cc. phosphate buffer (p_H 7.5) + 1 cc. 0.5 % acetaldehyde. The composition of the gas in apparatus A was: O₂ 5.3 %; N₂ 94.7 %; and in apparatus B: O₂ 5.3 %; N₂ 21 %; CO 73.7 %. Temperature 16°.

Fig. 2. Effect of CO on xanthine oxidase. Each apparatus contained 1 cc. oxidase solution + 1 cc. phosphate buffer (p_H 7.6) + 1 cc. (=1 mg.) hypoxanthine solution. The compositions of the gas mixtures were: in A, O₂ 5.2 %, N₂ 94.8 %; in B, O₂ 5.2 %, N₂ 20.9 %, CO 73.9 %. Temperature 17°.

This negative result was to be expected, in view of the fact that cyanide has no action on these systems.

On the other hand, the fact that a small concentration of cyanide inhibits the aerobic (but not the anaerobic) oxidation of succinate by the succinoxidase would naturally be explained, on Warburg's view, by assuming the inactivation of the hypothetical oxygen-transporting iron complex present in the system; we might therefore expect that this system would show inhibition by carbon monoxide. The curves given in Fig. 3 show quite definitely however that this is not the case, the CO producing no inhibition whatever.

The enzyme solution used in this experiment was prepared from very fresh ox muscle by mincing the muscle, washing very thoroughly with distilled water, extracting with $M/15$ alkaline phosphate solution, and centrifuging

the extract, in accordance with Ohlsson's [1921] method. The experiment was repeated a number of times with different enzyme preparations, and similar results were obtained. The very slight difference between curves *A* and *B* is clearly due to a small initial temperature difference; after the first few minutes the two velocities are identical throughout. Curve *C* shows for comparison the very striking inhibition produced by the addition of $M/1000$ cyanide.

Fig. 3. Effect of CO on succinoxidase. Each apparatus contained 2 cc. succinoxidase solution (in phosphate p_H 8) + 1 cc. sodium succinate solution (=2 mg. succinic acid). The compositions of the gas mixtures were: in *A*, O₂ 2.6 %, N₂ 97.4 %; in *B*, O₂ 2.6 %, N₂ 10.5 %, CO 86.9 %; in *C*, O₂ 21 %, N₂ 79 %. Curve *C* shows the effect of adding $M/1000$ KCN. Temperature 11°.

It is clear from these experiments that in the succinoxidase system the action of the cyanide is upon some substance which is not inactivated by CO.

These results make it clear that the inhibiting effect of carbon monoxide on the utilisation of oxygen by biological oxidation systems is (like that of cyanide) by no means universal, as Warburg seems to imply, since at least three typical biological oxidation processes are unaffected by it; and, secondly, they show that cyanide and carbon monoxide do not necessarily always inhibit the uptake of oxygen by acting in the same way or on the same substance. If it is assumed that cyanide inhibits the oxidation of succinic acid by inactivating an oxygen-transporting substance, and that carbon monoxide inhibits tissue respiration by acting in the same manner, it follows from these results that different oxygen-transporters must be concerned in the two cases.

SUMMARY.

Carbon monoxide has no inhibitory effect on the aerobic oxidation of aldehyde or hypoxanthine by the milk oxidase, or of succinic acid by the succinoxidase of muscle. Its action thus differs from that of cyanide, which strongly inhibits the latter reaction.

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