CXLII. CARBON MONOXIDE AS A TISSUE POISON.

By JOHN BURDON SANDERSON HALDANE.

From the Biochemical Laboratory, Cambridge.

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CARBON monoxide is relatively harmless to invertebrates, micro-organisms and plants. Thus Frankland [1888], Linossier [1889] and others, who found that very large amounts inhibited plant and bacterial growth, did not show conclusively that CO is any more poisonous than N₂. J. S. Haldane [1895] showed that cockroaches would live for long periods in a mixture of 80 % CO and 20 % O₂. Warburg [1926] however found that when the proportion of CO to O₂ in a gas mixture was raised to about 5, the O₂ consumption of yeasts and a coccus was diminished. He also showed that strong light neutralised the effect of CO. He concluded that CO combined with a catalyst in the cells, with which O₂ must combine before it can oxidise other substances, and that this catalyst was probably an iron compound analogous to haemoglobin. As the amount of CO needed to cause a given drop in the O₂ consumption is roughly proportional to the partial pressure of O₂, the analogy with haemoglobin can be carried further.

In the experiments here described Warburg's observation is extended to moths, seeds, and rats. Owing to the difficulty of obtaining a standard of respiration the general behaviour of the organism and not its O₂ consumption was observed. Wax moths (*Galleria mellonella*) from a culture kindly put at my disposal by Dr Keilin, were placed in a glass tube through which mixtures containing various amounts of O₂ and CO were passed. The tube containing the moths was kept in a water-bath at 20°, and the gas passed through sampling tubes before and after reaching the animals. Their contents were analysed after each experiment. If the quantities of O₂ differed by as much as 0.2 % the experiment was rejected. The CO content of one or both tubes was also determined by combustion.

In low percentages of O₂ the moths rapidly became sluggish in their movements, fell to the bottom of the tube, and after two or three minutes became immobile even when it was tapped. On readmitting air, even after an hour or more, they recovered completely. In larger but still inadequate amounts of O₂ various degrees of weakness were observed, ranging from inability to fly or climb to immobility save when stimulated. The symptoms were qualitatively the same whether they were due to O₂ want or to a combination of this with CO poisoning. There was little difference of susceptibility between

individuals, but in order to allow for this three moths were always used, and the behaviour of the majority recorded. Such differences occurred in twelve out of the forty-nine experiments on which Fig. 1 is based. Although various degrees of ataxia could be distinguished, behaviour is classified according as the moths were normal, ataxic, or wholly immobile even on stimulation. Six points recording experiments in the absence of CO have been omitted, as have the results of several experiments done under less rigid conditions which were, however, confirmatory.

Fig. 1.

+ Moths normal. o Moths ataxic. x Moths immobile.

In the absence of CO the moths were motionless in mixtures containing less than 1.0 % O_2 , normal in over 2.0 %. In presence of 80 % CO about 8.4 % O_2 is needed for mobility and 14 % for normal behaviour, while in intermediate concentrations of CO intermediate amounts of O_2 are required. The lines separating the three areas in the diagram are straight when the CO exceeds 30 % but bend downwards quite definitely at low percentages. The experiments were done in diffuse daylight or electric light, but no definite effect was produced by the proximity of a powerful lamp, probably because the moths are opaque.

The germination of cress (*Lepidium sativum*) seeds in various gas mixtures was next studied. The seeds were placed on wet sand in each of a series of bottles containing known gas mixtures, and their appearance noted daily. Since a preliminary series, which on the whole agreed with later results, showed evidence of leakage in one bottle, the bottles were immersed in a water-bath at room temperature in partial darkness. Two series of twelve bottles each made up in February and March gave quite consistent results. Thus in

the second series in the absence of CO the majority of seeds had split in three days in 8.9 % O_2 , in three and a half in 6.9 %, in four in 4.7 %, in eight in 3.0 %, while none split in sixteen days in 2.1 % or 1.4 % though all germinated on admitting air. In the presence of 70 % to 87 % of CO all had split in two and a half days in 19.2 % O_2 , in three in 15.3 %, in five in 10.7 %, but after sixteen days only a few had split in 6.2 % and none in 4.1 % or 2.6 %. All germinated on admitting air. By comparing the amounts of germination and growth it was possible to equate the effects of different gas mixtures. It was found that for equally rapid germination and subsequent growth between two and three times as much O_2 was needed in the presence of about 80 % CO as in its absence. No qualitative difference was noted between the effects of CO and of simple O_2 want. When the experiment was repeated in the summer on a smaller scale no change was noted in the susceptibility to O_2 want, but that to CO was somewhat increased, not all the seeds having split after five days in 83 % CO + 11.8 % O_2 . This may have been due to the higher temperature or to ageing of the seeds.

The experiments on rats were, of course, complicated by the presence of haemoglobin. Advantage was therefore taken of the fact that mammals can live on O_2 dissolved in their blood under high pressure even when almost all their haemoglobin is combined with CO [Haldane, J. S. 1895]. A small iron chamber was fitted with rubber washers so that its door could be made air-tight, a window for observation, and three taps, two of which were connected with cylinders containing O_2 and CO under pressure; the third could be opened to the outside. When a rat was placed in three atmospheres of O_2 and one of CO its behaviour was almost normal, but in some cases the movements of the hind limbs were rather clumsy. In two atmospheres of O_2 and one or half an atmosphere of CO movement was difficult, but there was little increase of breathing and no convulsions.

There are no exact data as to the affinities of rat's haemoglobin for CO and O_2 at body temperature. At 15° the ratio is about 280 : 1, that for a human haemoglobin being 400 : 1 [Anson, Barcroft, Mirsky and Oinuma, 1924], whereas at 37° the human value is about 250 : 1. If then the temperature coefficient of the equilibrium $HbO_2 + CO \rightleftharpoons HbCO + O_2$ is the same in rats and men, the rat's ratio at body temperature is about 175 : 1. Hence at three atmospheres pressure of O_2 and one of CO the rat's haemoglobin was about 98.3 % saturated with CO, the remaining 1.7 % being a very inefficient carrier of O_2 [Haldane, J. S., 1912]. Since a single atmosphere of CO is sufficient to nullify practically all the haemoglobin, the effects of any further addition of that gas can only be on the tissues, since, as will be seen below, the addition of a physiologically inert gas such as N_2 is without effect.

The following experiments, all on the same rat H, are typical.

July 9th

3.50	Pressure in chamber raised to total of 3.1 atmospheres of O_2
3.52	1 atmosphere CO added. Rat at first slightly excited, perhaps by the smell.
4.22	Rat normal. Excess of pressure over atmospheric has fallen from 48 lb. per sq. in. to 43. Lower pressure to 15 lb. Raise to 45 lb. with O_2 .

July 9th	
4.25-4.38	Pressure repeatedly lowered and then raised with O ₂ until the partial pressure of CO was reduced to 0.13 %. The rat was then taken out, and was unaffected.
July 11th	
3.2	Pressure raised to 3.1 atmospheres O ₂ .
3.4	1 atmosphere CO added.
3.6	2 atmospheres N ₂ added.
3.30	Pressure fallen from 77 to 64 lb. Rat normal. Pressure lowered and CO diluted as above.
4.0	Rat removed from chamber, quite normal.
July 13th	
4.55	Pressure raised to 3.1 atmospheres O ₂ .
4.57	Add 1 atmosphere CO. Rat normal.
5.0	Add second atmosphere CO.
5.1	Deep and rapid breathing.
5.2	Breathing rapid. Rat goes into violent convulsions.

Although the O₂ pressure was subsequently raised to 6 atmospheres the rat's breathing became shallow and it died.

Altogether eight experiments of this type were made. In all cases the addition of sufficient CO caused hyperpnoea and convulsions. If O₂ were at once added and the pressure quickly lowered so as to dilute the CO it was generally possible to save the animal's life. The rats used showed very different susceptibilities to high pressures of CO, all from the same cylinder. Thus while rat H died on exposure to 3 atmospheres of O₂ and 2 of CO, and rat F in 2 of O₂ and 2 of CO, rat B appeared practically normal after four minutes in this mixture, though it died on raising the CO pressure to 3 atmospheres. Finally rat D appeared little affected by four minutes' exposure to 2 atmospheres of O₂ and 3 of CO, though killed by an additional atmosphere of CO. These differences may of course be due to different relative affinities of the catalyst for CO and O₂, but it seems equally plausible to trace them to differences in the cerebral vaso-constriction caused by O₂ [Dautrebande and Haldane, J. S., 1921; Tinel, 1927]. No definite evidence was obtained that poisoning by CO at high pressure can be relieved by high pressures of O₂, but attempts to prove this were rendered difficult by the fact that convulsions due to acute O₂ poisoning set in at about 7 atmospheres pressure of O₂. It is, however, clear that CO has a poisonous action on rats apart from its combination with haemoglobin.

In order to test the possibility that the effects observed were due to impurities in the CO, the gas was made by two different methods. Most of the experiments were made with gas prepared by heating formic and sulphuric acids, and passing the gas evolved through soda or over soda-lime. The analytical figures obtained on combustion suggested that it contained about 2 % H₂. A sample prepared from oxalic and sulphuric acids had indistinguishable physiological properties. The point in Fig. 1 representing a mixture containing 67.1 % CO and 6.99 % O₂ was obtained with this gas. Two of the moths were quite immobile in it, one preserved reflex movements on tapping. Hence, as was to be expected, the mixture was just on the border-line of those producing immobility and ataxia.

All samples of CO had a definite smell generally compared to that of

garlic, but by one colleague to that of tar. This smell was not appreciably diminished by passing the gas over adsorbent charcoal or through bromine water and soda solution, which might be expected to remove large or unsaturated molecules. It is therefore likely that odour is a physiological property of CO [Haldane, J. S., 1921]. It is only observed in fairly high concentrations, which may of course be smelt with safety provided the operation is not repeated too often at short intervals.

DISCUSSION.

The experiments of Keilin [1927] on extracts of yeast, mammalian muscle, oatmeal, and potatoes, make it fairly clear that CO acts by combining with polyphenol or indophenol oxidase, or at least some component of that oxidase system. Since the amount of CO needed to produce a given degree of inhibition depends on the O_2 pressure it follows that both combine with it in the same way. Thus in the case of yeast saccharase, fructose competes with sucrose for the enzyme molecule, and therefore depresses hydrolysis more in weak than in strong sucrose solutions, whereas glycerol and other substances which do not so compete with sucrose depress hydrolysis to a degree nearly independent of the sucrose concentration [Michaelis and Pechstein, 1914]. In this respect CO is clearly comparable with fructose rather than glycerol. In view of the results of Keilin and the great speed with which O_2 must diffuse within a cell it is very unlikely that the substance sensitive to CO is a mere carrier of molecular O_2 like haemoglobin.

If CO combines with a catalyst X normally concerned in oxygen activation, the following reactions may be supposed to occur:

where A is an oxygen acceptor, possibly 2 H atoms from an activated molecule. Let x be the concentration of CO, y that of O_2 , $e - p - q$ that of X , p that of XO_2 , q that of XCO and a that of acceptor. Let k_1 , k_2 be the velocity constants of the first reaction, k_3 of the second, k_4 , k_5 of the third. Then when the reactions are proceeding steadily or in equilibrium, as the case may be, we have [Briggs and Haldane, J. B. S., 1925]

$$k_1(e - p - q)y = (k_2 + k_3a^2)p, \quad k_4(e - p - q)x = k_5q.$$

Eliminating q we find

$$p = \frac{e}{1 + \left(\frac{k_2 + k_3a^2}{k_1y}\right) \left(1 + \frac{k_4x}{k_5}\right)}.$$

Hence the velocity of oxidation,

$$v = k_3a^2p = \frac{k_3a^2e}{1 + \left(\frac{k_2 + k_3a^2}{k_1y}\right) \left(1 + \frac{k_4x}{k_5}\right)}.$$

If V be the velocity when the O_2 concentration is so high that v no longer depends on it, and if

$$K_{O_2} = \frac{k_2 + k_3 a^2}{k_1},$$

i.e. the Michaelis constant of the oxygen activator,

$$K_{CO} = \frac{k_5}{k_4},$$

i.e. the equilibrium constant of the third reaction,

$$\therefore v = \frac{Vy}{y + K_{O_2} \left(1 + \frac{x}{K_{CO}}\right)} \dots\dots\dots(1)$$

$$\therefore \left(\frac{V}{v} - 1\right)y = K_{O_2} + \frac{K_{O_2}}{K_{CO}} x, \left\{ \dots\dots\dots(2) \right.$$

or, $x + K_{CO} = \frac{K_{CO}}{K_{O_2}} \left(\frac{V}{v} - 1\right)y.$

Now if we suppose that a given degree of asphyxia as measured by behaviour or growth corresponds to a particular degree of reduction in the velocity of oxidation in certain cells, equation (2) should give the relation between the partial pressures of CO and O_2 required to produce it. For with a given degree of asphyxia a and hence K_{O_2} are presumably constant, though they may increase with increasing asphyxia.

Warburg showed that equation (1) did not hold for yeast, but that the initial effect of CO in diminishing oxidation was relatively less than the effect of larger amounts. This might have been due, among other things, to the existence of a component of the total oxidation not affected by CO, or to an increase with increasing asphyxia in the value of a , i.e. the amount of oxygen acceptors. Neither of these causes however would cause a deviation from the linear equation (2) such as was found in the case of *Galleria*. The relation however is nearly linear for large values of x , the equations for the two linear parts being $x + 25.0 = 6.9y$ and $x + 28.9 = 13.2y$. The agreement in the two values, 25 % and 29 % for the value of K_{CO} is as good as could be desired. Hence the catalyst behaves as if it were half saturated with CO in the absence of O_2 at a pressure of about 27 % of an atmosphere.

The deviation from linearity in low concentrations of CO may be due to several causes. In the first place, the dissociation curves of XO_2 and XCO may deviate from rectangular hyperbolae as do those of the corresponding haemoglobin derivatives. But since the presence of small amounts of CO in a gas mixture containing only enough O_2 partially to saturate the haemoglobin probably facilitates the formation of oxyhaemoglobin [Douglas, Haldane, J. S., and Haldane, J. B. S., 1911] such deviations from the law of mass action as might account for this effect would be in the opposite direction to those of haemoglobin.

It seems at least equally probable that the cells of the moth contain several catalysts of the type considered, having different affinities for CO and O_2 , only one however being the limiting factor to oxidation above a certain partial pressure of CO. This possibility is borne out by the very

different effects of CO on moths and cress. In 80 % CO the moth needs 7 or 8 times as much O_2 as when the O_2 is diluted with N_2 , the seed only about $2\frac{1}{2}$ times. It is very doubtful whether the difference of about 8° in the temperature will account for so large a difference, which is probably analogous to that between different haemoglobins. If the catalyst differs markedly in different cells of the same organism it is likely that measurements of O_2 consumption would yield a less simple result than qualitative experiments depending on a relatively small number of cells in the nervous system.

The actual O_2 pressures in the tissues during these experiments are of course unknown. Those of CO must be the same as in the gas mixture. As however for a given degree of asphyxia the rate of oxidation in the tissues is presumably constant, the O_2 pressures in the gas mixture must, if the O_2 reaches the tissues by diffusion, exceed that in the tissues by a constant amount, which must be less than 2 % of an atmosphere for the barely ataxic moths, less than 1 % for the barely immobile. The effect of allowing for this difference would be to lower the two curves in the figure by a small amount, constant for each curve. Calculations based on their upper portions would barely be affected, nor would such a correction account for their curvature.

The experiments on rats show that a CO-sensitive substance exists in the mammalian brain, as well as in the muscles where Keilin found it. Presumably it is of the same nature as the muscle catalyst, and hence as that in invertebrates and plants. If so CO, like cyanide, stimulates the respiratory centres by inhibiting oxidation there. The possibility that they are made sensitive to oxygen by a substance such as haemoglobin which changes its properties with the partial pressure of O_2 is rendered very unlikely.

The nature of the catalyst is of course unknown, though it may well be an iron-porphyrin derivative. Keilin's results show that it is a component of the polyphenol oxidase system, but it does not of course follow that the oxidation of polyphenols or related substances is a stage in every oxidative process inhibited by CO. Oxygen activated by the catalyst in question may be required for the oxidation of a variety of substances. As such catalysts have now been found in bacteria, yeasts, higher plants, insects and mammals, they are presumably present in the majority, if not all, of aerobic organisms.

SUMMARY.

1. The movements of a moth and the germination of cress seed are inhibited by CO. The greater the partial pressure of O_2 , the more CO is required.

2. Rats living on O_2 dissolved in their blood in presence of sufficient CO to combine with almost all their haemoglobin are killed by the addition of more CO, which must affect some substance in their tissues.

3. It is concluded that cells contain a catalyst of oxidation which is poisoned by CO. Its affinity for CO is determined in the case of the moth. Its affinities differ in different species, and perhaps in different tissues.

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