CARBON MONOXIDE AND OXYGEN UPTAKE BY THE TISSUES: A STUDY IN THE EARTHWORM

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Carbon monoxide can be noxious to man and a certain number of animals, which also depend on a blood haem pigment for the transport of oxygen. Except for its reaction with this pigment, however, CO would be a physiologically inert gas and not a tissue poison, such as cyanide. "Were not for its affinity for hemoglobin. ., carbon monoxide would be classed with nitrogen and hydrogen as a simple asphyxiant" (HENDERSON & HAGGARD 1943) There are many reports on the ability of intact animals, isolated organs or tissues to endure even high concentrations of CO (see MENDES 1948) HALDANE (apud HALDANE & PRIESTLEY 1935) demonstrated that a mouse can live in a high percentage of CO provided the pressure of oxygen is also increased, for the amount of O_2 which then enters the blood in simple solution eliminates the need for transportation by hemoglobin. All the toxic action of CO, therefore, would be exercised through the anoxemia resulting from the conversion of oxygen hemoglobin to carbon monoxide hemoglobin.

Carbon monoxide, however, has also affinity for the cell haem compounds, cytochrome and cytochrome oxidase. This affinity, though low, has often led to the admission that the gas could act also as a tissue poison, mainly when its relative pressure largely exceeds that of oxygen. This would particularly hold in cases where CO acts directly on cells or tissue preparations (WARBURG 1927) But in the intact animal, acting through respiratory organs and circulation, carbon monoxide would hardly reach the cells in sufficient amount to affect cell respiration. This would explain why so many

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animals are CO-insensitive over a large range of CO-percentages. The possibility that, in the intact animal, the toxity of CO be primarily due to cell block is even smaller in those who depend on a blood haem pigment for the O_2 transport. In man, a concentration of the gas sufficient to induce a fatal degree of saturation of hemoglobin would have no appreciable effect through its reaction with cytochrome (HENDERSON & HAGGARD l. c.) The ability of many organisms (including man) to burn CO to CO_2 at the expense of the respiratory oxygen (see MENDES l. c.), complicates still further the analysis of the action of CO.

The affinity of blood haem compounds for CO has been largely used in works to study whether in Invertebrates such compounds (hemoglobins or erythrocruorins and chlorocruorin) act as oxygen carriers or storers. In the course of these studies, the question frequently arose as to whether or not carbon monoxide, used in an improper percentage, besides affecting the pigment, depressed cell respiraton as well.

The CO-technique consists in physiologically suppressing the pigments by a certain amount of carbon monoxide. A comparison then of the respiratory rates of normal and CO-treated animals at decreasing oxygen tensions affords an indication of the role played by the pigments in the oxygen transport or storage. This technique, however, according to some (EWER & FOX 1940, JOHNSON 1942), must be used so that it does not affect cellular oxydations. In other words, there must be a proportional decrease of the CO tensions as one makes used of decreasing oxygen tensions, because otherwise the cytochrome system is affected an the respiratory depressions eventually observed cannot be ascribed solely to the suppression of the pigment. This view has been used mainly as a criticism of works in which CO, in a certain percentage (as a rule 20%), was employed throughout experments at decreasing oxygen tensions. At low O_2 tensions, the higher relative pressure of CO would favor its combination with the cell haem compounds, thus blocking the activity of the respiratory enzymes and carriers.

Now, this kind of criticism still remains on the theoretical ground. One of us (MENDES 1950), in a reinvestigation of a previous work (EWER & FOX 1940) on the function of chlorocruorin in Poly-

TABLE 1

The respiratory rates of the earthworm *Pheretima hawayana* at different oxygen tensions in the absence and in the presence of carbon monoxide (20% throughout or decreasing parallelly with the oxygen tension) 24 hour starving animals, in the dark, at 25°C and 36 strokes/minute. Mean local atmospheric pressure: 702 mm. Hg.

Experimental Series	N.° of experiments	Percentages of oxygen and carbon monoxide in the gas mixture		Rates of oxygen consumption c.mm. at N.T.P. per g per hr.			2nd hour mean rate as % of 1st hour	P_2	P_3
		1st hr.	2nd hr.	1st hr. Mean	2nd hr. Mean	P_1			
1	10	air	air	A_1 159 ± 49	B_1 139 ± 35	0.8	84 ± 05		—
	10	air	21% O_2 + 20% CO	A_2 146 ± 53	B_2 84 ± 25	0.01	60 ± 09		0.01
2	10	air	15% O_2	A_1 118 ± 20	B_1 101 ± 14	0.05	87 ± 12		0.7
	10	air	15% O_2 + 20% CO	A_2 102 ± 27	B_2 59 ± 23	0.01	56 ± 10		0.01
	10	air	15% O_2 + 15% CO	A_3 101 ± 20	B_3 58 ± 14	0.01	57 ± 09	0.9	0.01
3	10	air	10% O_2	A_1 116 ± 07	B_1 75 ± 07	0.01	65 ± 07		0.01
	10	air	10% O_2 + 20% CO	A_2 99 ± 09	B_2 57 ± 18	0.01	58 ± 17		0.01
	10	air	10% O_2 + 10% CO	A_3 103 ± 27	B_3 49 ± 15	0.01	48 ± 06	0.01	0.05
4	10	air	5% O_2	A_1 112 ± 34	B_1 46 ± 24	0.01	42 ± 17		0.01
	10	air	5% O_2 + 20% CO	A_2 107 ± 15	B_2 46 ± 14	0.01	43 ± 09		0.01
	10	air	5% O_2 + 5% CO	A_3 90 ± 14	B_3 44 ± 15	0.01	48 ± 12	0.3	0.01
5	10	air	2.5% O_2	A_1 98 ± 14	B_1 37 ± 10	0.01	38 ± 10		0.01
	10	air	2.5% O_2 + 20% CO	A_2 91 ± 17	B_2 33 ± 08	0.01	38 ± 09		0.01
	10	air	2.5% O_2 +2.5% CO	A_3 77 ± 04	B_3 28 ± 04	0.01	36 ± 08	0.5	0.01
6	10	air	1% O_2	A_1 105 ± 13	B_1 24 ± 07	0.01	24 ± 09		0.01
	10	air	1% O_2 + 20% CO	A_2 104 ± 20	B_2 20 ± 15	0.01	19 ± 07		0.01
	10	air	1% O_2 + 1% CO	A_3 93 ± 19	B_3 28 ± 23	0.01	34 ± 19	0.05	0.01

Pa = between "20% series" and "decreasing CO_2 series"

chetes, used 20% CO throughout the experiments. Despite that at all O_2 tensions used less respiratory depression was observed than in the first study, the results were criticized (EWER & FOX 1953) on the grounds that, at O_2 tensions lower than normal, 20% CO blocked cytochrome oxidase. An answer to this critcism is already published (MENDES 1957), in which the need of experimental work is emphasized.

This paper is a contribution to the solution of the problem. It reports the results of a study in which the earthworm was submitted to decreasing O_2 in the absence and in the presence of carbon monoxide kept at 20% or parallelly decreasing with oxygen.

MATERIAL AND METHODS

Specimens of *Pheretima hawayana,* collected in the Faculty garden, were used in the experiments. In the laboratory, they stayed before use 24 hours in the dark, in large Petri dishes lined with moistened filter paper in orden to get rid of as much earth as possible from the gut for the calculation of the body weight, and also to come closer to basal conditions. The respiration was measured in a WARBURG apparatus, in the dark, at 25°C and 36 strokes per minute.

Six experimental flasks, each containing one earthworm, were used in a experiment. In a first hour, the normal (at air) respiratory rate was measured for the six animals. Then, two of the flasks were perfused with N_2/O_2 mixtures of a desired O_2 tension and the other four with $N_2/O_2/CO$ mixtures with the same O_2 tension. In two of these four flasks, CO was kept at a constant tension (20%), whereas in the other two the CO tension varied with the O_2 tension. After the perfusion, new respiratory rates were determined in a second hour run.

The effects of using constant or decreasing CO as the oxygen tension decreased were ascertained in two ways. (a) From the comparison, in a same animal, of the respiratory rates in the first and in the second hours, thus attenuating the inconveniences of individual variation and size effects. (b) From the analysis of the absolute values measured in the second hour In both cases, the effects of a

more than two hours stay in the flasks and those of simply lowering the oxygen tension were also checked.

RESULTS

The following oxygen tensions (in percents, mean local atmospheric pressure ca. 702 mm Hg) were tested: 21, 15, 10, 5, 2.5 and 1 The results (Table I and the graphs of figs. 1 and 2), whether expressed in percents of first hour rate or as the absolute values of the 2nd. hour, show the following.

A two hour stay in the flasks did not significantly alter the respiration of the worms ($P_1 = 0.8$)

Pheretima poorly regulates respiration when the oxygen tension decreases. At 15% O_2, the respiratory rate is already significantly ($P_2 = 0.05$) lower than at 21%

Hemoglobin in *Pheretima* acts normally as an oxygen carrier, for at air O_2 tension, the respiratory rates of the CO-treated animals is highly significantly (P_1 and $P_3 = 0.01$) lower than that of normals.

At oxygen tensions lower than normal, down to 15% O_2 no significant differences in oxygen consumption were observed in the animals submitted to 20% CO as compared with those of the decreasing CO series. At 10% O_2, the respiratory rate in the presence of 20% CO was significantly ($P_2 = 0\ 05$) higher than in the presence of 10% CO.

From 5% O_2 downwards it is impossible in the three series to dissociate the effects of CO poisoning from those due to lack of oxygen.

DISCUSSION

Some of the results reported in this paper are a confirmation other previously obtained for *Pheretima*. Thus, the fact that hemoglobin in this earthworm acts normally as an oxygen carrier was already established by MENDES & VALENTE (1953) and agrees with the results obtained by JOHNSON (l. c.) in *Lumbricus herculeus*. That *Pheretima* is a poor regulator of respiration when the oxygen decreases had also been established before (MENDES & ALMEIDA 1962).

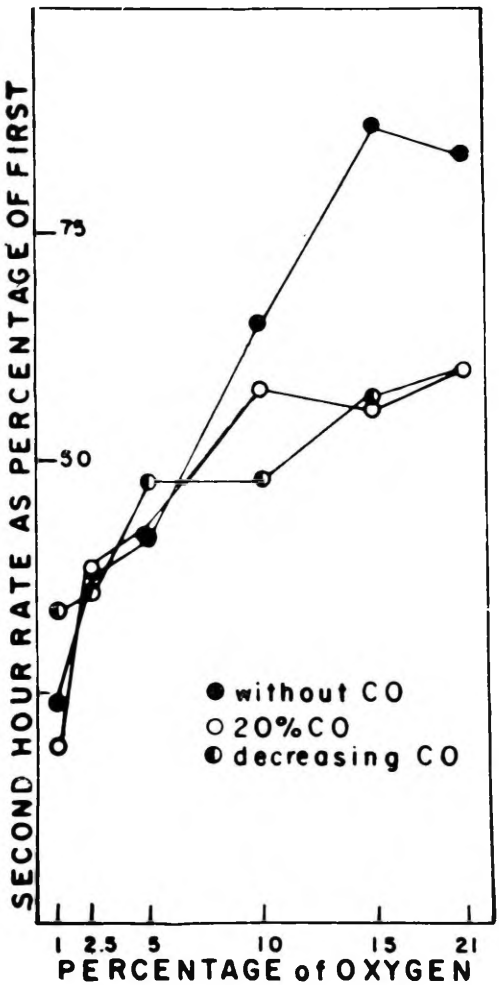

Fig. 1 — The respiratory rates of *Pheretima hawayana* at different oxygen tensions in the absence and in the presence of carbon monoxide (20% or parallelly decreasing with oxygen). The results are expressed in percents of the values obtained previously in normal conditions (air, no CO).

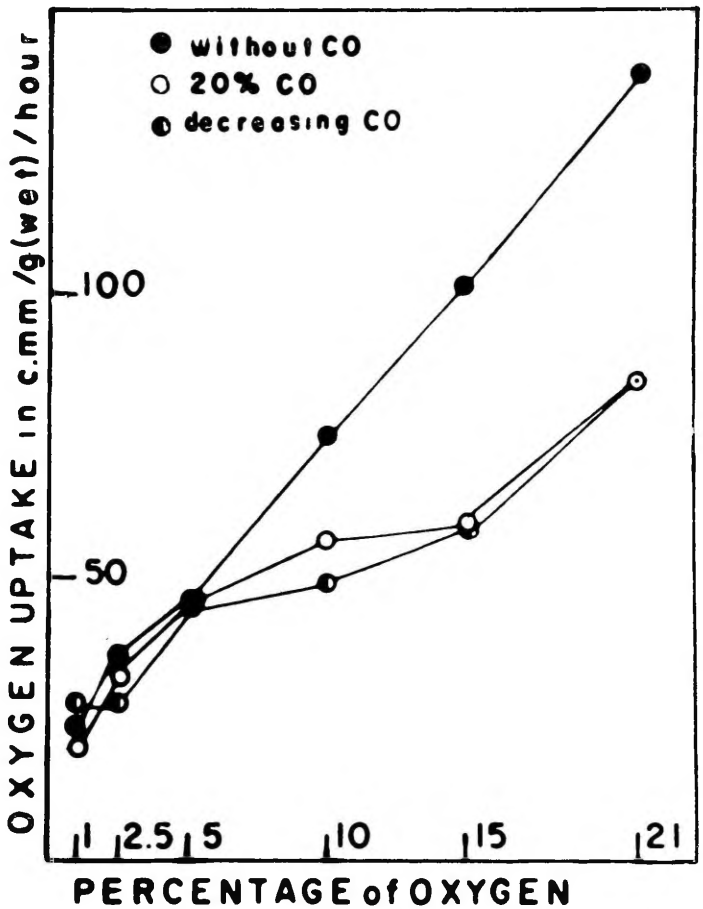

Fig. 2 — The respiratory rates of *Pheretima hawayana* at different oxygen tensions in the absence and in the presence of carbon monoxide (20% or decreasing parallely with oxygen).

At O_2 partial pressures lower than normal, *Pheretima* treated with 20% CO did not respire significantly less than that treated with CO decreasing at the same rate as oxygen. From 5% O_2 downwards it became impossible to distinguish the effect of CO from that due to oxygen impoverishment.

Of all the previous works in which the CO-technique was used in order to investigate the function of hemoglobin in earthworms, JOHNSON's (l. c.) is technically the best. In *L. herculeus,* the respiratory rate even without CO was lower in the second hour than in the first, at all O_2 concentrations tested. The rate of normal animals fell sharply below 76 mm (ca. 10% oxygen) The mean respiratory rate in the second hour in the presence of CO was lower than in its absence at all oxygen pressures above 8 mm, but this difference was significant only at 76 (10%) and 38 (5%) mm. of oxygen. That in *L. herculeus* it was still possible at 5% oxygen to distinguish between the effect of CO and that of O_2 impoverishment is probably linked to the fact that in JOHNSON's experiments the animals were not submitted to air in the first hour, but to an atmosphere containing the same O_2 percentage as in the second hour.

Our results indicate that, in *Pheretima hawayana,* the effects of using 20% CO+ lowered oxygen pressures are not significantly different from those induced by gas mixtures in which CO decreases parallelly with oxygen. Hence, in this earthworm, at least up to the 20% level of CO, no depressory effect of cellular respiration can be ascribed to carbon monoxide, even when the ratio CO/O_2 is as large as 20/1

As inspection of the curves of Fig. 1 and 2 reveals a slight tendency of the "20% CO" curve to seat above the "decreasing CO" curve. This tendency however, is not statistically significant and therefore cannot indicate an extra oxygen uptake to burn CO, which after 5% O_2 would decrease due to poor oxygen supply. In the case of the polychete *Spirographis spallanzani,* however when the results of experiments conducted at CO decreasing with oxygen (EWER & FOX 1940) are compared with those performed at CO kept a 20% (MENDES 1950), less respiratory depression is observed at all O_2 tensions in the latter work.

Both EWER & FOX (1940) and JOHNSON (l. c.) worried about the possibility that in their experiments carbon monoxide depressed also the activity of the respiratory enzymes. They therefore measured the respiration of slices of the animals in the absence and in the presence of CO. While in EWER & FOX's experiments, CO had no effect on the respiration, the rate of oxygen uptake of the slices of the earthworm in JOHNSON's study was significantly higher in the presence of the gas. To explain the results, JOHNSON suggested the possibility of the burning of CO by the tissues (FENN & COBB 1932) or the stimulation of the oxygen consumption by carbon monoxide (STANNARD 1936)

Neither EWER & FOX nor JOHNSON, however, did experiments, at the tissue level, in which there was a high relative proportion of CO to oxygen. Experiments somewhat in this direction were performed in this laboratory (PEREZ-GONZALEZ, KNAPP & MENDES 1960), in which body wall slices of *Pheretima* were submitted to gas mixtures containing 20% O_2 and increasing amounts of CO (20, 50 and 80%) In no case, the respiratory rate of the CO treated slices was significantly higher than in the controls ketp in air.

Thus, in *Pheretima,* at least up to 80% CO, the direct action of carbon monoxide does not seem to affect tissue respiration. This result in a way reinforces the conclusion from the data of this paper that carbon monoxide even in large CO/O_2 rations does not significantly affect cell respiration, when acting through the intact animal.

Of course, in view of the variability of reactions towards CO, as illustrated by the differences in behaviour of *Spirographis* and *Lumbricus* in the presence of carbon monoxide, the conclusion based on *Pheretima* cannot be extended to other animals. It is, therefore, advisable in any study involving the use of carbon monoxide, to perform experiments both at the organism and the tissue levels, before drawing conclusions as to the action of the gas on cellular respiration.

SUMMARY

1 This is a study to find out whether large CO/O_2 ratios, acting through the intact animal, affect cellular respiration, leading to an overall respiratory depression.

2. The respiratory rates of 24 hour starving specimens of *Pheretima hawayana* have been measured in a Warburg apparatus (one animal per flask), in the dark, at 25°C and strokes per min.

3. The rates measured in a first hour run, at air, were compared with those measured in a second, when the animals were submited to a certain lowered O_2 tension, in the absence or in the presence of carbon monoxide (20% or decreasing parallely with O_2)

4. The procedure allowed to check (a) the consequence of a two hour stay in the flask, (b) the responses to decreasing O_2, (c) the role of hemoglobin, (d) the effects on respiration of increasing CO/O_2 ratios as the oxygen decreased.

5 In normal conditions (air, no CO), the 2nd hour rate is not significantly different from that of lst hour; *P. hawayana* is a poor regulator of respiration; its hemoglobin functions normally as oxygen carrier.

6. At O_2 tensions lower than normal, down to 15% O_2 no significant differences in rates are observed in animals treated with 20% CO as compared with those submited to 15% CO. At 10% O_2, those treated with 20% CO respired significantly more than those exposed to 10% CO. From 5% O_2 downwards, it is impossible to distinguish between the eventual effect of CO and that due to oxygen impoverishment.

7 The results, thus, do not allow to conclude, at least for *Pheretima hawayana,* that, in experiments in which oxygen decreases from air to very low tensions, the use of 20% CO in the intact animal is likely to affect cellular respiration.

SUMÁRIO

1. Procurou-se determinar se relações CO/O_2 grandes, agindo através do animal intacto, afetam a respiração celular, conduzindo a uma depressão respiratória global.

2. As taxas respiratórias de espécimes, jejunos de 24 horas, de *Pheretima hawayana* foram medidas num aparelho de Warburg (um animal por frasco), no escuro, a 25°C e 36 agitações por minuto.

3. As taxas medidas numa primeira hora, no ar, foram comparadas com as medidas numa segunda hora, em que os animais foram submetidos a uma certa tensão abaixada de oxigênio, na ausência ou na presença de monóxido de carbono (20% ou decrescendo paralelamente com o oxigênio)

4. O procedimento permitiu verificar (a) a conseqüência de uma permanência de 2 horas no frasco; (b) as respostas à diminuição de oxigênio; (c) a função da hemoglobina; (d) os efeitos na respiração de relações CO/O_2 crescentes à medida que o oxigênio decrescia.

5 Nas condições normais (ar, sem CO), a taxa na 2a. hora não é significativamente diferente da da 1a. hora; *P. hawayana* é má reguladora da respiração; sua hemoglobina funciona normalmente como transportador de O_2.

6. Em tensões de oxigênio mais baixas que a normal, até 15% O_2 não se observaram diferenças significativas nos animais tratados com 20% CO, quando comparados com os submetidos a 15% CO. A 10% O_2, os tratados com 20% CO respiraram significativamente mais que os expostos a 10% CO. De 5% O_2 para baixo, é impossível distinguir entre o eventual efeito do CO e o devido ao empobrecimento em oxigênio.

7 Os resultados, assim, não permitem concluir, pelo menos em *Pheretima hawayana,* que, em experimentos em que o oxigênio decresce da tensão atmosférica para tensões muito baixas, o uso de 20% CO no animal intacto, é capaz d afetar a respiração celular.

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