CCX. TISSUE METABOLISM UNDER THE INFLUENCE OF CARBON MONOXIDE

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THE respiration of yeast cells which are saturated with glucose or other substrates such as ethyl alcohol or acetic acid is inhibited by carbon monoxide [Warburg, 1926, 2]. The effect is due to the CO combining with a component of the respiratory system of the cell.

Anaerobic fermentation of yeast and anaerobic glycolysis of tissues are the same in N_2 as in CO. Aerobic fermentation of yeast however, which according to the prevalent conception of the Pasteur effect is dependent on the magnitude of respiration, is only partly dependent on respiration in presence of CO. It increases with decreasing respiration but not in proportion to the decrease in respiration. In cases where respiration is only slightly inhibited by CO, the decrease in respiration cannot account for the increase in aerobic fermentation [Warburg, 1926, 2] which reaches nearly the same value as the anaerobic fermentation.

Warburg [1926, 2] has furthermore shown that the respiration of yeast which is inhibited by CO in the dark reappears again when the cells are exposed to light. Since the reappearance of respiration of yeast in light is accompanied by a decrease of aerobic fermentation, the influence of CO on respiration in yeast can be determined by measuring the changes of aerobic fermentation during the alternating periods in light and dark.

It is generally assumed that the respiration of other cells is inhibited in a similar way by CO. However, very few experiments are reported in which respiration of cells of higher animals under the influence of CO has actually been measured.

Warburg [1927] measured the aerobic glycolysis of several tissues in light and dark in presence of CO. He found more aerobic glycolysis in the dark than in light, and thence inferred that CO in the dark inhibited respiration of these tissues. Fujita [1928] has similarly inferred an inhibition of respiration of white blood corpuscles by CO as measured by their aerobic glycolysis. The metabolism of acetic acid bacteria measured directly in terms of oxygen uptake has been found to be inhibited by CO [Tamiya & Tanaka, 1930; Reid, 1931]. Respiration of *Aspergillus* was found to be inhibited by CO [Warburg & Kubowitz, 1929] as was also the respiration of frog's nerve [Schmitt, 1930; Chang & Gerard, 1931]. Runnström [1930] has shown that the respiration of fertilized sea urchin eggs in an atmosphere of 95% CO 5% O_2 is strongly inhibited, whilst the oxygen uptake of non-fertilized eggs is not affected by CO.

There is furthermore one experiment by Warburg *et al.* [1931] in which the influence of CO on the respiration of Morawitz-phenylhydrazine-red blood corpuscles has been determined. Respiration was found to be unaffected by CO in phosphate-NaCl solution but it was inhibited to about the same extent as that of yeast in Ringer bicarbonate.

Fenn & Cobb [1932, 1, 2] have studied the effect of CO on respiration of tissue slices of higher animals by direct measurement of oxygen uptake. A variety of tissues of rats and frogs has been examined by them, especially skeletal muscle, heart muscle, skin, liver and spleen. None of the tissues used showed any marked inhibition of respiration in 80% CO 20% O₂. On the contrary, increases of oxygen uptake of 160% and 98% were found in skeletal muscle and heart muscle respectively. Other tissues showed smaller or no increase and only frog's skin showed a slight decrease (14%) of respiration. They have also shown that the increased gas absorption observed in CO was due to the oxidation of CO to CO₂ which likewise occurs to an appreciable extent only in heart and skeletal muscle. The authors remark that their negative results with regard to the effect of CO on respiration do not necessarily contradict the theory of Warburg for they might merely mean that higher ratios of CO to O₂ should have been used.

Warburg [1927] examined liver, retina, chorion, embryonic tissue and tumours as to their reaction against CO and found certain differences of metabolism, according to periods in darkness and in light (Table I).

Table I. *Alternating manometer readings using different tissues in O₂/CO (in darkness (d) and in light (l)), in O₂/N₂ and in O₂*

Ringer bicarbonate, 0.2% glucose = R.B. Serum = S. From Warburg [1927].							
Tissue	...	...	Liver	Chorion	Retina	Embryo (rat)	
Medium	...	...	S.	S.	R.B.	S.	
Temp. (° C.)	...	...	25	37.5	37.5	37.5	
10% O ₂ , 5% CO ₂ , 85% CO:	20' (d)		- 8	+ 43	+ 73	30' (d)	+ 9.0
	20' (l)		- 11	+ 23	+ 56	30' (l)	+ 7.5
	20' (d)		- 4.5	+ 38	+ 71	30' (d)	+ 8.5
	20' (l)		- 11	+ 23	+ 47	30' (l)	+ 6.0
95% O ₂ , 5% CO ₂ :	20'		—	- 10	+ 36	30'	- 0.5
10% O ₂ , 5% CO ₂ , 85% N ₂ :	20'		—	+ 15	+ 46	30'	+ 5.5

The positive pressures were found always to be less in light than in darkness. Warburg concluded, applying the results and theory of his experiments with yeast to these tissues, that the high positive pressure in the dark is due to inhibited respiration and increased aerobic glycolysis, while the decrease of positive pressure in light is due to reappearance of respiration and diminished aerobic glycolysis. However, this does not follow conclusively from the figures given in his table because no actual measurement of respiration had been recorded.

Respiration can be measured by this method (using Ringer-bicarbonate, with 5% CO₂ in gas phase) only with two vessels with different volumes of fluid. High pressures in darkness and diminished pressures in light, as observed in one vessel, do not allow assumptions to be made with regard to the magnitude of respiration, because the same changes of pressure may result from changes in glycolysis only while respiration remains the same in both cases.

That this is actually the case will be shown in the following experiments.

Methods and material

Retina, allantois, chorion and liver have been examined. The methods used were the differential method of Warburg and the method of Dixon & Keilin. The gas mixtures contained 5% CO₂ and 5–10% O₂ in CO. The ratio O₂/CO varied from 1:8.5–1:18. The gas mixtures for the controls contained N₂

instead of CO. When working with Warburg's method the same precautions were taken as described in the previous paper for the experiments with low oxygen tension in order to ensure saturation of the fluids with CO₂ and O₂.

RESULTS AND DISCUSSION

The principal results of these experiments are shown in Table II. The oxygen uptake of retina and chorion is not inhibited by CO even in the high concentrations of CO which have been used. The Pasteur effect is inhibited completely in the case of retina ($Q_{O_2}^{O_2} = Q_{O_2}^{N_2}$) and strongly in the case of chorion. On those tissues CO therefore acts as ethylcarbylamine on tumours [Warburg 1926, 1]. In the case of allantois of 5 days old chicken embryos there is also no inhibition of respiration. Only in one case (14 days old embryo) (No. 3, Table II) is there an inhibition compared with the respiration in 95% O₂, but there is no inhibition if the figures obtained using low oxygen tension in presence of N₂ and of CO are compared.

The R.Q. (No. 2, Table II) is low. But the low oxygen tension accounts for this, as has been shown in the previous paper.

Table II.* *Metabolism of different tissues under the influence of CO.*

Warburg's differential method. Ringer bicarbonate, 0.2% glucose (except experiment N. 6).

Material	Vol. % of gas				Q_{O_2}	$Q_{O_2}^{O_2}$	R.Q.
	O ₂	CO ₂	N ₂	CO			
1. Allantois chicken, 5 days' incubation	95	5	—	—	12.6	1.1	—
	8	5	—	87	11.0	10.5	—
2. Same, 5 days	95	5	—	—	17.5	0	—
	7	5	—	88	21.8	16.0	0.72†
3. Same, 14½ days	95	5	—	—	13.0	0	—
	8	5	87	—	6.4	0	—
	8	5	—	87	6.7	3.4	—
4. Chorion (mouse)	95	5	—	—	15.7	6.4	—
	5	5	—	90	15.1	20.0	—
5. Retina (rat)	95	5	—	—	30.0	45.0	—
	10	5	—	85	29.0	79.0	—
	7	5	—	88	29.6	94.0	—
	5	5	90	—	30.0	68.0	—
	5	5	—	90	29.4	89.1	—
	5	5	—	90	29.9	87.4	—
6. Retina (rat) in Ringer phosphate 0.2% glucose	100	—	—	—	19.0	—	—
	5	—	95	—	19.0	—	—
	5	—	—	95	19.0	—	—

* For all tables: $Q_{O_2} = O_2$ uptake (μ l. O₂) per mg. dry weight of tissue per hr. $Q_{O_2}^{O_2}$ = aerobic glycolysis (μ l. CO₂ expelled from bicarbonate by acid production) per mg. dry weight of tissue per hr.

† Dixon-Keilin method.

Some experiments have been carried out with liver in Ringer-phosphate solution and various O₂/CO gas mixtures with absorption of CO₂ by caustic potash (10%). Special care has been taken to have the gas mixtures as free as possible from CO₂. CO, when prepared from sulphuric acid and formic acid, was passed through KOH and the final gas mixtures before being passed through the manometer was again purified with KOH. In addition, one manometer containing the same gas mixture and fluid but no tissues was used as control and the pressures of the experimental manometer corrected accordingly.¹

¹ A slight negative pressure in this manometer is due to the formation of potassium formate by KOH [Warburg & Negelein, 1928].

Table III. *Metabolism of liver (mouse) under the influence of CO in darkness*

Ringer bicarbonate, 0.2% glucose = R.B. or Ringer phosphate, 0.2% glucose = R.Ph.

Medium	Vol. % of gas				Q_{O_2}	$Q_G^{O_2}$
	CO ₂	O ₂	N ₂	CO		
R.B.	5	95	—	—	12.4	3.0
	5	8	87	—	4.2	5.4
	5	8	—	87	5.7	11.4
	5	20	—	75	8.5	8.3
R.Ph.		100	—	—	13.8	—
		20	80	—	10.7	—
		20	—	80	11.1	—
		10	90	—	9.2	—
		10	—	90	9.1	—

If the oxygen tensions were the same there was no inhibition of respiration in CO/O₂ compared with N₂/O₂ (Table III).

The pressure differences which have been observed by Warburg in darkness and in light may therefore be explained in the following way. The high pressure in the dark is due to complete inhibition of the Pasteur effect, whereby the aerobic glycolysis is increased to the figure of anaerobic glycolysis. Light reverses this inhibition. The Pasteur effect works again and aerobic glycolysis decreases to or approaches the level of aerobic glycolysis under low oxygen tension in N₂. That this is the case may be deduced from the figures of Warburg (v. Table I) where a very close agreement exists between the figures in CO (light) and in 10% O₂/N₂ but a great discrepancy occurs between the figures in CO (light) and the figures in 95% O₂. The latter should be closer to one another if there existed an inhibition of respiration by CO which is reversible in light.

Table IV. *Influence of light on the metabolism of different tissues*Ringer bicarbonate, 0.2% glucose. Warburg's differential method, *d* = dark, *l* = light.

Material	Vol. of gas			Dark or light 20' each	Q_{O_2}	$Q_G^{O_2}$	% increase of aerobic glycolysis in darkness
	CO ₂	O ₂	CO				
1. Allantois (chicken)	5	7	88	<i>d</i>	21.8	16.0	63
				<i>l</i>	18.2	9.8	—
2. Allantois (chicken)	5	8	87	<i>d</i>	11.0	10.5	64
				<i>l</i>	11.0	6.4	—
3. Liver (mouse)	5	10	85	<i>d</i>	8.2	5.2	300
				<i>l</i>	4.5	1.2	—
				<i>d</i>	4.0	3.0	220
				<i>l</i>	4.2	1.25	—
4. Liver (mouse)	5	20	75	<i>d</i>	8.5	8.3	565
				<i>l</i>	10.0	1.7	—

Table IV shows this change of aerobic glycolysis in O₂/CO under the influence of light and in Table V and Fig. 1 an experiment with liver is reported *in extenso*. Respiration is very small owing to the low oxygen tension and even becomes smaller with time but shows no dependence on the periods of light and darkness. Aerobic glycolysis, on the other hand, increases in the dark and decreases in light. The absolute differences are not very large but they are clearly apparent from the marked changes of the curve and from the manometer readings. The changes in each subsequent manometer reading resemble exactly those described by Warburg, namely less positive (or in this case, more negative) pressure in

Table V. *Protocol of experiment 3, Table IV*Liver, mouse, in Ringer bicarbonate, 0.2% glucose. 10% O₂, 5% CO₂, 85% CO. Temp. 38°.

	Vessel I	Vessel II	
	$V_G = 11.53$ ml.	$v_f = 11.90$ ml.	
	$V_F = 7.0$ ml.	$v_g = 3.0$ ml.	
	$K_{O_2} = 0.576$	$k_{O_2} = 0.888$	
	$K_{CO_2} = 0.871$	$k_{CO_2} = 0.968$	
20' dark	- 6.5	+ 7.25	
20' light	- 14.25	- 1.75	
20' dark	- 4.0	+ 4.25	
20' light	- 12.0	- 2.0	
	Dry weight = 9.53 mg.	Dry weight = 7.96 mg.	
		% increase of aerobic glycolysis in darkness	
	Q_{O_2}	$Q_G^{O_2*}$	
20' dark	6.93	5.49	260
20' light	5.10	1.53	—
20' dark	4.16	3.43	210
20' light	3.97	1.08	—

* R.Q. assumed = 0.75.

Fig. 1. Metabolism of liver (mouse) in Ringer-bicarbonate, 0.2% glucose, Warburg's differential method. 10% O₂, 5% CO₂, 85% CO. I, μ l. O₂ utilized per mg. dry weight of tissue; II, μ l. CO₂ per mg. dry weight of tissue expelled from bicarbonate by acid production.

light than in dark. But this change is, as Fig. 1 demonstrates, no change in respiration but only in glycolysis. Using 10 mg. tissue—the vessel-constants for CO₂ being between 0.8 and 1.0—an increase of 3 for $Q_G^{O_2}$ increases the pressure in 20 min. (dark) by 10 mm. The observed differences per 20 min. with and without light were actually of this order.

SUMMARY

1. The influence of carbon monoxide on the metabolism of a variety of tissues (retina, allantois, chorion, liver) has been studied manometrically.

2. The respiration was the same in O_2/CO as in O_2/N_2 gas mixtures. CO therefore does not seem to affect the respiration of these tissues.

3. On the other hand, replacement of N_2 by CO increases aerobic glycolysis to the level of the anaerobic glycolysis, thus indicating a complete inhibition of the Pasteur effect by CO .

4. The inhibition of the Pasteur effect by CO is partly or totally reversible under the influence of light, i.e. aerobic glycolysis decreases in light to its level in low oxygen tension.

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