CCXXXIX. THE INHIBITION OF RESPIRATION BY CYANIDE.

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DIXON AND ELLIOTT [1929] published a communication on the effect of cyanide on the respiration of animal tissues in which they claimed to have found that the maximum inhibition of respiration of animal tissues (kidney, liver, spleen) by cyanide was only 30–90 %. Alt [1930], working in Warburg's laboratory with Ringer-*M*/40 bicarbonate- CO_2 (0.03–0.05 atm. press.) medium, claimed to have found practically complete inhibition with animal tissues. He ascribed the low values of Dixon and Elliott to the influence of their medium, which had too high a phosphate concentration and a zero CO_2 pressure.

It was with a view to a closer investigation of the influence of the phosphate, bicarbonate, and CO_2 concentrations on respiration and on its inhibition that I carried out the following experiments.

I. METHODS.

Respiration was measured by Warburg's method as described by Dixon [1934]. The manometer flasks used were conical with an inner cup for retaining the solution for absorbing respiratory CO_2 . The medium used was that of Krebs [1933], modified in so far as it now contained $10^{-2} M$ phosphate. In order to make the measurements more accurate, large amounts of tissue were used when dealing with a high concentration of cyanide and an organ with a low oxygen uptake. Thus, for instance, with ox spleen and $10^{-2} M$ HCN it was necessary to use as much as 73 mg. dry weight of tissue, whilst 3.5 mg. were sufficient when no poison was used with rat kidney. It was found, however, both by Dixon and Elliott and by myself that respiration was independent of the volume in which the tissues were suspended. After each experiment the tissues were washed, dried at 100° to a constant weight and weighed. The oxygen consumption is expressed by the quotient Q_{O_2} , which is $\frac{\mu\text{l. O}_2 \text{ per hour}}{\text{dry wt. of tissue}}$. All measurements were made at 37.5° .

Technique of HCN poisoning.

Cyanide poisoning is reversible and therefore it is essential to prevent the KOH in the inner cup from absorbing HCN. Krebs [1935] has recommended certain solutions containing varying proportions of KCN and KOH which are so adjusted as to have a concentration of free HCN equal to that in the outer cup. When HCN (which was prepared by neutralising pure KCN with HCl) was used a KCN-KOH solution with a corresponding free HCN concentration was placed in the inner cup of the manometer vessel. This absorption is accelerated by the small squares of filter-paper which are always placed in the inner cups.

That these precautions are essential is clear from the figures in Table I, in which the results of experiments with KCN-KOH mixtures and KOH alone are recorded.

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Table I. *Showing the effect of absorption of HCN by KOH.*

Rat kidney slices with 0.5 % glucose substrate.

Concentration of cyanide	KCN-KOH in inner cup		KOH alone in inner cup	
	Q_{O_2}	% inhibition	Q_{O_2}	% inhibition
No HCN	—	—	—	—
$10^{-4} M$ HCN	— 19.4	2.0	— 19.8	0.0
$10^{-3} M$ HCN	— 4.0	79.9	— 18.3	7.5
$10^{-2} M$ HCN	— 3.1	84.3	— 6.1	69.1

This absorption of HCN will probably account to some extent for the low degree of inhibition found by Dixon and Elliott, and by Alt when he repeated their experiments, since these workers did not take the precautions described above. It may also be possible that the values found by Banga *et al.* [1931] for the inhibition of the respiration of pig heart muscle are too low.

II. INFLUENCE OF MEDIUM.

Influence on respiration.

Dickens and Simer [1931] have found that the respiration of a variety of tissues is the same in bicarbonate-saline medium as in phosphate-saline medium. Dixon and Elliott, however, used a pure phosphate buffer as medium in which the concentration was $M/10$, whereas the phosphate concentration in the medium of Dickens and Simer was $M/40$. The great variations and low values of Dixon and Elliott's figures for the respiration of various animal tissues suggest that a high phosphate concentration is harmful.

I carried out a number of experiments in which the respiration and its inhibition of rat liver and kidney slices were measured in Dixon and Elliott's medium and in the medium described in section I. The results of these experiments are recorded in Table II.

Table II. *Showing the influence of medium on respiration and on its inhibition by cyanide.*

Tissue	Concentration of cyanide	Pure phosphate medium		Saline medium	
		Q_{O_2}	% inhibition	Q_{O_2}	% inhibition
Rat liver	Control	— 8.8	—	— 11.1	—
	$10^{-4} M$	— 7.3	17.0	— 9.9	10.7
	$10^{-3} M$	— 2.5	71.6	— 2.1	81.1
	$10^{-2} M$	— 2.0	77.2	— 1.8	83.8
Rat liver	Control	— 7.9	—	— 10.4	—
	$10^{-4} M$	— 8.3	0.0	— 8.5	18.2
	$10^{-3} M$	— 3.4	57.0	— 2.1	79.9
	$10^{-2} M$	— 3.2	59.5	— 2.0	80.8
Rat kidney	Control	— 11.4	—	— 19.0	—
	$10^{-3} M$	— 2.4	79.9	— 2.9	84.7
	$10^{-2} M$	— 2.2	80.8	— 1.9	90.0

These figures show that the respiration in pure phosphate medium is lower than in saline medium with $M/40$ phosphate. Thus the respiration in phosphate medium for the two cases of rat liver and the one case of rat kidney is 79, 74 and 60 % respectively of the respiration in saline medium.

Influence on degree of inhibition.

In Table II it will be seen that with $10^{-2}M$ HCN the absolute values for the Q_{O_2} are very nearly the same in both media. $M/10$ phosphate inhibits the same part of the respiration as is affected by cyanide. The consequence is that the degree of inhibition is less.

III. MEASUREMENTS OF INHIBITION BY CYANIDE.

The respiration of several different organs and its inhibition by varying concentrations of cyanide were now measured. I found that the degree of inhibition increases rapidly with increasing concentrations of cyanide up to a concentration of $10^{-3}M$. At this stage the degree of inhibition ranges from about 75 to 85%. It is only very slightly increased by a further tenfold increase in cyanide concentration. The percentage inhibition is plotted against molar concentration of HCN in the typical curves in Fig. 1. The results of these experiments are recorded in Table III.

Fig. 1. Degree of inhibition produced by increasing concentrations of cyanide.

The degree of inhibition by $10^{-2}M$ HCN varies with different tissues and with different substrates from about 80 to 90%. An exception is seen in the case of Exp. 15 (guinea-pig kidney with pyruvate substrate), where the inhibition with $10^{-2}M$ HCN is only 46%. This is probably due to a reaction between pyruvic acid and the HCN (formation of cyanohydrin?).

IV. DISCUSSION.

Alt (see also Warburg [1931]) measured the inhibition of respiration of tissue slices suspended in bicarbonate-saline- CO_2 medium and found that there was an increase in degree of inhibition with increasing concentration of cyanide, reaching practically complete inhibition at a concentration of $10^{-2}M$. The production of lactic acid in the bicarbonate medium, however, would complicate the measurement of respiration. The low respiration associated with a high concentration of cyanide would be difficult to measure accurately, and it is possible that the difference between the results as found by his method and by mine is not real.

Table III. *Respiration, and its inhibition by cyanide, of various tissues.*

No.	Tissue	Added substrate	No HCN Q_{O_2}	$10^{-4} M$ HCN Q_{O_2}	$10^{-3} M$ HCN Q_{O_2}	$10^{-2} M$ HCN Q_{O_2}	% inhibition ($10^{-2} M$ HCN)
1	Sheep liver	0.5% glucose	- 2.6	- 2.4	- 0.55	- 0.57	78.1
2	Sheep kidney	0.5% glucose	- 14.0	- 9.8	- 2.1	- 1.8	87.1
3	Ox liver	0.5% glucose	- 1.8	- 1.4	- 0.86	- 0.7	61.1
4	Ox spleen	$10^{-2} M$ lactate	- 4.1	- 3.0	- 0.95	- 0.83	79.8
5	Ox spleen	None	- 3.8	- 3.0	- 0.98	- 0.84	77.9
6	Rat intestine	0.5% glucose	- 7.5	- 6.6	- 1.9	- 1.6	78.7
7	Rat brain cortex	0.5% glucose	- 15.0	- 9.8	- 2.6	- 1.6	89.3
8	Rat kidney	$10^{-2} M$ lactate	- 33.0	- 24.7	- 7.3	- 4.2	87.3
9	Rat kidney	None	- 20.6	- 18.7	- 3.1	- 2.2	89.3
10	Rat kidney	None	- 19.0	—	- 2.9	- 1.9	90.0
11	Rat kidney	0.5% glucose	- 19.8	- 19.4	- 4.0	- 3.1	84.3
12	Rat testis	0.5% glucose	- 8.0	- 5.8	- 1.3	- 1.0	87.5
13	Rat liver	None	- 10.4	- 8.5	- 2.1	- 2.0	80.8
14	Rat liver	None	- 11.1	- 9.9	- 2.1	- 1.8	83.8
15	Guinea-pig kidney	$10^{-2} M$ pyruvate	- 14.7	- 15.2	- 13.0	- 7.9	46.2
16	Guinea-pig kidney	None	- 13.0	- 12.7	- 2.7	- 2.3	82.3
17	Guinea-pig kidney	0.5% glucose	- 16.7	- 14.3	- 7.9	- 1.8	89.2

The low degree of inhibition observed by Dixon and Elliott is probably due to the combined effect of the two factors already discussed, namely the removal of HCN by the KOH in the inner cup and the inhibition of respiration by the high phosphate concentration of their medium.

As shown in Exps. 4 and 5 in Table III, the respiration of ox spleen is inhibited about 80% by $10^{-2} M$ HCN and 25% by $10^{-4} M$ HCN. This is not in agreement with the finding of Dixon and Elliott that there was no inhibition of the respiration of this tissue with as much as $3 \times 10^{-2} M$ HCN.

V. SUMMARY.

The respiration of liver, kidney and spleen slices suspended in $10^{-2} M$ phosphate-saline medium is inhibited to the extent of 75–85% by $10^{-3} M$ HCN and 80–90% by $10^{-2} M$ HCN.

The discrepancy between these results and those of other workers—especially the low values found by Dixon and Elliott—has been investigated.

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