

place of glucose have no effect on methemoglobin formation or its change to oxyhemoglobin.

Results *in vitro* with sheep blood also showed that methemoglobin formation is delayed if glucose is present, or if already formed, the addition of glucose will reconvert a part of it to oxyhemoglobin. The following experiment will illustrate this: to 4 cc. of washed r.b.c. suspended in 0.9% saline solution, there was added .05 cc. of M/20 phenylhydroxylamine, freshly prepared. R was then found to be 1.42, indicating 48% methemoglobin. This solution was divided into 2 parts: glucose was added to one part, and to the other part only saline. The glucose-containing blood now had only 18% methemoglobin present ( $R = 1.53$ ) while that with a saline alone remained unchanged at 48% ( $R = 1.42$ ). It has not been found possible to reduce *in vitro* all the methemoglobin to oxyhemoglobin. This was also found by Warburg, Kubowitz and Christian,<sup>3</sup> who state that unknown reactions take place.

Similar effects can be demonstrated using  $\text{NaNO}_2$  instead of phenylhydroxylamine. If glucose be added before the methemoglobin-producing substances, the formation of methemoglobin is delayed and the sample is still reddish when the control is brown.

These results show that glucose is effective in preventing methemoglobin formation, or after formation, in reducing it to hemoglobin which can then form oxyhemoglobin. It is suggested that the presence of glucose in the blood stream is responsible for the often observed failure of various agents to produce the expected proportion of methemoglobin. It is also suggested that injections of glucose be used clinically in cases where methemoglobin is present either as a result of pathological conditions or as a result of poisoning by such methemoglobin-producing substances as aniline dyes, nitrites, etc.

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### Action of Respiratory Catalysts and Inhibitors on Oxygen Consumption by Nitella.

EDWARD ROSS. (Introduced by S. C. Brooks.)

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The material used consisted of young, actively growing coenocytic cells of *Nitella clavata* collected from a large outdoor pool. The

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<sup>3</sup> Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1931, **242**, 170.

growing tips of several dozen stems were cut off and kept in a large beaker of pond water in the dark for 24 hours prior to use. These clusters of cells were from 2 to 10 mm. in diameter.

The manometric technique, originally described by Barcroft and Haldane, and subsequently employed extensively by Warburg, was used for measuring oxygen consumption. The material was immersed in pond water. Five per cent KOH was used in the inset to absorb  $\text{CO}_2$ , and the vessels containing the green plants were wrapped in an opaque black cloth to exclude light. Oxygen consumption was measured as a rate,  $Q_{\text{O}_2}$ , in cmm. per hour per gram wet weight.

The effects of NaCN and methylene blue, singly and combined, were studied, purely relative results being obtained when the agents were used separately. When methylene blue was added after the cyanide, all values were corrected for the percentage change of a control run at the same time. In every case the normal  $Q_{\text{O}_2}$  was established for the second hour of a 2-hour run. In the case of cyanide the vessels were then disconnected and 0.4 cc. of the proper NaCN concentration was added, and the  $Q_{\text{O}_2}$  of the second hour thereafter determined. When methylene blue was used alone the dye was poured into the plant environment from the side-arm after the normal  $Q_{\text{O}_2}$  had been established. When methylene blue was used in combination with NaCN, the percentage inhibition was first determined with cyanide alone, the methylene blue then added and the  $Q_{\text{O}_2}$  determined for the second hour thereafter, in conformance with the standard procedure. All inhibition and acceleration were expressed as percentage deviation from the normal as first established in each case.

The results are given in Table I, negative indicating the percentage decrease in oxygen consumption, and positive the increase.

TABLE I.

Concentration	Effects of NaCN Alone					Effects of Methylene Blue Alone			
	10 <sup>-1</sup> M	10 <sup>-2</sup> M	10 <sup>-3</sup> M	10 <sup>-4</sup> M	10 <sup>-5</sup> M	10 <sup>-2</sup> M	10 <sup>-3</sup> M	10 <sup>-4</sup> M	10 <sup>-5</sup> M
% deviation	-6%	-44	-46, -51	-29	-12	+107	+74	+43	+5
from normal	+35	-33	-42, -44	-20	-8	+123	+66	+48	-1
Aver.	+8	-39	-44, -51	-25	-10	+115	+70	+46	+2

Since the cells were definitely and irreversibly injured in  $10^{-1}$  M cyanide, the absence of inhibition is probably due to injury. Injury probably also decreases inhibition by  $10^{-2}$  M cyanide, though not to so great an extent. Normal oxygen consumption was resumed

when  $10^{-3}$  M cyanide had been washed out. There was, therefore, no irreversible injury at this or lower concentrations. Acceleration was perfectly reversible on washing out the methylene blue taken up from  $10^{-4}$  M solution; but after  $10^{-3}$  M it was not possible to wash out all the dye within a reasonable time. Injury cannot be surely excluded in the case of  $10^{-2}$  M solution of the dye.

The antagonism between cyanide and methylene blue has been tested by 3 experiments so far. In all of these  $10^{-3}$  M NaCN was used. The results, corrected for percentage changes in the controls, are given in Table II.

TABLE II.  
Effects of Methylene Blue Added after Cyanide.

Experiment	1	2	3
Effect of NaCN, $10^{-3}$ M	-44%	-33%	-36%
Concentration of dye added	$10^{-2}$ M	$10^{-3}$ M	$10^{-4}$ M
Resultant effect (0.0%—complete recovery)	-9%	-41%	-39%

The dye appears to have accelerating power only when its concentration is greater than that of the inhibitor. With this material the apparent acceleration may have been due to injury, investigation of which is in progress.