

THE EFFECT OF CERTAIN RESPIRATORY INHIBITORS ON THE RESPIRATION OF CHLORELLA.

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INTRODUCTORY REMARKS.

This paper presents experiments on the effect of hydrocyanic acid, hydrogen sulfide, and carbon monoxide on the respiration of the green alga, *Chlorella*. The subject is of interest because all three of these substances are known to inhibit respiration specifically and reversibly in various organisms.

Hyman,¹ in a paper on the effects of potassium cyanide on *Planaria*, has reviewed the cases where direct measurements of the effects of cyanides on respiration have been made. Negelein² has published experiments on the effects of hydrogen sulfide, and Warburg³ on the effects of carbon monoxide.

It has been shown in this laboratory that these three inhibitors of respiration do not check the respiration of *Chlorella*. Warburg⁴ has shown this for hydrocyanic acid, Negelein² for hydrogen sulfide; I have tried the effect of carbon monoxide.

As will be shown, this exceptional behavior of *Chlorella* vanishes when the alga is made heterotrophic.

Methods.

The methods used in this work were essentially the same as those of Warburg and Negelein in their work on *Chlorella*. The alga was cultivated as described by them,⁵ in a water thermostat lighted continuously with three 75-watt metal

¹ Hyman, L. H., *Am. J. Physiol.*, 1919, xlviii, 340.

² Negelein, E., *Biochem. Z.*, 1925, clxv, 203.

³ Warburg, O., *Biochem. Z.*, 1926, clxxvii, 471.

⁴ Warburg, O., *Biochem. Z.*, 1919, c, 268.

⁵ Warburg, O., and Negelein, E., *Z. physik. Chem.*, 1922, cii, 250.

filament lamps, about 30 cm. distant from the culture flasks. A slow stream of 5 per cent carbon dioxide in air was bubbled through the cultures. They were pure for the most part, but in control experiments with cells from cultures where no precautions were taken to exclude bacteria the same results were obtained as with cells from cultures known to be pure. The amount of *Chlorella* cells used so far exceeded that of any chance bacteria, that the latter did not affect the results.

Respiration was measured manometrically, in the dark. Measured quantities of cell suspension were pipetted into vessels of the type shown in Fig. 1. The vessels were connected by gas-tight joints with their respective manometers. The gas space above the cells, and the capillaries, as far as the manometric fluid, were filled with a mixture of 5 volumes per cent carbon dioxide in air before the

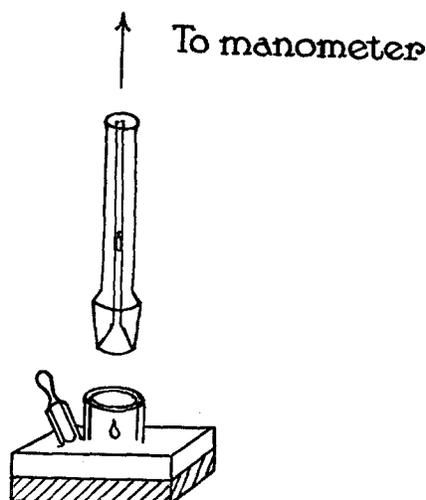


FIG. 1

system was closed. The vessels were shaken in a water thermostat at 20°C. in order to maintain a state of equilibrium between the gas in the space above the cell suspension and that dissolved in the suspension. It was always fast enough so that an increase caused no change in the results. At intervals the shaking was interrupted for the purpose of reading the manometers.

The principle involved here is that oxygen is less soluble in the cell suspension than carbon dioxide. Thus, if the cells, in respiration, consume oxygen and give off the same amount of carbon dioxide, the pressure in the gas chamber will decrease, causing a change in the manometer (*cf.* Warburg⁶ for a detailed discussion of this method).

⁶ Warburg, O., *Biochem. Z.*, 1924, clii, 51.

Experiments with Hydrocyanic Acid.

The experiments with hydrocyanic acid will be described first and most completely. They are typical of those performed with hydrogen sulfide and carbon monoxide.

The concentration of hydrocyanic acid was 10^{-4} normal in practically all experiments. This is a convenient concentration for purposes of comparison, as Negelein has used it also. Its effect on

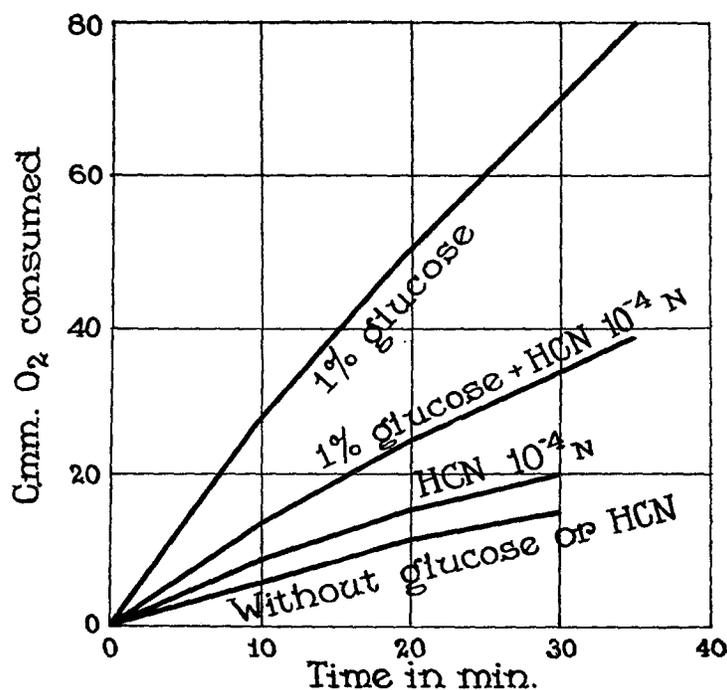


FIG. 2.

ordinary *Chlorella* cells (*i.e.* cells grown in an inorganic medium in bright light), is shown by the two lower curves of Fig. 2. Respiration is slightly accelerated. The two upper curves of Fig. 2 show the effect of the same concentration of hydrocyanic acid on the respiration of similar cells suspended in a solution containing 1 per cent glucose. It reduces their respiration over 50 per cent. Since all four curves represent the oxygen consumptions of the same amounts of cells, a com-

parison of the top and bottom curves shows that 1 per cent glucose about quadruples respiration.

Osterhout⁷ and Krehan⁸ have shown that hydrocyanic acid has definite effects on the permeability of living cells. Hence it might be argued that the acid merely checks the penetration of the sugar into the cells, and not the oxidation process inside them. It may be shown in various ways that this is not the case.

Cells may be allowed to remain in a medium containing sugar for some time before the addition of hydrocyanic acid. When it is thus later added, the resulting inhibition is the same as when it is added with the sugar.

Another method is to grow *Chlorella* in a medium containing 1 per cent glucose. For the experiment, the cells are centrifuged off and transferred to an inorganic medium. The respiration of such cells is checked 40 to 50 per cent by 10^{-4} normal hydrocyanic acid.

The most conclusive method is to take autotrophic cells, grown in an inorganic medium, allow them to stand in 1 per cent glucose solution until the sugar has had time to penetrate (about 15 minutes), and then return them to the inorganic medium in order to measure the respiration and the effect of hydrocyanic acid. Respiration is reduced 60 to 70 per cent.

Organic substances of various sorts other than glucose have been tried, to see whether they would render *Chlorella* respiration sensitive to hydrocyanic acid. Those which, like glucose, call forth a respiration of three to four times the normal, were used successfully. Checking by hydrocyanic acid was the same as in glucose. Such substances are fructose, galactose, and mannose. All act almost exactly alike. The effect of the sugar on the respiration is very independent of the concentration. Glucose, for example, accelerates the respiration about four times, whether it is present in 4/100 per cent or 4 per cent solution. The inhibition by hydrocyanic acid is likewise independent of the sugar concentration.

Other substances tried gave slight acceleration of respiration (in general less than double). Many indifferent substances will cause this slight acceleration. But no real sensitivity to hydrocyanic acid

⁷ Osterhout, W. J. V., *Bot. Gaz.*, 1917, lxiii, 77.

⁸ Krehan, M., *Internat. Z. physik.-chem. Biol.*, 1914, i, 189.

results. Although respiration may be checked slightly at first, it is soon accelerated, just as with ordinary cells. The substances tried were cane-sugar, arabinose, dioxyacetone, glycoll, mannitol, and lactic acid. Fig. 3 shows the effect of hydrocyanic acid on cells suspended in a solution containing 1 per cent glycoll. Glycoll was selected as typical of the indifferent substances.

Various authors (*e.g.* Hyman¹) have stressed the reversibility of the hydrocyanic acid inhibition. If the effect be irreversible, it is argued

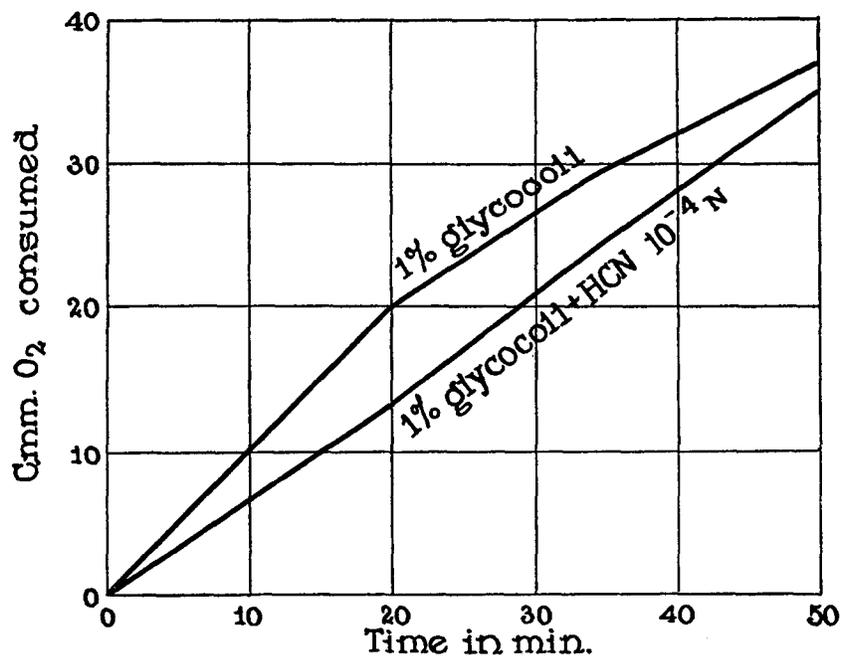


FIG. 3.

that the cells have been injured, and any inhibition of respiration cannot be regarded as specific. To test the reversibility in the case of sugar-containing *Chlorella* cells, the cells were subjected to the effect of hydrocyanic acid for 30 minutes, the checking of respiration measured, and then a stream of moist air was bubbled through the suspension, to remove the hydrocyanic acid. Respiration was then measured again, and found to be greater than in the control. The effect is therefore completely reversible.

Experiments with Hydrogen Sulfide.

Similar experiments were carried out with hydrogen sulfide and led to the same results as with hydrocyanic acid. Free hydrogen sulfide was formed in the cell suspension by the addition of an appropriate amount of $N/10$ solution of sodium sulfide. The solution

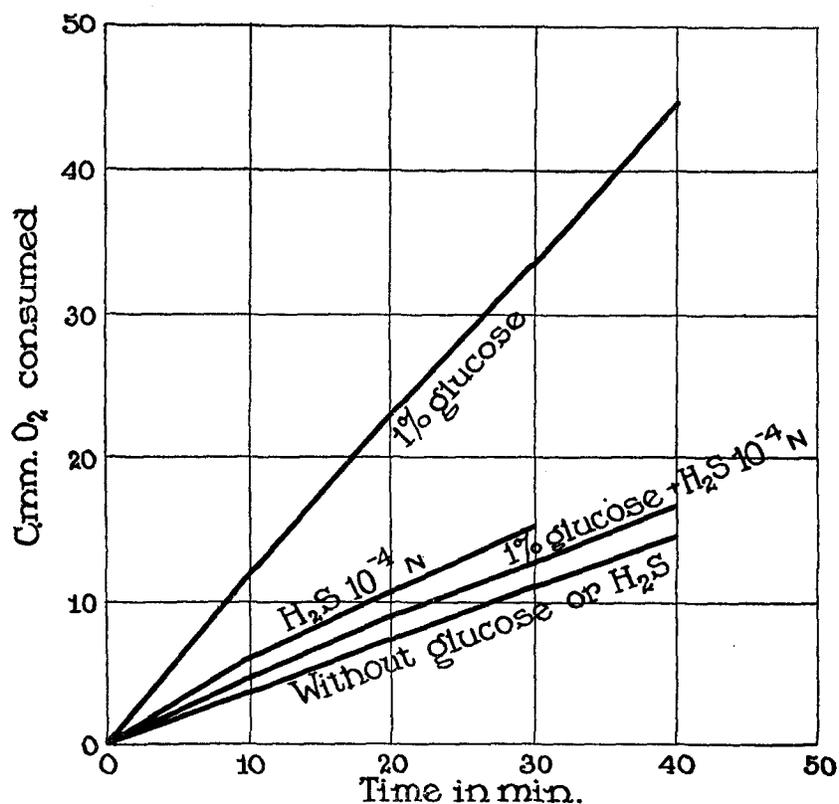


FIG. 4.

always contained acid phosphate which reacts with Na_2S to form free H_2S . (For the calculation of the correct amount of sodium sulfide to add in order to achieve a given concentration of hydrogen sulfide in the solution, see Negelein's paper on the effects of hydrogen sulfide.) Equilibrium between solution and the gas chamber is established, a large amount of hydrogen sulfide remaining in the gas chamber.

Fig. 4 shows the effect of hydrogen sulfide on ordinary *Chlorella* cells and on cells suspended in 1 per cent glucose solution. It is essentially the same as Fig. 1, which shows the corresponding curves for hydrocyanic acid.

The hydrogen sulfide effect was tested for reversibility, and it was found, like that of hydrocyanic acid, to be completely reversible. After removal of the hydrogen sulfide, respiration was greater than before it had been added.

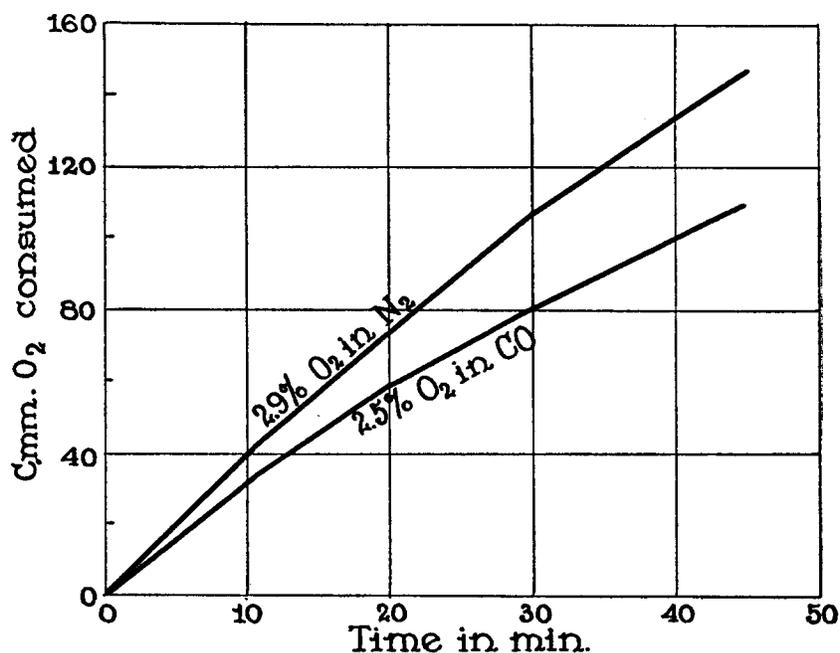


FIG. 5.

Experiments with Carbon Monoxide.

In the experiments with carbon monoxide, a mixture of oxygen and carbon monoxide replaced the usual 5 per cent carbon dioxide in air used in the gas space. As control the same amount of oxygen in nitrogen was used. Fig. 5 shows the effect of a mixture of approximately 2.5 volumes per cent oxygen in carbon monoxide on *Chlorella* suspended in a solution containing 1 per cent glucose. No curves are

given for the effect of carbon monoxide on ordinary *Chlorella* cells, for it does not affect their respiration.

The inhibition of respiration by carbon monoxide is completely reversible. Carbon monoxide was removed by passing the oxygen-nitrogen mixture through the gas chamber.

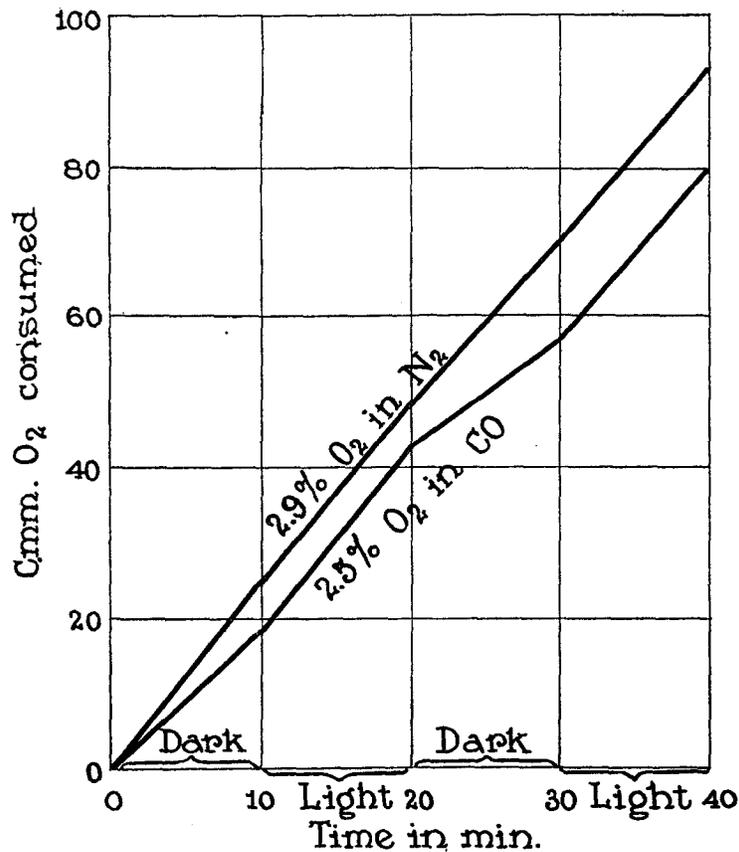


FIG. 6.

Warburg³ has shown that the inhibition of yeast respiration by carbon monoxide practically vanishes in light. This experiment cannot be performed with ordinary *Chlorella*, as photosynthesis takes place in the light. But yellow non-photosynthesizing *Chlorella*, practically chlorophyll-free, may be produced in a medium containing

1 per cent glucose and of low iron content. Such cells are completely heterotrophic, and their respiration is inhibited by hydrocyanic acid and hydrogen sulfide. Respiration is practically the same in light as in darkness. Fig. 6 shows the effect of successive periods of light and darkness on the respiration of such cells in 2.5 per cent oxygen in carbon monoxide, and in 2.9 per cent oxygen in nitrogen.

SUMMARY.

Chlorella, when made heterotrophic by means of certain sugars, respire like other heterotrophic cells when subjected to the respiratory inhibitors, hydrocyanic acid, hydrogen sulfide, and carbon monoxide.

Whether the case of *Chlorella* is typical for green cells in general remains to be seen. Experiments with various other green organisms are being carried out, in hope of settling this point.

My thanks are due to Professor Otto Warburg for suggestion and criticism during this work.