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English translation of the 1927 doctoral thesis (in German)# of Robert Emerson, a pioneer in Photosynthesis*

On the Effect of Hydrogen Cyanide, Hydrogen Sulfide and Carbon Monoxide on the Respiration of various Algae. Inaugural Dissertation for Obtaining a doctorate.

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English translation of the 1927 doctoral thesis (in German)[#] of Robert Emerson, a pioneer in Photosynthesis*

by Hartmut Lichtenthaler^a and Lars Olof Björn^b

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Note: We refer the readers, upfront, to Emerson (1927), as well a paper by Fu et al. (2019) that deals with the application of this area of research.

Emerson, R. (1927) The effect of certain respiratory inhibitors on the respiration of *Chlorella*. *J Gen Physiol* 10 (4): 469-477

Fu, W., Gudmundsson, S., Wichuk, K., Palsson, S., Palsson, B.O., Salei-Ashtiani, K., and Brynjolfsson, S. (2019) Sugar-stimulated CO₂ sequestration by the green microalga *Chlorella vulgaris*. *Sci.Total Environm.* 654, 275-283.

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*Invited and edited, before submission to *J Plant Sci. Res.*, by Govindjee Govindjee (Department of Plant Biology and Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; email: gov@illinois.edu)[#] also see the preceding “Letter to the Editor”.

[#]Emerson, R. (1927) Über die Wirkung von Blausäure Schwefelwasserstoff und Kohlenoxyd auf die Atmung verschiedener Algen. Friedrich Wilhelms-Universität zu Berlin, 32 pages

On the Effect of Hydrogen Cyanide, Hydrogen Sulfide and Carbon Monoxide on the Respiration of various Algae

Inaugural Dissertation for Obtaining a doctorate

Approved by the Faculty of Philosophy of the Friedrich-Wilhelms University of Berlin

by Robert Emerson from New York, U.S.A.

Day of Promotion: 11th October 1927

Referees: Professor Dr. Hans Kniep and Prof. Dr. Ludwig Diels

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Translated in English in 2020 by Hartmut Lichtenthaler and Lars Olof Björn

It had been shown in Warburg's laboratory that, with the exception of *Chlorella*, the respiration of various cells and tissues is specifically and reversibly inhibited by hydrogen cyanide, hydrogen sulfide and carbon monoxide. These substances do not act according to their adsorption constants like narcotics, but at much lower concentrations. According to the calculations of Warburg¹⁾ a 1/10 normal hydrogen cyanide would be required to inhibit the respiration via adsorption to the same degree as the weakest narcotics do. In reality the respiration is already strongly inhibited by a 1/10000 normal solution. According to its adsorption constant hydrogen cyanide should have no measurable effect at this concentration.

The adsorption constants of hydrogen sulfide and carbon monoxide are of the same order of magnitude as that of hydrogen cyanide. The inhibition of respiration generally caused by these substances are hence unique and not due to abnormal adsorption phenomena.

The behavior of the green alga *Chlorella* with respect to these substances is clearly an exception. Warburg²⁾ has shown that hydrogen cyanide does not only fail to block the respiration of *Chlorella*, but even enhances the respiration. Later Negelein³⁾ also demonstrated that hydrogen sulfide does not induce an inhibition but rather causes an enhancement.

For this noteworthy behavior of the respiration of *Chlorella* there is presently no explanation. My

own experiments demonstrate that several other green algae respond in a similar way. In the present investigation it is shown, however, that the respiration of *Chlorella* responds completely normally when the cells have been treated beforehand in a particular way: that is the respiration is strongly inhibited by small amounts of hydrogen cyanide, hydrogen sulfide and carbon monoxide. In addition, the respiration of several other algae has been studied.

I. Survey on previous Experiments with Hydrogen Cyanide, Hydrogen Sulfide and Carbon monoxide

1. The effect of hydrogen cyanide

That hydrogen cyanide is strongly poisonous for living organisms is well known already since a long time. In 1857, Claude Bernard has described the effect of cyanide on blood containing organisms in his "*Leçons sur les effets des substances toxiques*". He realized that after a cyanide poisoning the blood of veins was red. He also knew that cyanide inhibits the alcoholic fermentation.

Since then many researchers have worked in this field. However, only relatively few careful and further measurements have been performed with living cells. In this context I will concentrate on those experiments in which the effect of cyanide on the respiration (oxygen consumption, and evolution of carbon dioxide) has been measured directly. The rather

numerous observations on the impact of cyanide on streaming of protoplasm, catalase activity, growth and development of spores, although being quite interesting, do not play any role in this context, because it is not known how these processes are related to respiration. Reviews of these topics are given in an article by Hyman⁴.

It has been shown in a number of cases that the respiration of animal cells is fairly sensitive towards cyanide. In sponges, Hyman⁵ already found a respiration inhibition in a 10^{-5} normal KCN solution. Below this concentration (5×10^{-6} normal) he found already some acceleration. 10^{-4} normal KCN resulted in an inhibition of respiration by ca. 70%. He also obtained similar results with planaria⁶. Vernon⁷ measured the oxygen consumption of the kidney and found it strongly reduced by cyanide (0.1-0.2%). During his investigation the cyanide concentration decreased steadily and the oxygen consumption increased continuously; for this reason it is not possible to clearly indicate the percentage effect. Warburg⁸ observed a 50% inhibition of the respiration of blood of geese by 5×10^{-4} normal cyanide and a 70% inhibition of that of sea urchin eggs by 10^{-4} normal cyanide solution.

For the effect of cyanide on the respiration of plants there exist only few measurements. Meyer⁹ has determined the respiration of shoots of *Tropaeolum* with and without cyanide. He observed a strong inhibition by cyanide. Due to the facts that he worked with relatively concentrated solutions and did not find a recovery of the respiration after removal of cyanide, one must assume that the cells in his experiments had been partially damaged. In the case of yeast, Mayer had found a reversible inhibition of respiration and fermentation by cyanide.

Schroeder¹⁰ chose *Aspergillus* as investigation object and measured CO_2 evolution and oxygen consumption under normal conditions and in the presence of KCN. Both processes were strongly and reversibly inhibited by a 10^{-3} normal KCN solution.

Negelein¹¹ investigated the respiration of fermenting yeast and found a full inhibition by 10^{-4} normal cyanide solution. Finally, Warburg¹²

investigated the respiration of *Chlorella* and observed no inhibition by cyanide, instead a strong acceleration even at relatively high cyanide levels. Only at a 1/10 normal solution, when cyanide already acts as narcotic, could he find some inhibition.

2. The effect of hydrogen sulfide

The effect of hydrogen sulfide on living processes has been investigated much less than that of cyanide. Already in 1933 Berzelius mentioned in his Handbook of Chemistry some observations of Thénard. Thénard observed that the presence of small amounts hydrogen sulfide in the air were deadly for horses, birds or dogs. From Thénard's observations Negelein calculated that a concentration of not more than 5×10^{-5} molar hydrogen sulfide in the blood serum is deadly.

Moreover, Negelein¹³ dealt with the effect of hydrogen sulfide on diverse life processes. He investigated respiration and fermentation of yeast as well as the reduction of nitrate, the CO_2 assimilation and respiration in *Chlorella*. In each case he found for hydrogen sulfide a similar effect as for cyanide. Finally, he compared the effect of both agents for 10^{-4} molar solutions and found utmost congruence. The respiration of yeast was inhibited and the respiration of *Chlorella* was enhanced.

3. The effect of carbon monoxide

Claude Bernard¹⁴ recognized carbon monoxide as a strong blood poison and compared its effect with that of cyanide. He added that 1/6 carbon monoxide in the atmosphere has no impact on the alcoholic fermentation. Moreover he mentions that carbon monoxide inhibits the germination of cress seeds, and also mentions that the same seeds germinate quite normally when the seeds are brought back to normal air. From this follows that plants are much more insensitive as compared to blood containing animals. Wehmer¹⁵ mentions that a 50% mixture of carbon monoxide in air has no damaging effect on barley and cress seedlings. The growth may have been perhaps somewhat hindered.

However, Warburg¹⁶ was the first who found an inhibition of respiration by carbon monoxide, particularly at a relatively high carbon monoxide

pressure. He applied gas mixtures of oxygen and carbon monoxide and used the same amounts of oxygen mixed with nitrogen as control. His experiments showed that the respiration of baker's yeast is inhibited to about 60% in a mixture of 10% oxygen in carbon monoxide. In a *Micrococcus* and in baker's yeast he found similar inhibitions of respiration. The respiration of red blood cells of birds was only little inhibited.

Moreover, he indicates the following: "In the presence of carbon monoxide and oxygen the respiration-ferment is portioned between both gases. For this reason, a certain carbon monoxide pressure inhibits respiration the more the lower the oxygen pressure". The inhibition is not dependent upon the absolute amount of carbon monoxide, but upon the ratio O_2/CO .

According to my experiments, a mixture of 5% oxygen in carbon monoxide has almost no effect on the respiration of *Chlorella*. An enhancement, if it should exist, is practically not measurable. Clearly, neither do cyanide and hydrogen sulfide nor carbon monoxide inhibit the respiration of *Chlorella*. Cyanide and hydrogen sulfide even enhance the respiration. As is shown on the following pages this unique behavior of *Chlorella* disappears when the algae are brought into a sugar-containing solution of a proper concentration.

II. Description of Methods

1. Cultivation methods for algae

Algae were cultivated according to the method of Warburg and Negelein¹⁷⁾ in flasks with integrated gas inlet pipes. The flasks contained 250 to 300 ml nutrition solution and were kept in a glass bowl which was continuously illuminated from below via three 75 watt metal filament lamps. The temperature was kept constant within 1.5°C using a water stream flowing through the bowl. For the cultivation of *Oscillatoria* the streaming water was beforehand warmed up with a gas flame and the temperature was thus kept between 26 and 27°C. For the different Chlorophytes the temperature was kept between 21 and 22°C with unheated regular tap water. The culture flasks were connected with each other via rubber hoses and a

gas mixture of ca. 5% CO_2 in air was blown through the flasks. About one liter gas per hour was led through the flasks.

The nutrient solution applied was that of Warburg and Negelein¹⁷⁾, however, only half as strong. It was made in the following way:

50 ml were taken from the solutions A, B and C and 0.5 ml from solution D and diluted with tap water up to one liter. The tap water of the institute

Stock solution A	$MgSO_4 \cdot 7H_2O$	50g in distilled water 1000 ml
Stock solution B	KNO_3	25g in distilled water 1000 ml
Stock solution C	KH_2PO_4	25g in distilled water 1000 ml
Stock solution D	$FeSO_4 \cdot 0$	28g in distilled water 100 ml

contains, according to Warburg and Negelein 2.4×10^{-3} moles of calcium per liter and 1×10^{-6} moles of iron per liter. Hence, the final solution had the following composition:

$MgSO_4$	0.01	molar
KNO_3	0.0125	molar
K_2PO_4	0.009	molar
Ca	0.0024	molar
Fe	0.000006	molar

This nutrient solution we have termed Knop's solution. When it is sterilized in an autoclave, one obtains a flaky, white sediment, which, however, disappears when an air CO_2 mixture is blown through the solution.

The algal material was taken from agar stock cultures grown in daylight and inoculated into the sterile culture flasks. After the algal cells had multiplied well they were centrifuged off and used for experiments. The experiments were not carried out under sterile conditions. However, errors caused by bacteria are negligible. The amounts of algal cells being brought into the measuring vessels (ca. 0.2 cm^3 cells per 7 cm^3 suspension solution) were so large that a few accidental bacteria could not affect the results. That bacteria could multiply to any extent within the brief time of an experiment in an inorganic

suspension culture is impossible. During the normal procedure the measuring vessels were taken from the drying closet (temperature 115°C) shortly before their filling and also the centrifuge tubes, etc. Hence, one could expand the tests to more than four hours, even in cases with 1% glucose-containing solutions. When, after the experiment, the algal cells had been centrifuged off, the supernatant was not turbid from bacteria. No experiment with a sugar containing solution was performed longer than four hours. Most assays lasted ca. 30–120 minutes.

Since the respiration intensity depends upon the light level at which the cells were cultivated, one has to take the following into account. When the cells are taken directly from the artificially illuminated cultures they show a small yet easily measurable respiration, which from culture to culture is fairly constant. When the cells are kept at room temperature in the dark, the respiration slowly decreases until it is no longer measurable. Cells cultivated under diffuse daylight in general exhibit a lower respiration rate than the more strongly illuminated cells that I have used.

The influence of various substances depends on the intensity of the respiration. Glucose enhances the respiration of illuminated cells fourfold. However, when one takes ‘strongly’ starved cells which either had been in darkness for a long time or were grown in a weaker light, glucose can increase respiration hundred fold.

All results being described here refer to the respiration of light grown cells.

For the respiration measurements the cell suspension cultures of the culture flasks were once or several times washed with nutrient solution in the centrifuge and then pipetted into the experimental vessels.

2. The types of algae

For my culture of *Eudorina* I thank Dr. Belar. The other algae were provided by Miss Canabaeus of the Prussian Institution for water hygiene. I wish to express here my sincere thanks to Miss Canabaeus.

1. *Chlorella sacharosa*. Cells oval, width 2,4 – 6 μm , length 4.8 – 7.2 μm . Without pyrenoid,

chromatophore lobular, membrane thin.

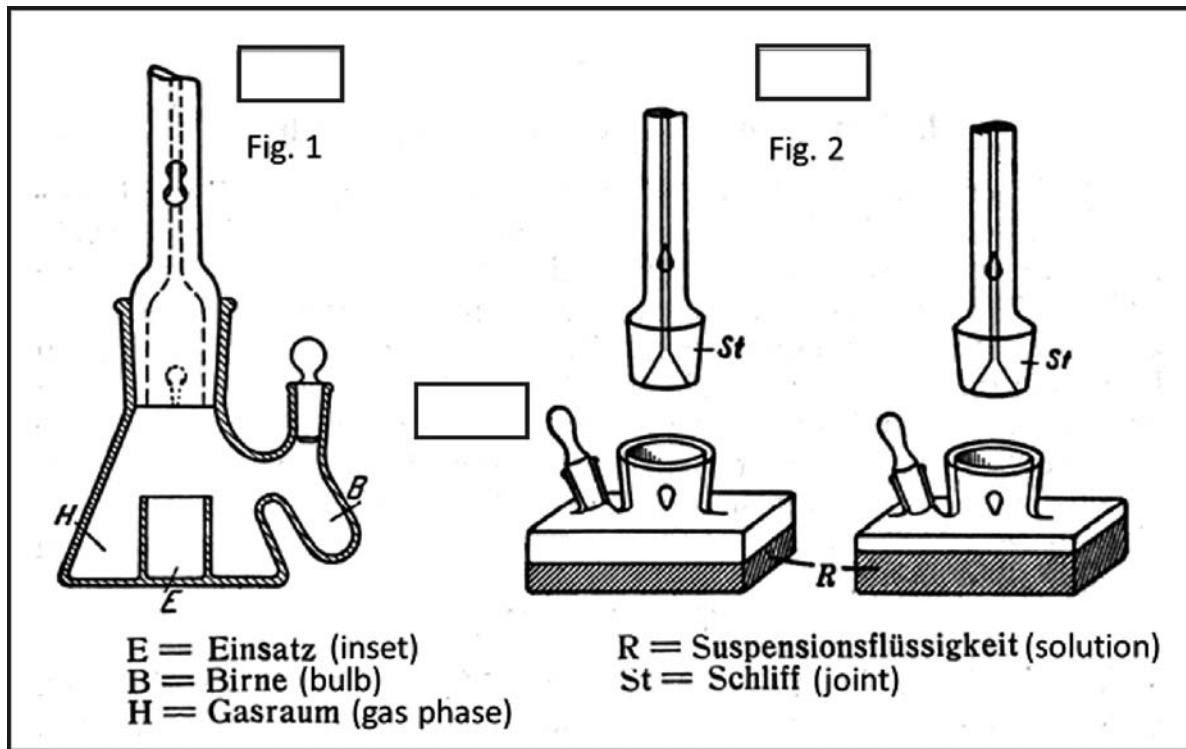
2. *Chlorella pyrenoidosa*. Cells globular, diameter 3 – 4.8 μm . Pyrenoid large and distinct, chromatophore bell-shaped, covering almost the whole wall surface. Membrane thin.
3. *Chlorella vulgaris*. Cells globular, diameter 4.8 – 10.8 μm . Pyrenoid not clear, chromatophore bell-shaped, membrane thin.
4. *Stichococcus bacillaris*. Cells cylinder-like, width 1.8. – 2.4 μm , length 4 – 7.2 μm . Tender peel, 1 – 2 pyrenoids, ends rapidly constricted.
5. *Oscillatoria implicissima*. Cell breadth 7.2 – 8.4 μm , height 3 – 5 μm . Not constricted, unclearly granulated, end cells not pointed, but bluntly rounded.

3. Measuring methods

The respiration was determined manometrically using a method carefully described by Warburg¹⁸⁾. Two different vessels which are shown in Figs. 1 and 2 were used. In the vessels of Fig. 1 the CO_2 formed by respiration was absorbed by potassium hydroxide in the inset well “E”, whereas in the vessels of Fig. 2 the respiration CO_2 remained in the suspension solution.

The vessels were filled with precisely measured amounts of cell suspension cultures. Then each vessel was connected to its manometer and was shaken in a water thermostat in the dark. Shaking is required because gas phase and suspension solution must be in equilibrium at anytime. Equilibrium was always attained so fast that a change of the speed had no influence on the results, i.e. the results were fully independent of the shaking speed. This had to be verified in each experiment. In general a speed of ca. 80 excursions per minute was sufficient.

The shaking was interrupted after defined time intervals (e.g. every 10 minutes) in order to read the manometer values. Together with the suspension vessels one vessel without cells but filled with some suspension solution was placed as barometer into the thermostat water. The manometer of this vessel was regularly measured with the others in order to correct



for changes in the atmospheric pressure. The mm³ of consumed oxygen were then determined using the corrected level changes.

4. Calculation of the oxygen consumption

During respiration, carbon dioxide is formed and oxygen is consumed. Our main interest was in the oxygen consumption. When working with vessels according to Fig. 1 where the CO₂ being formed is continuously absorbed by potassium hydroxide, we can directly calculate the oxygen consumed from the readings of the pressure changes. We have a closed gas space, whose volume and temperature remain constant. Hence, the consumed oxygen is proportional to the pressure change. V, the volume of the whole vessel until the sealing liquid in the manometer, and V_F, the volume of the cell suspension are known in each case.

$$V - V_F = V_g,$$

Where, V_g is the volume of the gas phase to the sealing liquid. We have to consider the solubility of gases in the cell suspension. When a pressure change of h millimeter occurs, the consumed O₂ amount, X_{O₂} (at 0° C and normal pressure) is:

$$h \left[\frac{V_g \frac{273}{T} + V_F \times \alpha_{O_2}}{P} \right]$$

Here T is the absolute temperature of the thermostat, α_{O₂} is the Bunsen absorption coefficient of the gas (oxygen) at T⁰, and P is the normal pressure of the sealing liquid in millimeter (a Brodie solution 10 000 millimeter corresponding to 760 millimeter mercury served as sealing liquid). When V_G and V_F are expressed in cubic millimeter, then one obtains X_{O₂} in cubic millimeter.

For a certain liquid volume and defined temperature the value in parenthesis, mentioned above, remains constant. Hence, we can write: X_{O₂} = h k_{O₂}. The factor k is always positive. When h is negative, then X becomes negative, i.e. gas will be consumed. However, if h is positive, then X will be positive, i.e. gas had been produced.

This way of calculation is only valid when only one gas is consumed or produced; this happens in our case only when CO₂ produced via respiration will be absorbed by potassium hydroxide. However, when one

wants to measure the respiration in the presence of hydrogen cyanide or hydrogen sulfide, it is better to work without potassium hydroxide, because both are absorbed by potassium hydroxide. In this case one uses the vessels shown in Fig. 2. In this case the reading of the pressure change “h” corresponds to the sum of pressure changes by the consumed oxygen (h_{O_2}) and the produced CO_2 (h_{CO_2}).

$$h = h_{O_2} + h_{CO_2}$$

Moreover: $X_{O_2} = h_{O_2} \times k_{O_2}$

$$X_{CO_2} = h_{CO_2} \times k_{CO_2}$$

Here, of interest is mainly X_{O_2} ; since we have four unknowns and only three equations, we cannot directly determine X_{O_2} . When, however, we consider that h is dependent on V_g and V_f we can perform two measurements under the same conditions: one where V_f is relatively large and V_g relatively small, and the other where V_f is relatively small and V_g is relatively large. When we mark all values where V_f is large with upper case letters, and with lower case letters when V_f is small, then we obtain six equations:

We may have in the vessels either equal cell amounts or equal cell concentrations.

$$\begin{aligned} H &= H_{O_2} + H_{CO_2} \\ X_{O_2} &= H_{O_2} \cdot K_{O_2} \\ X_{CO_2} &= H_{CO_2} \cdot K_{CO_2} \\ h &= h_{O_2} + h_{CO_2} \\ x_{O_2} &= h_{O_2} \cdot k_{O_2} \\ x_{CO_2} &= h_{CO_2} \cdot k_{CO_2} \end{aligned}$$

When we work with equal cell amounts, then the following holds:

$$X_{O_2} = x_{O_2}$$

$$X_{CO_2} = x_{CO_2}$$

In total, then, we have eight equations. These yield:

$$X_{O_2} = \frac{hk_{CO_2} - HK_{CO_2}}{\frac{k_{CO_2}}{k_{O_2}} - \frac{K_{CO_2}}{K_{O_2}}} \quad (1)$$

$$X_{CO_2} = \frac{hk_{O_2} - HK_{O_2}}{\frac{k_{O_2}}{k_{CO_2}} - \frac{K_{O_2}}{K_{CO_2}}} \quad (2)$$

However, it is much more correct to work with equal cell concentrations. In this case we have:

$$X_{O_2} = \frac{V_f}{v_f} \cdot x_{O_2}$$

$$X_{CO_2} = \frac{V_f}{v_f} \cdot x_{CO_2}$$

and

$$X_{O_2} = \frac{\frac{V_f}{v_f} \cdot h \cdot k_{CO_2} - HK_{CO_2}}{\frac{k_{CO_2}}{k_{O_2}} - \frac{K_{CO_2}}{K_{O_2}}} \quad (3)$$

$$X_{CO_2} = \frac{\frac{V_f}{v_f} \cdot h \cdot k_{O_2} - HK_{O_2}}{\frac{k_{O_2}}{k_{CO_2}} - \frac{K_{O_2}}{K_{CO_2}}} \quad (4)$$

The oxygen consumption was calculated either according to equation (1) or using equation (3), depending on whether equal cell amounts or equal cell concentrations have been used. In general, I have worked with equal concentrations.

5. Composition of the gas space

For assays using the vessels with potassium hydroxide in the inset (center well) simply air was applied in the

gas phase. In other cases a gas mixture of 5% CO₂ in air was blown through the gas space and the suspension saturated with it. In assays with carbon monoxide, that had been performed in vessels with the potassium hydroxide inset, special gas mixtures were applied as indicated below.

6. Addition of hydrogen cyanide, hydrogen sulfide or carbon monoxide

Hydrogen cyanide had always been freshly prepared by mixing ten cm³ of a 1/10 molar KCN solution with one cm³ normal sulfuric acid. This solution was diluted by distilled water as needed, and placed into the vessels with the cell suspensions. Simultaneously, the same amount of distilled water was given into the control vessels.

Hydrogen sulfide was produced within the suspensions by addition of calculated amounts of sulfide. The suspension solution always contained acidic phosphate in large amounts in relation to the added sulfide amounts. Hence, all of the sodium sulfide was immediately converted to hydrogen sulfide. However, since a considerable amount of hydrogen sulfide immediately diffuses into the gas space of the vessel, one has to apply some extra sodium sulfide, in order to maintain a defined concentration of hydrogen sulfide in the gas space. According to Negelein¹⁹⁾ I have calculated

$$n = cx 10^{-3} \left[\frac{v_g \frac{273}{T} + V_f \times \alpha_{H_2S}}{\alpha_{H_2S}} \right]$$

where, n means the number of moles of Na₂S which must be brought into the vessel in order to produce in the solution a concentration of hydrogen sulfide of c moles per liter.

Carbon monoxide was always mixed in the gas space with a defined amount of oxygen. In this case about the same amount of oxygen was mixed with nitrogen and lead into the control vessel. However, it turned out that the respiration of all investigated algae was independent of the oxygen pressure, at least between the limits of 3 – 20 percent by volume. Carbon monoxide was mixed with oxygen in a glass ball of

ca. 800 cm³ volume filled with mercury.

7. Determination of the reversibility of the inhibition

Each respiration inhibition was checked for reversibility. The inhibiting substance was removed by replacing the carbon monoxide oxygen mixture by the same amount of oxygen in nitrogen. Hydrogen cyanide and hydrogen sulfide were flushed out by bubbling air through the solution. The cell suspensions were brought into a Folin's flask and air was sucked through it via a water-jet pump. Sometimes, hydrogen cyanide and hydrogen sulfide were also removed by washing out, by using a centrifuge. However in such cases a small amount of cells were lost. For this reason, flushing the gases out was preferable.

It should be mentioned here that all respiration inhibitions described here were partially reversible. After removal of hydrogen cyanide and hydrogen sulfide the respiration was always higher than normal. Only in the case of *Oscillatoria* it was not possible to fully remove the inhibition caused by hydrogen cyanide.

EXPERIMENTAL PART

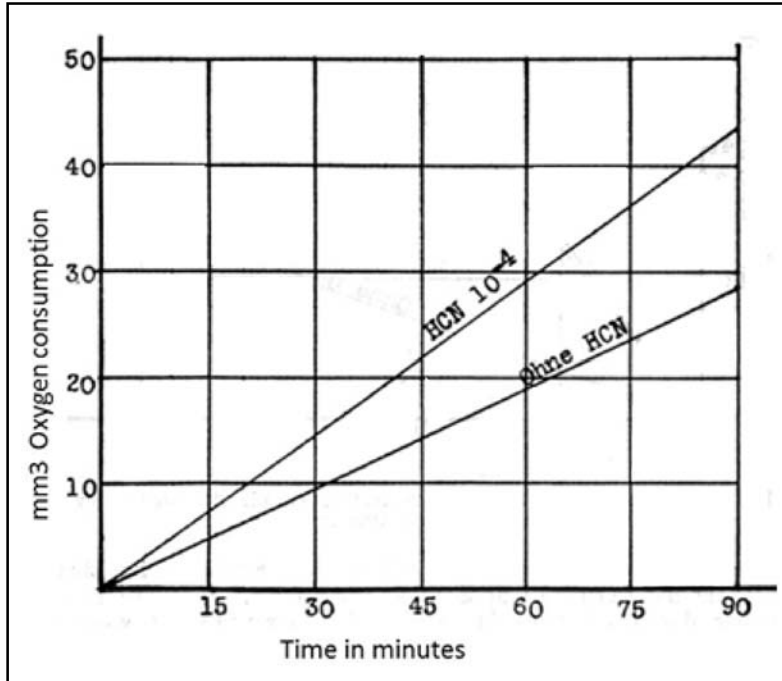
1. Assays with *Chlorella*

Assays were performed with three different *Chlorella* species, *C. pyrenoidosa*, *C. saccharosa* and *C. vulgaris*. The latter was best suited for the experiments described here. This *Chlorella* respire, at 20°C, about 2.5 mm³ oxygen per hour per mg dry matter. As Warburg has described, hydrogen cyanide does not inhibit respiration, but enhances it. The enhancement by 10⁻⁴ molar hydrogen cyanide is shown in Curve 1. [Note : Emerson used the word "Curve" for what we will now call "Diagram" or better "Figure", we have left the original word for historical reasons.] One should not attach importance to this enhancement because the respiration of *Chlorella*, as is well-known, is accelerated by diverse substances. There the effect is quite unspecific. Essential is only that hydrogen cyanide does not cause an inhibition.

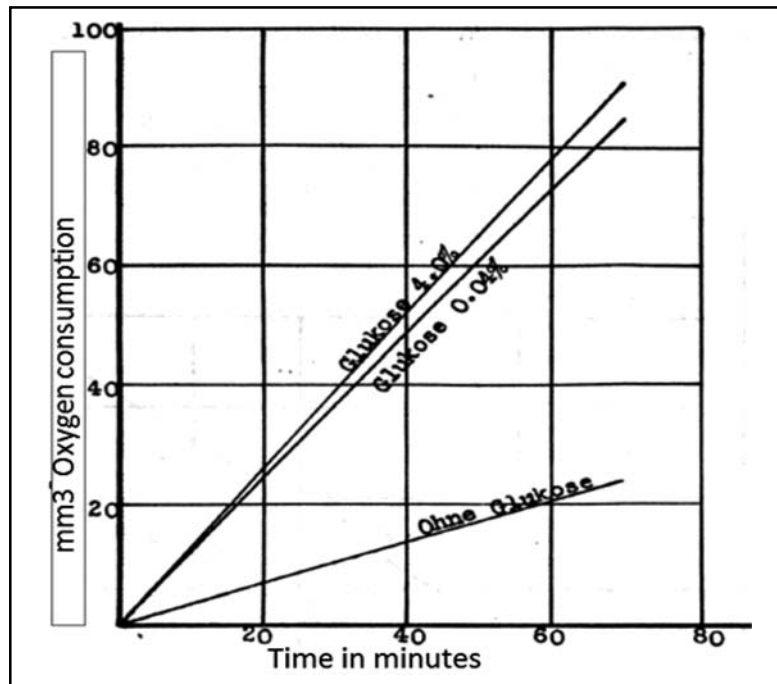
Some substances are, however, able to specifically enhance the *Chlorella* respiration, in particular glucose. Because the cells of the *Chlorella*

species used were particularly permeable to glucose, the respiration immediately increased fourfold, after they had been placed into a glucose containing solution.

This was a great advantage here because in the other species the respiration enhancement only occurs after



Curve 1. Influence of hydrogen cyanide (HCN 10⁻⁴ molar) on the respiration (without added sugar) of *Chlorella*. [Ohne HCN = control without HCN].



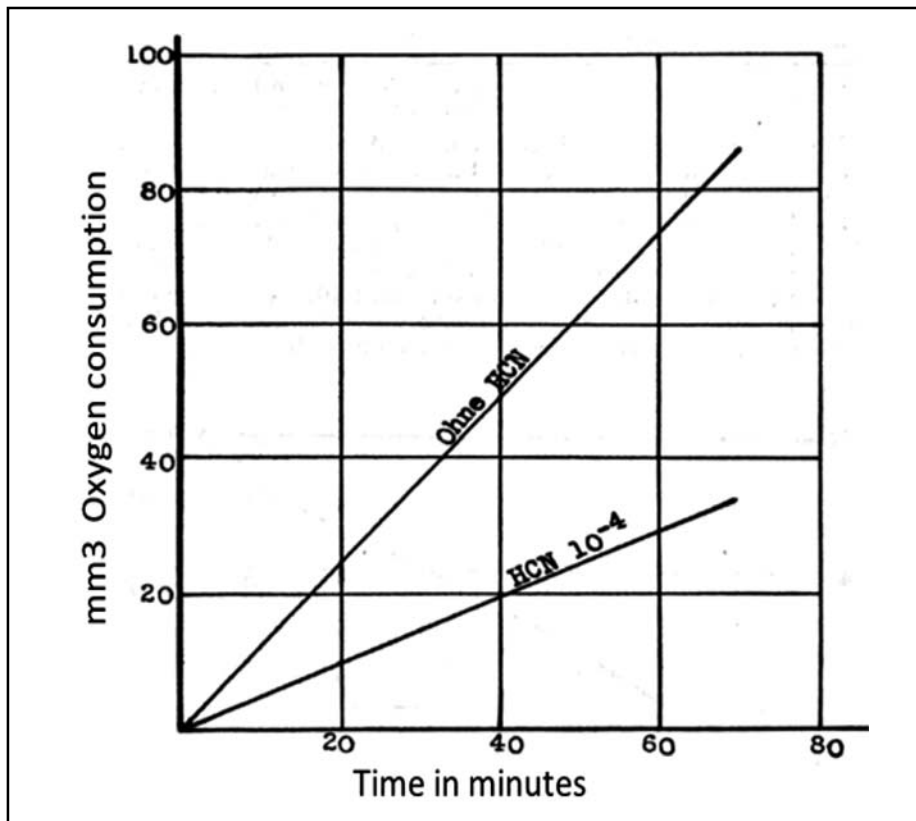
Curve 2. Influence of the glucose concentration (glucose 4.0% and 0.04%) on the increase of the respiration of *Chlorella*. [Ohne Glukose = without glucose].

four hours.

As can be seen from Curve 2 the respiration increase is fairly independent from the glucose concentration. It remains between 0.04 to 4.0% glucose always about the same. This fourfold increased respiration can be easily inhibited by hydrogen cyanide. A 10^{-4} molar solution, which does not inhibit the normal respiration, inhibits the enhanced respiration to 60 %.

According to assays of Osterhout²⁰) and Krehan²¹ hydrogen cyanide has certain effects on the

permeability. Therefore, it appears to be possible that there does not occur a real respiration inhibition, but an inhibition of the import of the sugar. However, that this is not the case, and that here it is really an inhibition of the oxidations within the cells, can easily be demonstrated by saturation of the cells with glucose before hydrogen cyanide is added. One can grow the cells in sugar-containing nutrition solution (Knop solution with 1% glucose), centrifuge them and transfer them in pure inorganic solution. They respire then as strongly as in glucose-containing solution, and the respiration is inhibited by 10^{-4} molar hydrogen cyanide to ca. 60%.



Curve 3: Influence of hydrogen cyanide (HCN 10^{-4} molar) in *Chlorella* cells suspended in 1 % glucose. [Ohne HCN = control without HCN].

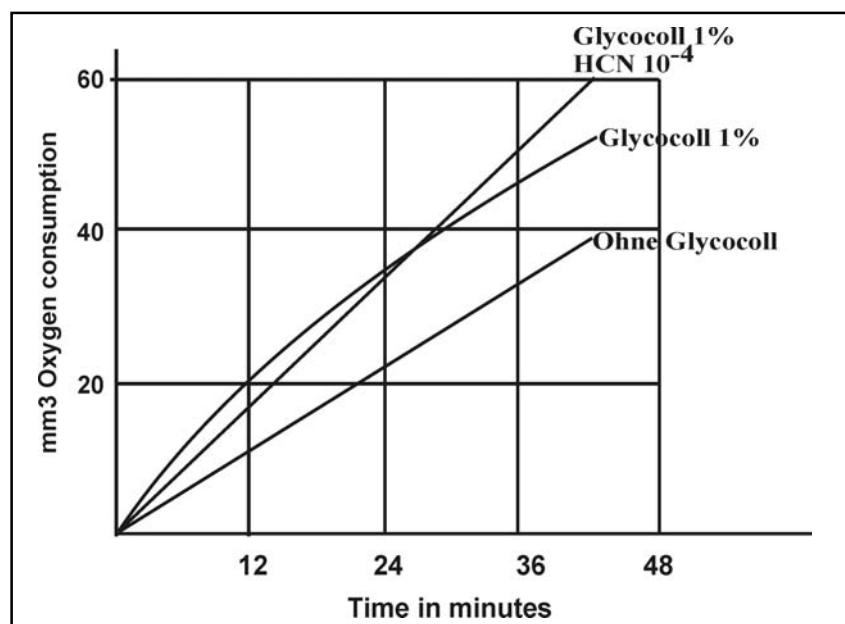
Table 1. Influence of glucose concentration on the effect of hydrogen cyanide on *Chlorella* during 1 h.

Glucose concentration	4%		0.04%	
HCN concentration (molar)	0	10^{-4}	0	10^{-4}
O ₂ consumption in mm ³	79.1	30.0	78.0	31.0
Inhibition. of O ₂ consumption by HCN	62%		60%	

It is still better to let the autotrophically (in inorganic solution) grown cells stand for some time (15–20 minutes) in a solution of 2% glucose, and then transfer them back to the inorganic solution, in order to measure the effect of hydrogen cyanide. One obtains again an inhibition by 10^{-4} molar HCN of about 60%.

Within the boundaries mentioned above (0.04 to 4%) the inhibition by hydrogen cyanide is quite independent of glucose concentration (cf. Table 1).

Various other compounds, which do not specifically speed up respiration, are also unable to render them sensitive to hydrogen cyanide. A number of substances were tested: Glycocoll (glycine),



Curve 4. Influence of hydrogen cyanide (HCN) 10^{-4} molar on *Chlorella* cells suspended in 1% glycocoll (glycine). [Ohne glycocoll = without glycocoll].

mannitol, arabinose, dihydroxyacetone, lactic acid (as lithium salt), and saccharose. All these substances elicit a small increase in respiration rate. This, however, is usually transient, as can be seen from Curve 4. In such cases one does not see any inhibition by 10^{-4} molar hydrogen cyanide. The effect for *Chlorella* cells in 1% glycocoll is displayed in Curve 4.

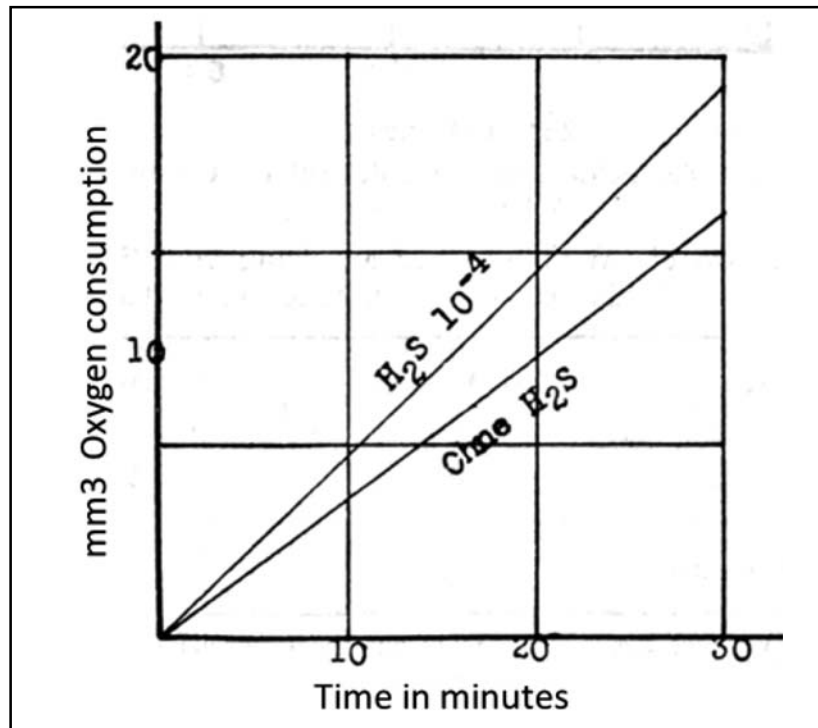
On the contrary, all hexoses gave the same effect as glucose. Experiments were carried out with fructose, galactose, and mannose. The respiration always increased about four times, and this increased respiration was inhibited by about 60% by 10^{-4} molar hydrogen cyanide.

Hexose diphosphate occupies a position intermediate between the hexoses and the non-specific compounds. It approximately doubles the respiration rate, and the inhibition by hydrogen cyanide is correspondingly

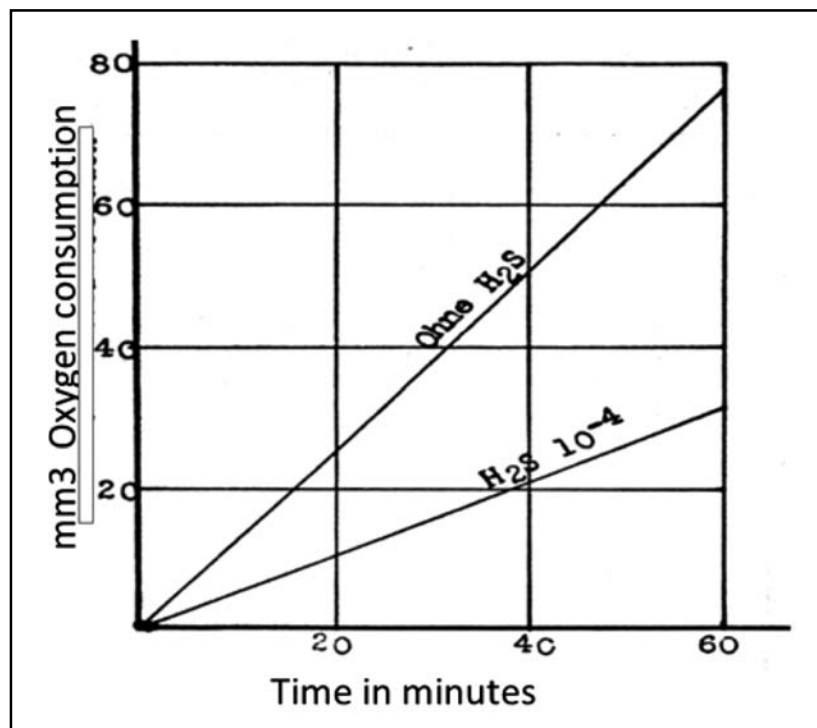
lowered as compared to glucose, but still obvious. 10^{-4} molar hydrogen cyanide inhibits to about 40%.

The conditions for hydrogen sulfide are the same as for hydrogen cyanide. As found by Negelein, hydrogen sulfide increases the respiration rate of *Chlorella* at concentrations that clearly inhibit already the respiration of yeast cells. The effect of 10^{-4} molar H_2S is shown in Curve 5. The respiration increase caused by glucose is again inhibited by H_2S to about 50%, cf. Curve 6.

Also carbon monoxide, which, as found by Warburg, reversibly inhibits respiration of yeast, has initially no effect on *Chlorella*. Based on Table 2, in which the effect of a mixture of 95% CO and 5% O_2 is shown, one could perhaps conclude a small inhibition, although not to the extent of that caused by HCN or H_2S .



Curve 5. Influence of hydrogen sulfide (H_2S 10^{-4} molar) on the respiration of *Chlorella* without glucose. [Ohne H_2S = without H_2S].



Curve 6. Influence of hydrogen sulfide (H_2S 10^{-4} molar) on the respiration of *Chlorella* in 1% glucose. [Ohne H_2S = without H_2S].

Table 2. Effect of carbon monoxide on the respiration of *Chlorella* without glucose

Gas mixture	5% O ₂ in N ₂	5% O ₂ in CO
Time in minutes	90 ¹⁾	90 ¹⁾
O ₂ consumption in mm ³	34.2	37.2
[¹⁾ Warburg, Biochem.Zschr. 119 , 148, 1921.]		

In Table 3 the effects of two different CO and O₂ mixtures are shown. In 95% CO+5% O₂ the inhibition is about 15%, while in 97.5% CO+2.5% O₂ it is about 30%. Here the CO concentration has been varied very

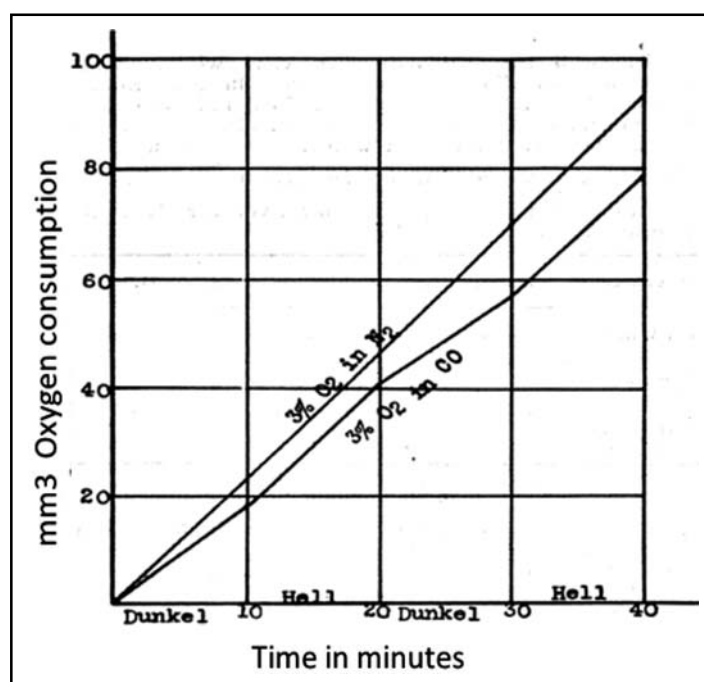
little, whereas the O₂/CO ratio has been changed considerably. From this one can conclude that the degree of inhibition depends on the O₂/CO ratio, and not on the concentration of CO.

Table 3. Effect of carbon monoxide on the respiration of *Chlorella* in 1% glucose

Gas mixture	5% O ₂ in N ₂	5% O ₂ +95% CO	3% O ₂ +97% CO
Time in minutes	30	30	30
O ₂ consume. in mm ³	28.2	23.5	19.5
% inhibition by CO	-	16.5	31

Warburg²²⁾ found that the CO inhibition of respiration by yeast was largely annihilated by light. As I have explained in earlier work²³⁾, a similar effect of light can be shown for *Chlorella*. For this experiment yellow, non-assimilating *Chlorella* cells

were used, since normal cells immediately start to assimilate in light. Yellow, almost chlorophyll-free *Chlorella* cells showing almost the same gas exchange in light as in darkness can be obtained by cultivation in iron-poor sugar-containing nutrient solution.



Curve 7. Influence of carbon monoxide (3% O₂ in CO) on the respiration of *Chlorella* in the dark (Dunkel) and the light (Hell). Control vessel: 3% O₂ in N₂.

Curve 7 shows the effect of changing “light” and “dark” periods on the respiration of such cells with and without CO. At each light or dark change the CO-curve shows a small kink. In the light the respiration is nearly normal, but it is inhibited in the dark.

The small oxygen partial pressure alone does not play any role for the respiration rate. Table 9 shows the oxygen consumed by *Chlorella* cells in 1% glucose solution with air and in a mixture of 3% oxygen+97% nitrogen.

From these experiments the following conclusions can be drawn:

Respiration of *Chlorella* increases fourfold in glucose solution. This increased respiration is inhibited to 60% by H₂S and CO, which are otherwise without effect.

HCN, H₂S, and CO are all compounds which specifically inhibit respiration. It is of interest to compare their effects to that of indifferent (ineffective) compounds. Also narcotics, which inhibit the respiration of all kinds of cells, including *Chlorella*, affect the respiration to a much greater degree when it is increased by glucose. At a concentration by which the respiration in glucose is already inhibited to 50%, elicits on the respiration without glucose only an acceleration.

According to Warburg, narcotics only produce an effect upon being absorbed. They displace the fuels from the sites of combustion. In order to demonstrate the role of adsorption for the inhibition, two members of a homologous series were used, ethyl and phenyl urethane.

Table 4. Effect of narcotics on the respiration of *Chlorella* cells.

Narcotic	Suspension liquid	Time, min.	O ₂ -consumption, mm ³	Effect of the narcotic
–	Knop, no glucose	30	20.6	
Ethyl urethane, 2%	Knop, no glucose	30	24.2	Acceleration 17.5%
–	Knop, no glucose	40	21.4	
Phenyl urethane, 0.1%	Knop, no glucose	40	32.8	Acceleration 55%
–	Knop+1% glucose	30	81.1	
Ethyl urethane, 2.5%	Knop+1% glucose	30	43.5	Inhibition 45%
–	Knop+1% glucose	30	44.6	
Phenyl urethane, 0.1%	Knop+1% glucose	30	29.0	Inhibition 35%

Their effects on *Chlorella* cells with and without glucose are shown next to one another in Table 4. Concentrations which only accelerate respiration when it is not stimulated by glucose are already sufficiently potent to strongly inhibit, e.g., avian blood cells; cf. Warburg²⁴. Thus, *Chlorella* is usually less sensitive to narcotics than other cells are.

About one hundred times more ethyl urethane than phenyl urethane is needed for inhibiting respiration to the same degree. Correspondingly, phenyl urethane is much more strongly adsorbed.

Thus, the glucose-stimulated *Chlorella* respiration exhibits a much greater sensitivity to all

the investigated inhibitors, specific or unspecific.

Experiments with *Eudorina* and *Stichococcus*

In addition to the three *Chlorella* species mentioned above, the respiration was investigated in two other chlorophyceans, *Eudorina* and *Stichococcus*.

Stichococcus that had been cultured in a purely inorganic solution had a respiration at 20° C of about 5 mm³ O₂ per hour per mg dry weight. It was very much increased by 10⁻⁵ to 10⁻² molar HCN. As can be seen from Table 5, it is increased by 10⁻⁴ molar HCN to 94%, i.e. almost doubled. From this one could assume that this acceleration would disappear in the

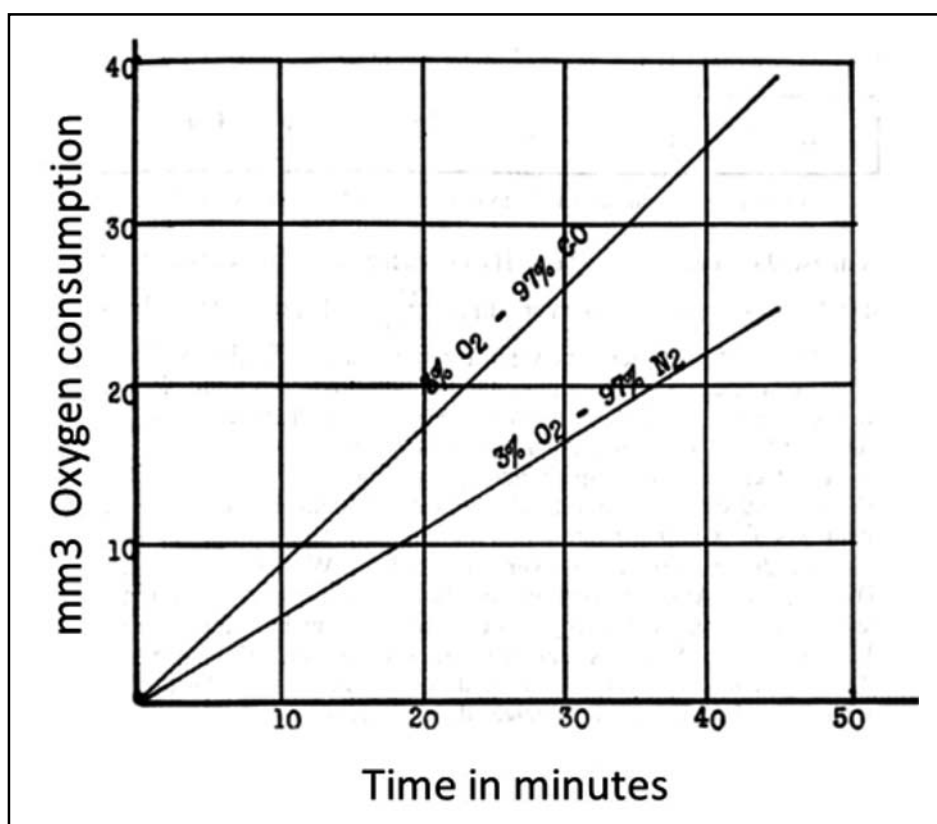
presence of sugar, as is the case for *Chlorella*. However, it has not been possible to achieve this sugar-induced respiration increase with *Stichococcus*, which is so easy with *Chlorella*. After four hours in 2% glucose, the respiration rate of *Stichococcus* increased by only 30%. As already mentioned, the experiments with glucose were no longer continued. Sucrose and various hexoses were also tried, but without a clear success. In addition, it has been assayed to accelerate respiration by small amounts of ethanol, this however without success. Finally, *Stichococcus* was grown in

glucose-containing solution. In this way one obtains cells that at 20°C respire about 8 mm³ O₂ per mg dry weight, that is not even twice as much as normal cells. One can glean from Table 5 the effect of the 10⁻⁴ molar hydrogen cyanide on such cells. The respiration increase is about 50%, i.e. only half as large as for purely autotrophic cells.

Also carbon monoxide has a stimulating effect on respiration of *Stichococcus*. The effect of 97% CO in O₂ is shown in Curve 8. The increase is about 50%.

Table 5. Effect of hydrogen cyanide (HCN) on the respiration of *Stichococcus*.

	Cells grown inorganically		Cells grown in 2% glucose	
HCN molarity	0	10 ⁻⁴	0	10 ⁻⁴
Time in minutes	50	50	45	45
O ₂ -consumption in mm ³	41.0	79.0	67.0	101.0
HCN-effect	94% respiration rate increase		50% respiration rate increase	



Curve 8. Influence of carbon monoxide (97% CO + 3% O₂) on the respiration of *Stichococcus*. Control vessel. 97% N₂ + 3% O₂.

Thus, it has not been possible to inhibit respiration of *Stichococcus* by those hydrogen cyanide concentrations that very clearly inhibit the glucose-stimulated respiration of *Chlorella*. However, it would perhaps be possible to inhibit respiration of *Stichococcus*, were it possible to stimulate it by glucose as is the case with *Chlorella*.

At 20°C *Eudorina* respire about 5 mm³ O₂ per hour per mg dry weight. The respiration rate is increased by about 30% by 10⁻⁴ molar HCN, but even 10⁻³ molar suffices for achieving some inhibition; cf. Table 6.

Carbon monoxide does not inhibit respiration, but increases it. 95% CO in O₂ increases respiration by about 20% (Table 7).

The respiratory inhibition of *Chlorella* by carbon monoxide described above depends on the O₂/

CO ratio. The acceleration of the *Eudorina* respiration by carbon monoxide is not dependent on this ratio, but from the concentration of carbon monoxide. In 20% O₂ and 80% CO the respiration is accelerated to almost the same extent as in 3% O₂ and 97% CO. For this effect of CO, the O₂ concentration is of no importance. From Table 9 it is evident that the partial pressure of oxygen alone does not affect the respiration.

The three different chlorophycean algae (*Chlorella*, *Eudorina*, *Oscillatoria*) behave in essence similarly. The normal respiration is neither inhibited by CO nor by HCN. On the contrary, it is usually more or less accelerated. When it is possible to increase respiration considerably, as is the case with *Chlorella*, this respiration will be clearly inhibited by a concentration that does not inhibit the ordinary respiration.

Table 6. Influence of hydrogen cyanide on the respiration of *Eudorina*.

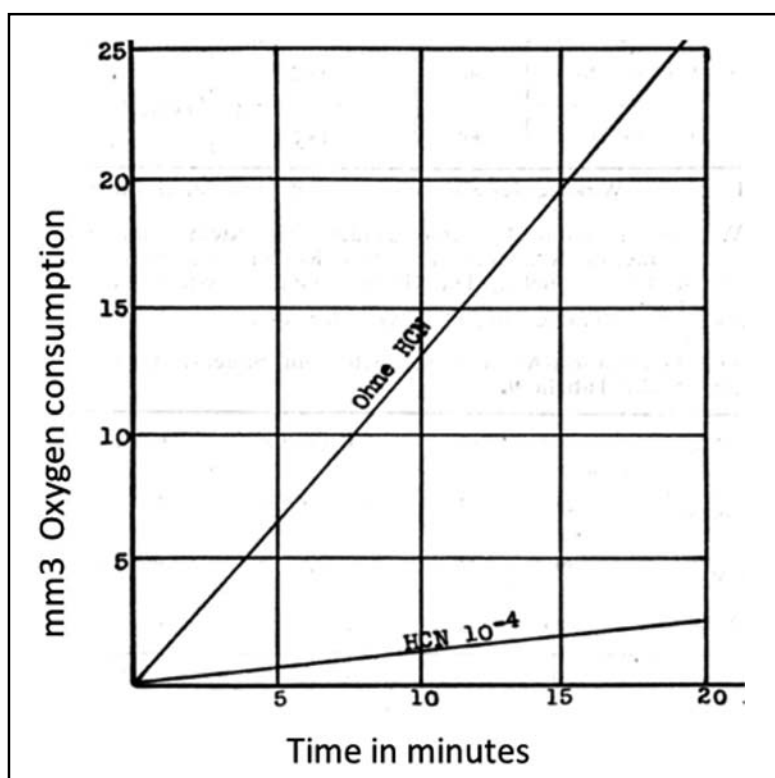
HCN (molar)	0	10 ⁻⁴	10 ⁻³
Time (min.)	30	30	30
O ₂ consumption (mm ³)	49.7	62.9	42.0
HCN effect	–	Acceleration 27%	Inhibition 12%

Table 7. Effect of carbon monoxide on the respiration of *Eudorina*.

Gas mixture	Time (min.)	O ₂ -consumption (mm ³)	CO-effect
3% O ₂ in N ₂	60	50.1	
3% O ₂ in CO	60	60.9	Acceleration 21%
20% O ₂ in N ₂	40	41.3	
20% O ₂ in CO	40	51.8	Acceleration 25%

For comparison, a completely different alga, *Oscillatoria*, [initially and also by Emerson 1927 regarded as a blue-green alga belonging to Chlorophyceae, is today known as a cyanobacterium] was investigated. Its respiration proved to be very sensitive to hydrogen cyanide. It was cultivated just

as *Chlorella*, in a purely inorganic solution. Despite this 10⁻⁴ molar HCN inhibits respiration by 80%; cf. Curve 9. Thereby, respiration is of about the same magnitude as for *Chlorella*. At 25° C, it amounts to about 2.5 mm³ oxygen consumption per mg dry weight per hour.



Curve 9. Influence of hydrogen cyanide (HCN 10⁻⁴ molar) on the respiration of *Oscillatoria*. [Ohne HCN = without HCN].

Also carbon monoxide is easily able to inhibit respiration, but the effect is very small. With a mixture of 3% O₂ and 97% carbon monoxide the inhibition is about 10%; cf. Table 8.

percent carbon monoxide in 10% oxygen does not inhibit any more. The inhibition is again dependent on the O₂/CO ratio, and not on the concentration of carbon monoxide.

As can be expected, this small inhibition disappears at higher oxygen concentration. Ninety

The respiration of *Oscillatoria* is also independent of oxygen pressure; see Table 9.

Table 8. Effect of carbon monoxide on *Oscillatoria* respiration.

Gas mixture	Time (min.)	O ₂ -consumption (mm ³)	CO-effect
3% O ₂ +97% N ₂	30	16.6	
3% O ₂ +97% CO	30	14.2	Inhibition 12%
10% O ₂ +90 % N ₂	30	18.2	
10% O ₂ +90 % CO	30	18.7	No inhibition

Table 9. The effect of oxygen pressure on respiration of three different algae.

Alga type	<i>Chlorella</i>		<i>Eudorina</i>		<i>Oscillatoria</i>	
% O ₂ in N ₂	20	3	20	3	20	3
Time (min.)	30	30	30	30	30	30
O ₂ consumption (mm ³)	34.0	34.1	31.2	30.9	16.5	17.9

Final remarks

The main conclusions of the present work are as follows:

1. Respiration of *Chlorella* is very much (four times) increased by hexoses.
This increase is probably due to the combustion of hexoses by the cells.
2. The combustion of the sugar in the algal cells is inhibited by hydrogen cyanide, hydrogen sulfide, and carbon monoxide. The respiration increase elicited by sugar behaves normally, as it is inhibited by these substances, as is, e.g. the respiration of yeast.
3. Narcotics inhibit the respiration increase elicited by glucose to a much higher degree than the normal respiration.
4. The respiration of other algae investigated here is not inhibited by hydrogen cyanide and carbon monoxide, but accelerated as is the respiration of *Chlorella* that has not been stimulated by glucose.

5. One can give no special importance to this acceleration since the respiration of *Chlorella* can be accelerated by many indifferent compounds²⁵. Almost any foreign substance, combustible or not, is able to, more or less, accelerate the respiration of *Chlorella*.
6. An explanation is still lacking for the strange fact that hydrogen cyanide, hydrogen sulfide, or carbon monoxide normally do not inhibit respiration of green algae.
7. This behavior of green algae is no general property of autotroph organisms. The normal respiration of *Oscillatoria*, which is an autotroph as is *Chlorella*, is readily inhibited by hydrogen cyanide or carbon monoxide.

Protocol I

The experiment shows that respiration of *Chlorella* in glucose-containing solution is inhibited by hydrogen sulfide. Vessels according to Fig. 2, experimental temperature 20°C, in each vessel 0.15 cm³ cells, suspension liquid Knop's solution + 1% glucose, assay time 60 min., in the gas phase 5% CO₂ in air.

H ₂ S, moles/liter	Volume, cm ³				Vessel constant				Pressure change, mm		X _{O₂} , mm ³
	V _f	V _g	V _f	V _g	K _{O₂}	K _{CO₂}	k _{O₂}	k _{CO₂}	H	h	
0	7	4.86	3	10.69	0.47	1.07	1.01	1.26	-87.3	-11.2	-77.0
10 ⁻⁴	7	5.5	3	11.6	0.53	1.13	1.09	1.34	-16.3	+15.6	-43.0

Inhibition by 10⁻⁴M H₂S = 100x(77-43)/77%=44%

Protocol II

This experiment shows that carbon monoxide inhibits *Chlorella*-respiration in glucose-containing solution. Vessels according to Fig. 1, experimental

temperature 20°C, in the inset 0.3 cm³ 5% KOH, in each vessel 0.07 cm³ of cells. Suspension liquid Knop's solution + 1% glucose, duration of experiment 60 minutes.

Gas mixture	VG, cm ³	VF, cm ³	K _{O₂}	H, mm	X _{O₂} , mm
3% O ₂ 97% N ₂	14.07	3.3	1.32	-48.0	-63.5
3% O ₂ 97% CO	13.22	3.3	1.24	-28.6	-35.5

Inhibition by carbon monoxide 100x (63.5-35.4)/63.5 = 44%

Protocol III

This experiment shows that hydrogen cyanide accelerates *Stichococcus* respiration to about 50 %. Vessels according to Fig. 2, suspension liquid Knop's solution, duration of experiment 30 minutes.

Density of suspension: 0.14 cm³. Cells in 7 cm³. Equal cell concentration in all vessels. Cells cultivated in Knop's solution + 2% glucose. Gas phase: 5% CO₂ in air.

H ₂ S, moles/liter	Volume, cm ³				Vessel constant				Pressure change, mm		O ₂ consumption, mm ³
	V _f	V _g	V _f	V _g	K _{O₂}	K _{CO₂}	k _{O₂}	k _{CO₂}	H	h	
0	7	5.5	3	11.6	0.53	1.13	1.09	1.34	-65.8	-9.3	-50.4
10 ⁻⁴	7	4.86	3	10.69	0.47	1.07	1.01	1.26	-109.2	-13.0	-78.4

Acceleration of respiration by HCN $100 \times (78.4 - 50.4) / 50.4\% = 56\%$

Protocol IV

This experiment shows that the *Eudorina*-respiration is accelerated by carbon monoxide.

Vessels according to Fig. 1, experimental temperature 20°C, in the central well 0.3 cm³ 5% KOH, in each vessel 0.1 cm³ of cells, suspension liquid Knop's solution. Duration of experiment 60 minutes.

Gas mixture	VG, cm ³	VF, cm ³	K _{O₂}	H, mm	X _{O₂} , mm
3% O ₂ +97% N ₂	14.07	3.3	1.3	-38.0	-49.4
3% O ₂ +97% CO	13.22	3.3	1.24	-49.1	-60.9

Acceleration by carbon monoxide $100 \times (60.9 - 49.1) / 49.1\% = 23\%$

Protocol V

This experiment shows that hydrogen cyanide inhibits *Oscillatoria*-respiration very much.

Vessels according to Fig. 1. Experimental temperature 25°C, in central well (inset) 0.3 cm³ 5% KOH, suspension liquid Knop's solution, in the gas phase air.

Reference (control)

V _G , cm ³	V _F , cm ³	K _{O₂}	Time, min.	HCN, moles/liter	H, mm	X _{O₂} , mm ³
13.22	3.3	1.24	30	0	-33.6	-41.3
			30	0	-29.5	-36.0

Decrease in the reference (control) up to $100 \times 36.0 / 41.3\% = 87\%$

Experiment

V _G , cm ³	V _F , cm ³	K _{O₂}	Time, min.	HCN, moles/liter	H, mm	X _{O₂} , mm ³
14.07	3.3	1.3	30	0	-56.6	-73.3
			30	10 ⁻⁴	-7.5	-9.75

$73.3 \times 0.87\% = 64\%$..., [X_{O₂} x inhibition in control]

Inhibition by HCN = $100 \times (64 - 9.75) / 64 = 86\%$

Protocol VI

Alga	mg d.w. per 0.1 cm ³ fresh matter	O ₂ consume per mg dry weight per hour
<i>Chlorella vulgaris</i>	11.5	2.8 mm ³
<i>Eudorina</i>	10.3	5.6 mm ³
<i>Stichococcus</i>	17.6	5.3 mm ³
<i>Oscillatoria</i> *	21.8	2.6 mm ³

*at 25°C

Finally, I express my sincere thanks to Professor Otto Warburg as well as to the assistants in his department, and to Mr. Erwin Negelein.

Curriculum vitae

I, Robert Emerson, was born on the 4th of November 1903 in New York, N.Y.

After a seven year attendance at the "Ethical Culture" School in New York and completion of the 17th year of my life I went to Harvard College. There I studied natural science, in particular biology, and in June 1925, I was awarded the degree of Master of Arts. During my university studies I visited the biological laboratory at Woods Hole in Massachusetts. In the summer of 1925, I received an Atkins Fellowship and worked in the biological laboratory of Soledad, Cuba.

In September 1925, I travelled to Germany, where I continued my studies at the Friedrich Wilhelm University and the Kaiser Wilhelm Institute for Biology in Berlin.

Robert Emerson

[Note: Full references are available from Govindjee (gov@illinois.edu)]

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