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LIGHT-DEPENDENT CHLORIDE ABSORPTION IN VALLISNERIA LEAVES

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CONTENTS

CHAPTER 1

INTRODUCTION

The origin of this investigation lies in the phenomenon studied by ARISZ *et al.* (1943, 1947) that light enhances the absorption of chloride in Vallisneria leaves, and that this light influence does not depend on the presence of carbon dioxide in the medium. The present investigation was carried out with the purpose to gain a better insight into this influence of light on chloride absorption.

The main result was the finding that Vallisneria leaves are capable of utilising the energy of light absorbed in the chloroplasts as a source of the energy required in chloride absorption as well as in photosynthetic carbon assimilation. Furthermore, it was found that in the light, under conditions largely preventing cytochrome oxidase activity chloride absorption is by no means abolished, which proves that chloride absorption in Vallisneria leaves needs not necessarily be coupled with respiration as far as this process is mediated by cytochrome oxidase.

A stimulation of salt absorption by light was found earlier in a number of organisms: in *Elodea canadensis*, *Ceratophyllum demersum*, *Myriophyllum* spec.: INGOLD (1936), and in *Nitella clavata:* HOAGLAND *et al.* (1924, 1927). In none of these cases, however, carbon dioxide

was excluded from the medium. It is therefore not impossible that the whole effect of light in these cases was due to photosynthetic carbohydrate formation. The stimulation of potassium absorption from seawater in *Valonia macrophysa* by light, found by JACQUES and OSTERHOUT (1934), was ascribed by the authors to the increase in the pH of the medium caused by photosynthesis. SCOTT and HAYWARD (1954) found that in the light *Ulva lactuca* excretes sodium ions. This process according to the authors is not inhibited by substances which inhibit photosynthetic carbon assimilation. Presumably, therefore, in this case there is a more direct influence of light on the process, similar to what was found with Vallisneria. In none of these investigations the salt absorption was studied in light of varying wavelengths. There is, therefore, no convincing evidence that in any of these cases chlorophyll absorbed the light active in salt absorption, as was found in the experiments with Vallisneria reported here.

According to KESSLER (1955) the reduction of nitrate in *Scenedesmus* is greatly stimulated by light in the absence of carbon dioxide. Also, BONGERS (1956) found that in normal *Chlorella* cells nitrate reduction occurs in the light only; the process competes with carbon dioxide reduction for reducing power generated in the chloroplasts. Perhaps these processes too have something in common with the light-dependent chloride absorption in Vallisneria leaves.

In the following a survey of the contents of the paper is given.

A description of material and methods (Chapter 2) is followed by a Chapter (3) on the velocity with which chloride absorption in Vallisneria leaves responds to exposure to light. The reaction to light was found to be quite rapid: it took about five minutes before the absorption rate was maximal.

In the next Chapter (4) the action spectrum will be treated, a subject discussed more fully in an earlier paper: VAN LOOKEREN CAMPAGNE (1957). From the action spectrum it was concluded that the light active in chloride absorption is absorbed by chlorophyll. This pointed to a more or less close connection between photosynthesis and chloride absorption.

Therefore, in Chapter 5 the reciprocal interaction between carbon assimilation and chloride absorption was studied. In agreement with Arisz's earlier findings the presence of carbon dioxide had no influence on chloride absorption, but bicarbonate ions had an inhibiting effect. Chloride had no effect on either carbon dioxide or bicarbonate assimilation. These data were thought to indicate that carbon assimilation products or intermediates are not involved in the chloride absorption process.

Consequently the energy required in chloride absorption must be supplied and utilised in another form. By means of inhibitors an attempt was made to get some indications as to the form of metabolic energy involved. Carbon monoxide (Chapter 6) and cyanide (Chapter 8) yielded data liable to more than trivial interpretation, as did oxygen withdrawal (Chapter 7).

The other inhibitors tested were hydroxylamine, 8-hydroxyquinoline,

fluoride, azide, 2,4-dinitrophenol, arsenate, arsenite, mono-iodo-acetate, phenylmercuri-nitrate, ethylenediaminetetraacetate.

These substances apparently caused reversible or irreversible changes in the cytoplasm and did not show any evidence of a more specific influence on the chloride absorption mechanism. They were, therefore, unsuitable for the purpose outlined above.

The inhibition of chloride absorption by bicarbonate finally, gave rise to some observations concerning the photosynthetic assimilation of bicarbonate as compared to carbon dioxide assimilation (Chapter 9). This chapter also contains a rectification of an earlier paper in which it was stated that there is no photosynthetic assimilation of bicarbonate in Vallisneria leaves: VAN LOOKEREN CAMPAGNE (1955).

The implications of the experimental data are discussed in Chapter 10 in connection with data from literature.

CHAPTER 2

MATERIAL AND GENERAL METHODS

Plants of *Vallisneria spiralis* L. (*Hydrocharitaceae*) were grown in concrete culture basins which were about 50 cm deep, were filled with deionised water and contained a 10 cm bottom layer of clay rich in organic matter. They were artificially illuminated during 15 hours daily with about 2800 meter-candles light from Philips HO 450 watt mercury vapour lamps; the temperature was 20–25° C.

Leaves to be used in an experiment were selected for healthy appearance and complete absence of injuries, and cut into 4 × 50 mm strips. The leaf strips were arranged into sets of eight (about 500 mgm fresh weight), and inserted into perspex frames which fitted in the experimental vessels described later on. They were kept overnight in the dark in water from the culture basin; after his treatment wound effects have largely disappeared: ARISZ (1957 I).

All photosynthesis experiments were carried out in a 17.5 ml closed perspex vessel containing eight leaf strips mounted in a perspex frame (Fig. 1). During the experiments the vessel was placed in a water bath at 24° C. The contents were stirred by turning the vessel back and forth by a mechanical device.

In the earlier experiments the oxygen content of the solution was determined before and after photosynthesis had taken place according to the micro-Winkler method: VAN DAM (1936), VAN LOOKEREN CAMPAGNE (1955). Later on a simple volumetric method was developed yielding results of the same accuracy, and permitting the time course of oxygen evolution to be followed. To this end a capillary calibrated glass tube was attached to the lower outlet tube of the vessel (Fig. 2). The upper outlet tube was closed after filling the apparatus with the experimental solution up to the capillary tube, leaving about 2 ml of air in the vessel.

Volume changes of the air space in the vessel can be read from the calibrated capillary tube. The hydrostatic pressure inside the vessel

has to be kept constant in order to prevent a. variations in the total gas pressure in the vessel, b. variations in the total volume of the vessel itself, perspex being rather flexible, c. variations in the volume of the piece of bicycle valve rubber tubing which connects the vessel to the calibrated glass tube. To this end the liquid meniscus in the calibrated tube is kept at a constant distance above the water level

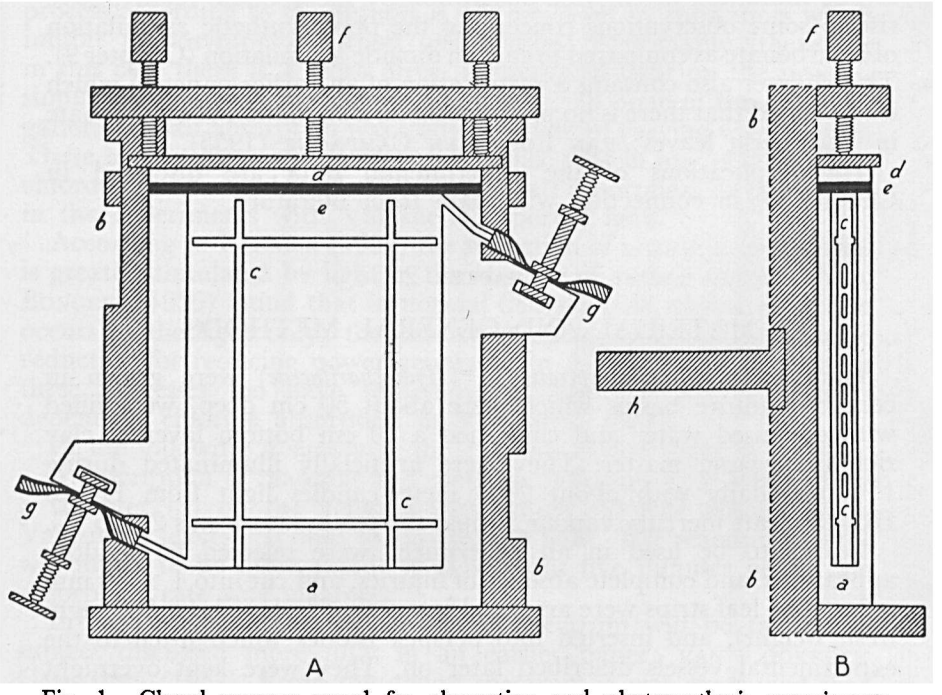


Fig. 1. Closed perspex vessel for absorption and photosynthesis experiments. Internal dimensions 8 × 50 × 80 mm. A. Front view; B. Longitudinal section. The perspex vessel a. is mounted in a brass frame b. The perspex holder c. with the leaf strips (not shown) is placed in the vessel. The vessel is sealed by a lid d. provided with a rubber strip e. and pressed firmly on the vessel by means of the screws f. Filling and emptying the vessel are accomplished by means of the rubber tubes g., which can be closed by means of screw clamps. By means of the axis h. the frame b. is placed in a shaking device which is inserted into the constant-temperature bath. By this device the vessel is turned back and forth in the plane perpendicular to the axis h.
(From van Lookeren Campagne 1955)

of the constant temperature bath in which the vessel is immersed. This is done by lowering the calibrated tube over the same distance over which the meniscus had risen in the calibrated tube.

The apparatus thus constitutes a volumetric device with an uncompensated manometer, which at the same time serves as a volumeter. From the volume changes recorded at constant total gas pressure the oxygen production can be calculated according to an appropriate equation. In this equation the fact should be taken into account that all gases present are to some extent dissolved in the liquid phase,

and that the amounts dissolved are directly proportional to the partial pressures of the gases concerned. This is necessary because the volume of the liquid phase is about 7.5 times as large as the gas phase. In literature (Warburg and Negelein, and Dixon, quoted in UMBREIT

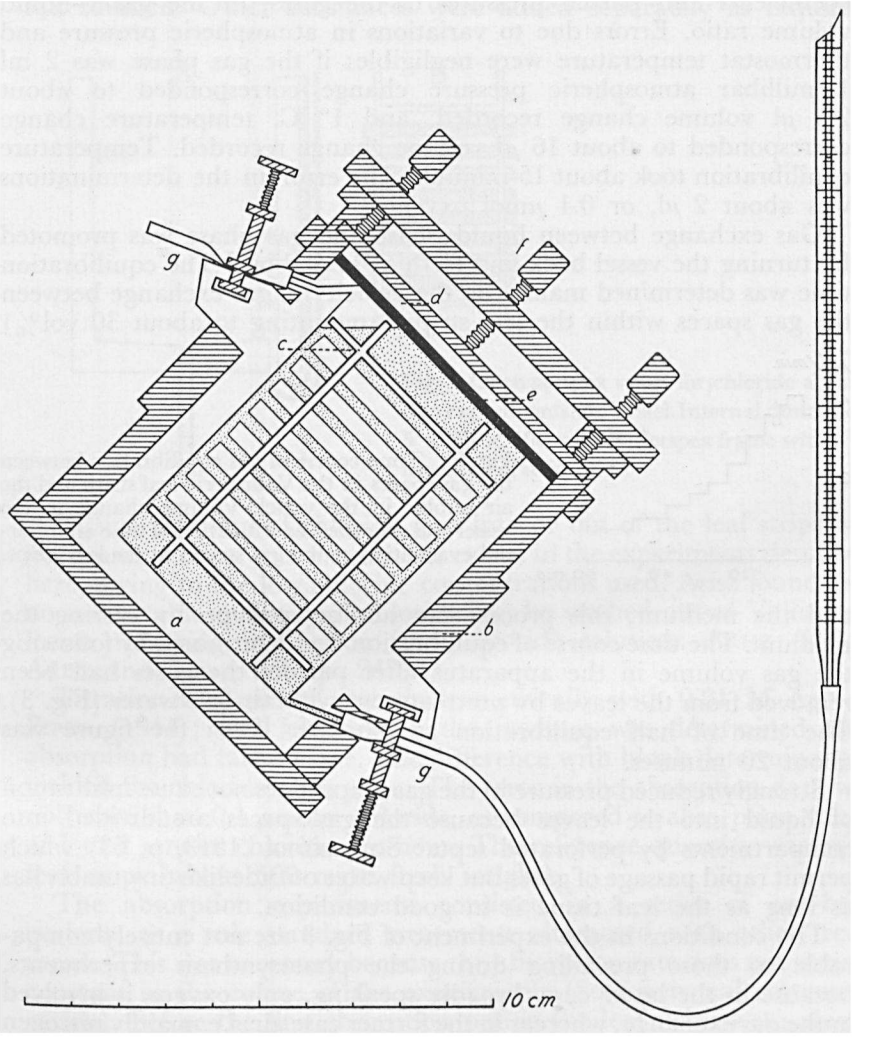


Fig. 2. Arrangement for volumetric oxygen determination. The calibrated capillary glass tube is connected to the lower outlet tube of the vessel containing the leaf strips. The experimental solution, which fills the vessel for the greater part, also serves as an indicator liquid in the calibrated tube. For explanation of the letters see Fig. 1.

et al. (1949); Cunningham and Kirk, quoted in GLICK (1949); YOCUM and BLINKS (1954); KOK (1955) no equation was found which met this requirement.

Therefore an equation was derived as described in the Appendix,

which gives the ratio of oxygen produced to volume change recorded at constant total gas pressure. Under the conditions of the experiments this quotient was according to calculation surprisingly insensitive to variations in the values of the determining factors such as solubility coefficients and partial pressures of the gases, or the gas-to-liquid volume ratio. Errors due to variations in atmospheric pressure and thermostat temperature were negligible: if the gas phase was 2 ml 1 millibar atmospheric pressure change corresponded to about 2.5 μl volume change recorded, and 1° C. temperature change corresponded to about 16 μl volume change recorded. Temperature equilibration took about 15 minutes. The error in the determinations was about 2 μl, or 0.1 μmol oxygen.

Gas exchange between liquid phase and gas phase was promoted by turning the vessel back and forth as said above. The equilibration time was determined mainly by the velocity of gas exchange between the gas spaces within the leaf strips (amounting to about 30 vol%)

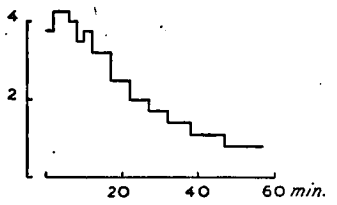


Fig. 3. Time course of gas equilibration between the gas spaces in the Vallisneria leaf strips and the air bubble in the vessel. Volume change in the vessel was recorded as a function of time after partial evacuation of the gas spaces in the leaf strips.

and the medium; this process can not be sped up by stirring the medium. The time course of equilibration was determined by following the gas volume in the apparatus after part of the gases had been removed from the leaves by pretreatment with air-free water (Fig. 3). The time of half equilibration as estimated from the figure was about 20 minutes.

Strongly reduced pressure in the gas spaces does not cause infiltration of liquid into the leaves because the gas spaces are divided into compartments by perforated septa: SOLEREDER (1913, p. 63) which permit rapid passage of gases but keep water outside like tiny umbrellas as long as the leaf tissue is in good condition.

The conditions in the experiment of Fig. 3 are not entirely comparable to those prevailing during the photosynthesis experiments, because in the latter case, broadly speaking, only oxygen is involved in the gas exchange, whereas in the former case air, i.e. mainly nitrogen was involved. As the solubility of oxygen is twice the solubility of nitrogen in water, and the diffusion velocity of both gases in water is approximately equal the equilibration time for oxygen alone is probably appreciably shorter than for air. This obtains only of course when the gas solubility and the diffusion velocity in Vallisneria leaf tissue are about the same as in water, which is to be expected: KROGH (1919).

The chloride absorption experiments were mostly carried out in four vessels of the type shown in Fig. 4. These vessels were filled from

a 25 ml pipette; stirring was provided by aeration. The method was established by ARISZ (1956).

In most experiments the solution was 5×10^{-4} M KCl, to which 2.5×10^{-4} M $CaSO_4$ was added in order to prevent injury by a single salt solution. Other substances were added separately as required.

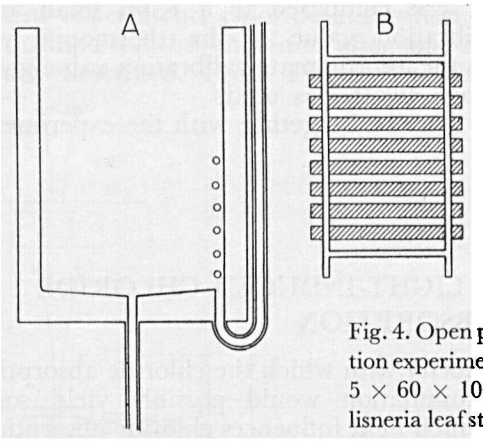


Fig. 4. Open perspex vessel for chloride absorption experiments. A. Vessel. Internal dimensions $5 \times 60 \times 100$ mm; B. Perspex frame with Vallisneria leaf strips.

Passive diffusion of chloride ions into or out of the leaf strips was not found to occur to a measurable extent in the experiments described here, owing to the low chloride concentrations used. Arisz found that no measurable quantity of chloride can be washed out of Vallisneria leaves following incubation in a chloride solution: ARISZ (1954), ARISZ and SOL (1956, p. 242).[1])

Titration was carried out electrometrically with 0.01 M $AgNo_3$: PIPER (1944); total chloride in the medium was determined after absorption had taken place. The difference with blank determinations yielded the chloride absorption. The error in the absorption data was not more than 0.1 μmoles chloride, as compared to absorption values of 1 to 2 μmoles chloride per hour. The reference quantity was eight leaf strips (about 500 mgm fresh weight).

The absorption experiments consisted in a series of one-hour periods; as a rule standard treatments alternated with other treatments. This was necessary because the absorption rate does not remain constant in the course of the experiments (SOL, personal communication). Before the first absorption period the leaf strips were always exposed to light in the experimental chloride solution during about 45 minutes. This was done because during this time the chloride absorption rate is often much lower than later on.

As said above the leaf strips were kept overnight in the dark in water from the culture basin before they were used in an experiment. Following this pretreatment in the dark the chloride absorption rate is in most cases relatively steady, whereas following pretreatment in

[1]) The apparent free space in Vallisneria leaves was found to be 4%: A. Kylin, Physiol. Plant. *10*: 732 (1957) (note added in proof)

the light the chloride absorption rate often strongly decreases with time (SOL, personal communication).

The light source used in most experiments was a Philips SO 140 watt sodium vapour lamp. Light intensity was measured with a General Electric selenium-cell photometer reading in foot-candles. In the case of coloured light [1]) the intensity was recalculated in ergs cm^{-2} sec^{-1}; to this end the photometer was compared to a Kipp small area thermopile. The exact calibration value of the thermopile was unknown; in the calculations the approximate calibration value given by the factory, 0.16 μvolt per μwatt was used.

Special techniques will be described together with the experiments in which they were used.

CHAPTER 3

TIME COURSE OF LIGHT-INDUCED CHLORIDE ABSORPTION

It was thought that the velocity with which the chloride absorption responds to changes in illumination would possibly yield some indication as to the way in which light influences chloride absorption. Therefore the time course of chloride absorption at light-dark transitions was followed by continually measuring the electric conductivity of the medium. The same technique was used by LUNDEGÅRDH (1949). The experimental arrangement is shown in Fig. 5. A 4 × 100 mm leaf strip was inserted into a slot-shaped perspex vessel measuring 1 × 4.2 × 120 mm. Subsequently the vessel was closed and immersed into a water bath at 24° C. About 1.5 ml of a 2×10^{-4} M potassium chloride solution was pipetted into the apparatus. The solution was circulated by means of air bubbles as shown in Fig. 5. This resulted in a rapid mixing: within one minute all concentration differences were levelled out. Conductivity was determined at two minutes' intervals by means of a Philips Philoscop GM 4140 connected to two platinum wire electrodes. The measuring current was generated by a Philips low-frequency oscillator GM 4260 at 2 Volts and 1000 cycles/sec. Conductivity change (arbitrary units) was used as a measure of chloride absorption.

A typical experiment is shown in Fig. 6.

It can be seen that light-induced chloride absorption starts abruptly some five minutes after the light is turned on; when the light is turned off the absorption stops almost immediately. This rapid response indicates that light has a direct effect on the chloride absorption process proper and not on membrane permeability. The lag period is not perceptibly dependent on light intensity. As shown in Fig. 24 (Chapter 9) photosynthesis behaves in essentially the same way during the induction period. It seems, therefore, as if carbon dioxide fixation and light-induced chloride absorption are during the

[1]) In preliminary experiments by Mr J. H. Mook a Christiansen light filter was used, which was kindly procured by Kema (Arnhem).

induction period limited by a common process. This is not unreasonable as in both processes the light is absorbed by the chlorophyll system (Chapter 4). The point will be treated in the Discussion (Chapter 10).

The experiment in Fig. 6 clearly shows that the accuracy of the conductivity measurements is too low to permit usable quantitative determinations of the chloride absorption to be made. In all subsequent experiments chloride absorption was, therefore, determined by titration of the medium after one-hour absorption periods as already described. From Fig. 6 it is also clear that the induction

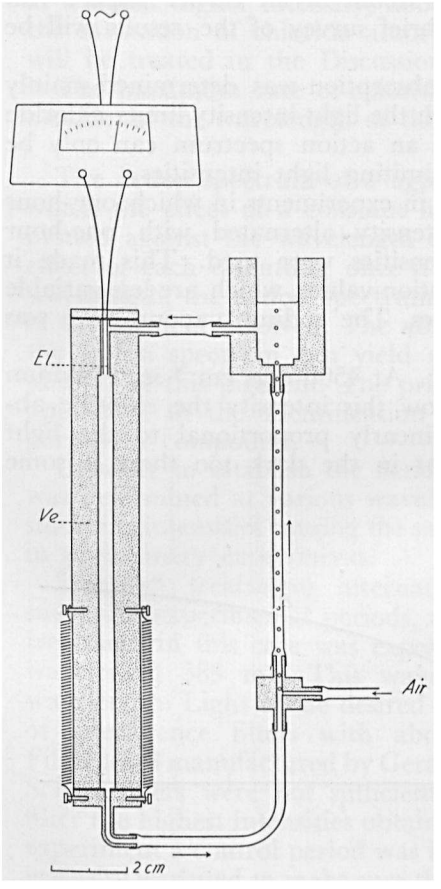


Fig. 5. Arrangement for determination of electric conductivity in the medium during chloride absorption. Vessel Ve (internal dimensions 1 × 4.2 × 100 mm) with a 4 × 100 mm Vallisneria leaf strip and two platinum wire electrodes El which are connected to a Philips Philoscop resistance meter (measuring current 1,000 cycles/sec. a.c.); circulation of the medium (1.5 ml) effected by ascending air bubbles.

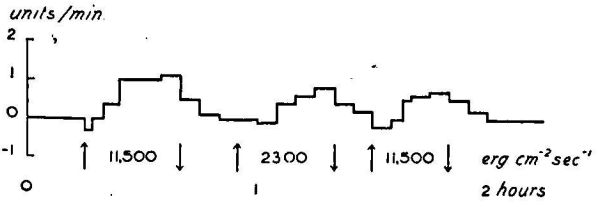


Fig. 6. Time course of light-induced chloride absorption in Vallisneria leaves. 2×10^{-4} M KCl + 10^{-4} M $CaSO_4$; sodium light; 24° C. (↑ light on; ↓ light off).

period is too short to influence the measurement of steady-state chloride absorption seriously if the absorption period is at least one hour.

CHAPTER 4

LIGHT CURVE AND ACTION SPECTRUM

These subjects were treated in detail in an earlier paper: VAN LOOKEREN CAMPAGNE (1957). A brief survey of the results will be given here.

The light curve of the chloride absorption was determined mainly in order to know the range in which the light intensity limits chloride absorption. This was necessary as an action spectrum can only be determined in the range of rate-limiting light intensities.

The light curve was determined in experiments in which one-hour periods with a standard light intensity alternated with one-hour periods in which other light intensities were used. This made it possible to calculate relative absorption values, which are less variable than the absolute absorption values. The sodium vapour lamp was used as light source.

The result is given in Fig. 7 (A). At 4500 ergs cm^{-2} sec^{-1} sodium light saturation was reached. Below this intensity the chloride absorption was more or less rectilinearly proportional to the light intensity. It should be noted that in the dark too there is some

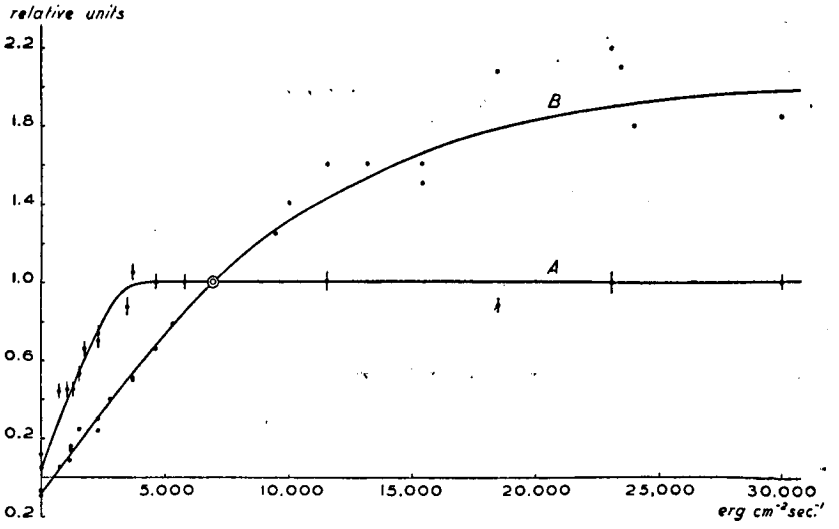


Fig. 7. Light curve of chloride absorption (A) and of photosynthesis (B) in Vallisneria leaves. Chloride absorption from 10^{-3} M KCl + 2.5 × 10^{-4} M $CaSO_4$; photosynthesis from 10^{-3} M CO_2 + 10^{-3} M $KHCO_3$ + 2.5 × 10^{-4} M $CaSO_4$; 24° C. Reference light intensity 7.000 ergs cm^{-2} sec^{-1} sodium light. (Partly from van Lookeren Campagne 1957)

chloride absorption. This absorption in the dark may be appreciably higher in other experiments (Figs. 11, 14, 15). The light intensity at which light saturation is reached is not always the same: it may shift in the course of weeks or months. Mr. J. H. Mook, using non-monochromatic light obtained with Schott filters, found similar curves (unpublished).

For comparison the light curve of photosynthesis, also shown in Fig. 7 (B), was determined in an analogous manner using the micro-Winkler method of oxygen determination. Light saturation is reached at a much higher intensity, about six times as high as required for the saturation of chloride absorption. The implications of this fact will be treated in the Discussion (Chapter 10).

The maximum rate of chloride absorption was found to be independent of the wavelength of the light used (experiment not shown).

The action spectrum of a light-dependent process is the curve in which the effect of a constant light intensity (in quantum units) is plotted against the wavelength of the light. If we assume that the effect of each quantum, once it is absorbed, is independent of the wavelength, the action spectrum represents the absorption spectrum of the pigment involved. The absorption spectrum and consequently the action spectrum can yield useful information on the chemical identity of this pigment. The principle was found by Warburg, who applied it in the identification of cytochrome oxidase as an iron porphyrin compound.

In order to establish the action spectrum the chloride absorption was determined at various wavelengths using equivalent light intensities: i.e. intensities causing the same rate of absorption, as determined in preliminary experiments.

Standard treatments alternated with other treatments in the successive experimental periods, as described in Chapter 2. Standard treatment in this case was exposure to a constant light intensity at wavelength 589 mμ. This wavelength was used as the reference wavelength. Light of the desired wavelengths was obtained by means of interference filters with about 10 mμ half-peak width, type Filtraflex B manufactured by Gerätebauanstalt Balzers (Liechtenstein). Schott filters were not sufficiently selective; with the Christiansen filter the highest intensities obtainable were not high enough. In each experiment a control period was included during which a higher light intensity was used to make sure that during the other periods the light intensity had actually been limiting the chloride absorption.

The intensities which caused identical chloride absorption rates were calculated in relative quantum units, the intensity at 589 mμ being put equal to unity. The reciprocals of these values are a measure of the efficiency with which light is utilised in chloride absorption. In Fig. 8 (A) these reciprocals are plotted as a function of a wavelength, thus yielding the action spectrum.

For comparison the same procedure was followed to establish the action spectrum of photosynthesis. The curve found coincided exactly

with the action spectrum of chloride absorption. This proves that the chlorophyll system absorbs the light effective in the induction of chloride absorption by Vallisneria leaves.

As a further confirmation of this conclusion the light transmission of a Vallisneria leaf was determined (Fig. 8 (B)). No correction for scattering was applied.

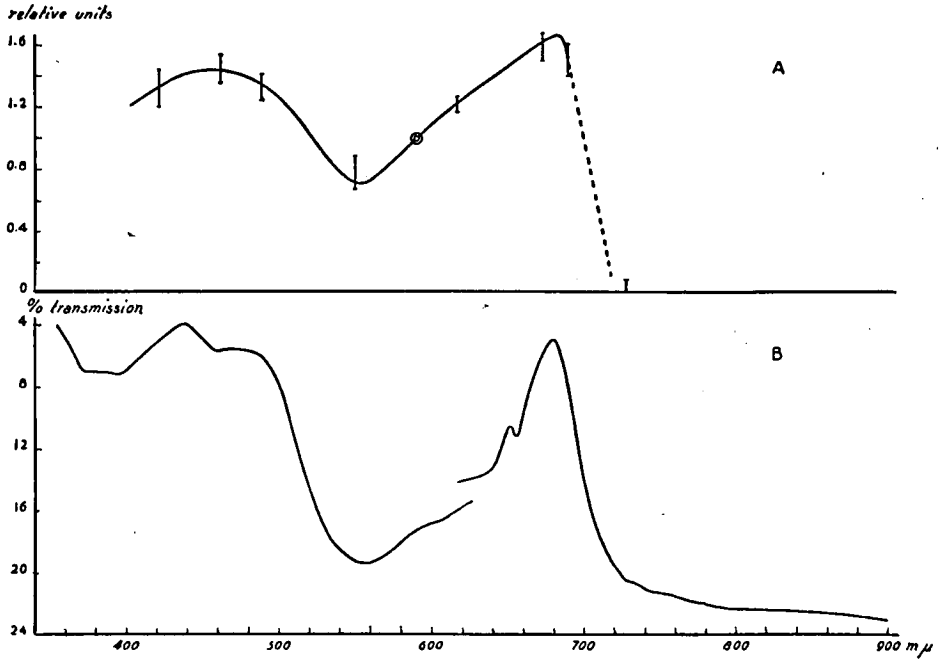


Fig. 8. A. Action spectra of chloride absorption and photosynthesis in Vallisneria leaves. Reference wavelength 589 mμ. Variation of calculated values indicated by the vertical lines. B. Transmission spectrum of a water-logged leaf of Vallisneria. No correction was applied for scattering.
(From van Lookeren Campagne 1957)

It should be mentioned here that essentially no carbon dioxide was present in the vessel during chloride absorption. This suggests that carbon dioxide assimilation is in no way involved in the chloride absorption process. More evidence for this supposition will be given in the next chapter.

CHAPTER 5

CHLORIDE ABSORPTION AND PHOTOSYNTHETIC CARBON ASSIMILATION

As shown in the previous chapter chlorophyll is involved in light-induced chloride absorption. When this was found the first question was, of course, whether there is a direct interaction between photo-

synthetic carbon assimilation and light-induced chloride absorption. So it was tried to find out if the presence of carbon dioxide had any influence on the rate of chloride absorption. This attempt met with a complication: in preliminary experiments more than once results were obtained which suggested that at a pH lower than 5.5, especially in media buffered with phosphate, chloride absorption was depressed. Therefore, it was thought better to prevent the occurrence of low pH values in the medium. Consequently when the medium was aerated with air containing more than a trace of carbon dioxide bicarbonate was added to the medium in order to raise the pH to above 6 or higher. If now, as was really found, under these conditions the chloride absorption is diminished the problem presents itself whether this inhibition is caused by the presence of carbon dioxide molecules or the presence of bicarbonate ions.

It was attempted to solve this problem by comparing the influence of varying the carbon dioxide concentration while the potassium bicarbonate concentration was kept constant, and vice versa. Experiments carried out according to this principle met with two difficulties: Firstly, it was found that the bicarbonate concentration did not remain constant during a one-hour absorption period: depending on the pH the leaf strips presumably exchanged hydrogen ions against other cations, or vice versa, thereby converting bicarbonate into carbon dioxide or vice versa. This process becomes troublesome at bicarbonate concentrations from 10^{-3} M downward.

Secondly no equilibrium with regard to carbon dioxide concentration existed between the solution in the vessels and the gas bubbling through. The carbon dioxide concentration actually established in the medium depended on the light intensity, i.e. the rate of photosynthesis, as was tentatively concluded from the pH value of the medium at the end of the absorption periods.

These facts must be borne in mind when the results of the experiments are considered. Examples are shown in Figs. 9, 10, 11. The final bicarbonate concentrations given in the figures should be considered as approximate values; they were calculated from the pH, determined with a glass electrode, and from the estimated carbon dioxide concentration.

From Fig. 9 it may be seen that at low concentrations of both carbon dioxide and bicarbonate there is no inhibition of chloride absorption at all.

In Fig. 10 an experiment is given showing that at a constant carbon dioxide concentration bicarbonate ions at the higher concentrations

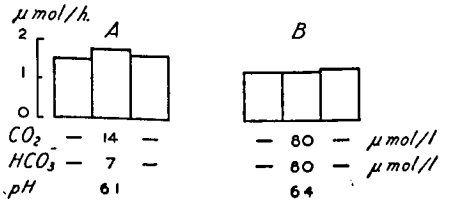


Fig. 9. Chloride absorption in the presence of carbon dioxide and bicarbonate at low concentrations. 5×10^{-4} M KCl + 2.5^{-4} M $MCaSO_4$; 24° C.; 4500 ergs cm^{-2} sec^{-1} sodium light; pH values mentioned are final pH values.

have a markedly inhibiting influence on chloride absorption, in the light as well as in the dark. Phosphate and nitrate, to the contrary, have but a slight, or no effect, Sol (unpublished).

No attempt was made to establish the quantitative relationship between the bicarbonate concentration and the inhibition of chloride absorption, because the inhibitions observed were too variable.

Olsen (1953) and Steward and Preston (1941) also found a specific inhibitory effect of bicarbonate on salt absorption.

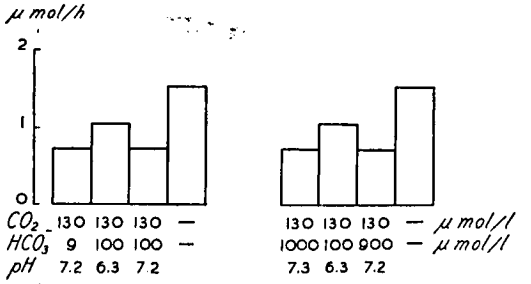


Fig. 10. Chloride absorption at constant carbon dioxide concentration and varied bicarbonate concentration. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; sodium light; pH values mentioned are final pH values.

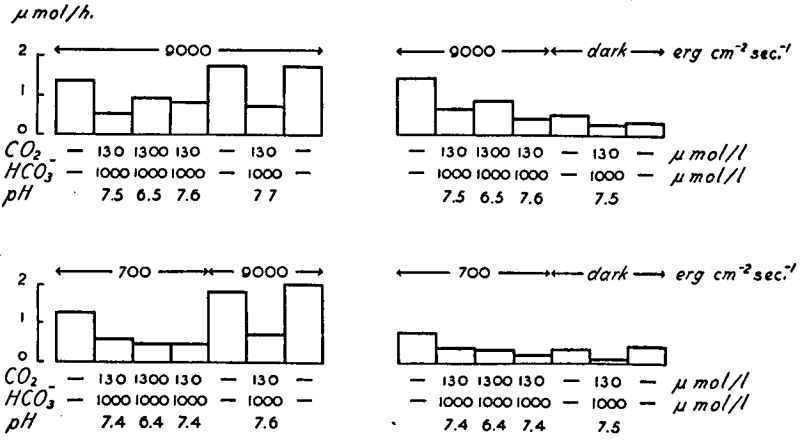


Fig. 11. Chloride absorption at constant bicarbonate concentration and varied carbon dioxide concentration. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; sodium light; pH values mentioned are final pH values.

Carbon dioxide, on the other hand, has but little influence (Fig. 11), even at high carbon dioxide concentration and limiting light intensity (700 ergs cm^{-2} sec^{-1}): at the lower light intensity it appears to inhibit somewhat, at the higher intensity (9000 ergs cm^{-2} sec^{-1}) it slightly stimulates chloride absorption. Perhaps there is some competition between chloride absorption and carbon dioxide reduction at the limiting light intensity, whereas at the higher light intensity the respiration-dependent chloride absorption is possibly stimulated somewhat by products or intermediates of photosynthesis (cf. Discussion).

In any case it is clear that carbon dioxide reduction cannot be very

directly involved in any part of the chloride absorption process. It is supposed, therefore, that after being transformed into chemical energy in the chloroplasts, the light energy is not utilised in the chloride absorption process as a carbon dioxide reduction product or intermediate, at least not to any appreciable extent.

In order to obtain some evidence as to the mechanism of the inhibition of chloride absorption by bicarbonate ions it was tried whether chloride ions also inhibited photosynthetic bicarbonate assimilation, a process discussed in Chapter 9. In the experiments carried out to this end the utilisation of bicarbonate was determined by measuring the pH before and after a 7 hours' period of incubation with a Vallisneria leaf piece.

Each of a series of test tubes was provided with 20 ml of a 3×10^{-3} M potassium bicarbonate solution to which either no, or 6×10^{-3} M, or 3×10^{-2} M potassium chloride was added, a 10 cm piece of Vallisneria leaf being subsequently introduced into each, some blanks excepted. The test tubes were well closed during the experiments. pH was measured with a Beckman type H pH meter. To this end the solution from each test tube in turn was transferred to a small beaker and after the measurement was poured back into the test tube.

From the pH the ratio of carbonate to bicarbonate concentration could be calculated according to the equation $\frac{CO_3^{=} \times H^+}{HCO_3^-} = K_2 = 10^{-10.3}$. As the initial bicarbonate and carbonate concentrations were known it was possible to calculate the final carbonate concentrations with the assumption that the cation concentration remained unaltered. The calculated increase in carbonate was taken as a measure of bicarbonate utilisation. The results of the experiment are shown in Table I. At the lower concentration chloride has no detectable effect on bicarbonate utilisation; at the higher concentration chloride has an apparently depressing effect, but here the pH measurement is not entirely reliable as a consequence of the high salt concentration. Possibly the effect, if it is real at all, is of merely osmotic nature, and not specific for chloride, cf. ARISZ and SOL (1956); ARISZ and SCHREUDER (1956 II). At any rate there is no convincing evidence that chloride ions interfere with photosynthetic bicarbonate utilisation.

Mutual interference between chloride ions and bicarbonate ions at a carrier somewhere at the outer boundary of the cytoplasm cannot be, therefore, the cause of the inhibition of chloride absorption by bicarbonate, but an interaction situated at the tonoplast, for instance, is quite possible, as in that case no influence of chloride on bicarbonate utilisation in the chloroplasts could possibly be expected, even if there is competition.

The inhibition of light-induced chloride absorption cannot lie in an enzyme system it has in common with photosynthesis, because photosynthesis has never been found to be impaired in the presence of bicarbonate.

TABLE I

Influence of chloride on bicarbonate utilisation in photosynthesis. Medium 20 ml $KHCO_3$ 3×10^{-3} M + $CaSO_4$ 5×10^{-5} M incubated for 7 hours, with a leaf piece 10 cm long. Light intensity 16,000 ergs cm^{-2} sec^{-1} sodium light. Temperature 24° C.

KCl, M	—						6×10^{-3}					3×10^{-2}					
pH initial	9.4^3	9.3^8	9.4^0	9.4^1	9.4^1	9.4^1	9.3^7	9.3^7	9.3^6	9.4^0	9.3^8	9.2^2	9.2^1	9.2^1	9.2^0	9.2^2	9.2^0
pH final	9.4^3	10.1^8	9.9^2	9.9^4	9.8^7	10.0^3	9.9^9	9.9^1	9.7^4	10.0^0	10.0^0	9.5^7	9.6^6	9.5^8	9.5^4	9.7^2	9.2^0
$CO_3^{=}$, μmoles initial			6.3						6.6					6.3			
$CO_3^{=}$, μmoles final	18.0	13.5	14.1	12.6	15.6		15.6	14.1	11.4	15.6	15.6	11.1	12.3	11.4	10.8	13.5	
$\triangle CO_3^{=}$, μmoles	11.7	7.2	7.8	6.3	9.3		9.3	7.8	5.1	9.0	9.3	4.8	6.0	5.1	4.5	7.2	
leaf width, mm	8^5	7	6^5	6	7		8	6^5	5^5	8	7^5	7^5	7^5	6	6^5	6	
$\triangle CO_3^{=}$, μmoles/h $\times$ 16 cm^2	3.2	2.4	2.7	2.4	3.1		2.7	2.7	2.1	2.6	2.9	1.5	1.9	2.0	1.6	2.8	
mean $\pm$ standard deviation of the mean			2.8 ± 0.2						2.4 ± 0.2					2.0 ± 0.3			

The phenomenon reminds one of the inhibition by bicarbonate of the respiration of plant roots studied by MILLER and THORNE (1956). There is a difference: the chloride absorption in Vallisneria is rapidly inhibited without time lag, whereas the respiration of root tips is not inhibited during the first hour in a medium containing bicarbonate. The difference is not necessarily essential because the time lag with root tips is probably caused by slow penetration of bicarbonate: in extracts of root tips the oxidation of cytochrome c is inhibited at the moment bicarbonate is added: MILLER and EVANS (1956). These authors suppose that the inhibition of respiration in roots is caused by an inhibition of cytochrome oxidase, but it is not clear whether they mean by the term "cytochrome oxidase" the terminal oxidase only or the whole enzyme system involved in the oxidation of cytochrome c. At any rate the site of inhibition can be anywhere between cytochrome c and oxygen in the respiratory reaction chain.

An influence of chloride in the medium on carbon dioxide assimilation was not found. It could, indeed, hardly be expected as even bicarbonate assimilation is not affected by chloride.

CHAPTER 6

INFLUENCE OF CARBON MONOXIDE

Several authors have shown that "active" salt absorption in plants is strongly inhibited by carbon monoxide: ORDIN and JACOBSON (1955), MIDDLETON (1955), SUTTER (1950), WEEKS and ROBERTSON (1950). They always used nonphotosynthetic organs or tissues, in which active salt absorption is necessarily dependent on the only available source of metabolic energy, *viz.* the respiration. Consequently, when carbon monoxide brings the respiration to a stand-still by blocking the ultimate oxidase there is no more respiratory energy available, and salt absorption must necessarily also come to a stand-still.

With photosynthetic cells and tissues the situation is different. Here, metabolic energy can be derived from light energy in the chloroplasts. In the present study it has already been demonstrated that Vallisneria leaves can utilise energy derived from light in chloride absorption.

As GAFFRON (1935) proved that steady-state photosynthesis is not inhibited by carbon monoxide the conversion of light energy into metabolic energy is evidently not influenced by carbon monoxide. So it should be possible to check whether the chloride absorption process proper is inhibited, and consequently whether autoxidisable heavy-metal compounds play a part in this process.

KRALL (1955) and GAFFRON (1935) found an inhibition of photosynthesis during an induction period after a pretreatment in the dark with carbon monoxide. According to the authors the effect was completely the same as that obtained with anaerobic pretreatment in

the dark. It does not prove the indispensability of a heavy metal enzyme, and of oxygen in steady-state photosynthesis. Whittingham (1956) ascribes the prolonged induction period after anaerobic incubation in the dark to the accumulation of fermentation products, as does FRANCK (vide RABINOWITCH, monograph). During incubation in the dark with carbon monoxide, fermentation products will accumulate as well, so it may be expected that the effect on photosynthesis during the induction period is the same as with the anaerobic pretreatment. Therefore the assumption of any special effect of carbon monoxide on a reaction specific for photosynthesis is not justified.

Before discussing the experiments the technique used will be described. The arrangement consisted in the closed vessel described above (Fig. 1) and a water-cooled 500 watt projection lamp as a light source: VAN LOOKEREN CAMPAGNE (1957, Fig. 2). It is known that iron-carbonyl compounds are decomposed in light of the shorter wavelengths, but not in red light: WARBURG (1926, 1949). By comparing the influence of red and white light it is, therefore, possible to discriminate between iron-carbonyl and other carbonyl compounds. Red light was obtained by means of a Schott RG 5 glass filter transmitting only on the long wavelength side of 660 mμ. The light intensity could not be measured exactly because the Schott filter was frosted, but it was certainly not limiting the chloride absorption.

Carbon monoxide was generated by adding formic acid to hot sulfuric acid; it was purified by bubbling through alkali. The purified gas was stored over water. A gas mixture containing a small quantity of oxygen was prepared in the vessel in the following way: the vessel containing already the leaf strips was first filled with pure carbon monoxide, subsequently three quarters by volume of the gas was replaced by the chloride solution. The solution was saturated with air, consequently the resulting composition of the gas mixture after equilibration was calculated to be 1.7 % oxygen, 95 % carbon monoxide and the rest nitrogen. The calculation was not checked by analysis of the gases.

The first experiment was an indication that also in Vallisneria photosynthesis in red light is not inhibited by carbon monoxide. Oxygen was determined volumetrically. The gas phase was about 4.5 ml, therefore the error due to variations in atmospheric pressure and temperature was much larger than it was with the normal 2 ml gas phase. The light intensity was low: 5000 ergs cm^{-2} sec^{-1} red light. In the presence of carbon monoxide the oxygen production was 4.4. $\pm$ 0.4 μmoles per hour; without carbon monoxide 4.3 $\pm$ 0.3 μmoles per hour. Oxygen consumption in the dark, by the way, was not measurably inhibited by carbon monoxide, but perhaps the volume decrease recorded was not caused by the disappearance of oxygen only but partly by the disappearance of carbon monoxide, this gas possibly being hydrated to formic acid or oxidised to carbon dioxide.

When it was thus demonstrated that photosynthesis was unaffected it was tried whether carbon monoxide had any effect on chloride

absorption. From Fig. 12 it is clear that carbon monoxide indeed inhibited chloride absorption in red light, but the inhibition was by no means total. In the dark chloride absorption was totally inhibited

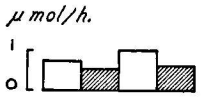
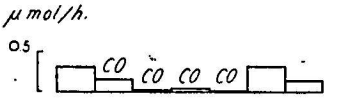


Fig. 12. Inhibition of chloride absorption by carbon monoxide in red light. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C; gas phase either air or 95 % CO +1.7 % O_2 + 3.3 % N_2.

(Fig. 13), in complete agreement with the data in literature (see above). The small absorption during the first period with carbon monoxide is probably not significant. Presumably some absorption occurred during the first few minutes after the vessel was filled, as this had to be done in the light.

Fig. 13. Influence of carbon monoxide on chloride absorption in the dark. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; gas phase either air or 95 % CO + 1.7 % O_2 + 3.3 % N_2.



The possibility existed that also in the light part of the chloride absorption is respiration-dependent, as it is in the dark, and that in the light only this respiration-dependent part of the chloride absorption was inhibited. Therefore, in a number of experiments the rate of uninhibited chloride absorption in the dark was compared to the degree of inhibition by carbon monoxide in red light. Examples are shown in Fig. 14. In some experiments the quantitative agreement was remarkable (A), but in other experiments the decrease in absorption in red light was significantly larger than the unhibited absorption in the dark (B). This can be explained in two ways: either the light-induced chloride absorption is slightly sensitive to carbon monoxide, or the respiration-dependent absorption is more or less

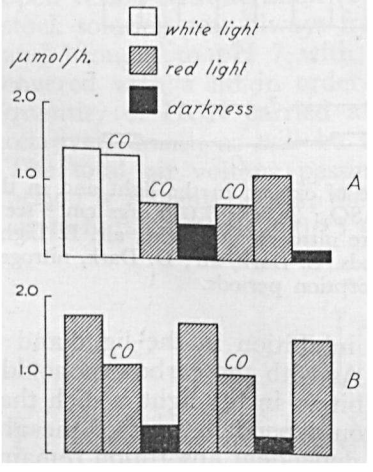


Fig. 14. Carbon monoxide inhibition of chloride absorption. Light-reversibility, and comparison with absorption in the dark. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; saturating red light; gas phase either air or 95 % CO + 1.7 % O_2 + 3.3 % N_2. A and B are separate experiments.

enhanced in the light, possibly by transference of intermediates of photosynthetic carbon dioxide assimilation or of reduced substances like DPNH from the sites of photosynthesis (chloroplasts) to the sites of respiration (mitochondria). The second explanation is considered the more likely one, because a sensitivity of the light-induced absorption process to carbon monoxide does not agree very well with the insensitivity of photosynthesis.

The substance or substances combining with carbon monoxide contain iron, as the inhibition present in red light is reversed by white light (Fig. 14A). The light-reversibility of the inhibition of absorption in the dark cannot, of course, be proved with certainty: any light causes light-dependent chloride absorption which obscures the expected reversion of the inhibition of the respiration-dependent absorption.

CHAPTER 7

ANAEROBIC CHLORIDE ABSORPTION

As the carbon monoxide experiments suggested that the light-dependent part of chloride absorption does not require the presence of free oxygen the influence of very low partial pressures of oxygen on chloride absorption was studied. The contents of the vessels were rendered and kept oxygen-free by aeration with pure nitrogen. The vessels were covered with a lid in order to keep air out. The nitrogen used for aeration had no measurable oxygen content, as was checked with reduced methylene blue; even when nitrogen was bubbled through for hours no blue colour reappeared.

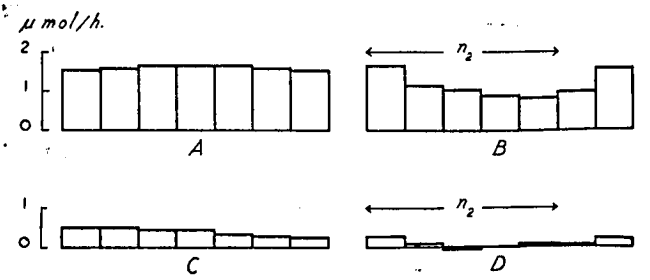


Fig. 15. Chloride absorption in the absence of oxygen in the light and in the dark. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; 7,000 ergs cm^{-2} sec^{-1} sodium light. Aeration with either air or pure nitrogen. A. Light; air, B. Light, nitrogen during the first five absorption periods. C. Dark, air; D. Dark, nitrogen during the first five absorption periods.

The experiments indicate a partial inhibition in the light and a total inhibition in the dark (Fig. 15). As with the carbon monoxide experiments the magnitude of the inhibition in the light is such that it is conceivable that only a respiration-dependent part of the absorption is inhibited, whereas the light-dependent absorption remains

unaffected. In some experiments the inhibition gradually becomes much larger in the course of the experiment, which is probably caused by a gradual inactivation of an enzyme system somehow involved in the absorption mechanism.

The inhibition starts about one hour after the removal of oxygen and disappears also about one hour after oxygen is admitted again. Although highly improbable, the possibility exists that the removal of oxygen did not take less than one hour. It is, however, impossible that the penetration of oxygen through the tissue, when oxygen is admitted again, took more than a few minutes (cf. Fig. 3). It would seem probable, therefore, that the oxygen deficiency primarily results in the inactivation of the respiratory enzyme system only. This would be in agreement with the results of the carbon monoxide experiments.

The absence of a complete inhibition of chloride absorption does not, however, prove with certainty that free oxygen is not required in the absorption process. For notwithstanding all precautions to keep the partial pressure of oxygen as low as possible complete removal of all traces of oxygen could not be achieved, because Vallisneria leaves produce some oxygen in photosynthesis from the carbon dioxide liberated in fermentation. Diffusion through the tissue is not rapid enough to carry this oxygen and carbon dioxide away with sufficient efficiency.

CHAPTER 8

INHIBITION WITH CYANIDE

The other inhibitor yielding results worth mentioning was cyanide (strictly speaking hydrocyanic acid, HCN). Its action on chloride absorption is instantaneous and immediately and totally reversed upon removal of the inhibitor (Fig. 16). For these experiments the open vessels with aeration (Fig. 4) were used. The hydrocyanic acid stock solution was always freshly prepared from potassium cyanide and brought to pH 7 with sulfuric acid. The vessels were loosely covered with a lid in order to prevent evaporation of HCN. The quantity of HCN carried away with the aeration air stream was relatively small, as will be evident from the following calculation: The total air volume passing through the solution in a one-hour period is 200 ml, or less; the volume of the solution is 25 ml, so the air-to-liquid ratio is 200/25 = 8, or less. The partition coefficient of

Fig. 16. Influence of 10^{-4} M hydrocyanic acid on chloride absorption. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; 7,000 ergs cm^{-2} sec^{-1} sodium light.

HCN between air and water at 18° C. is about 0.005 (molar units) at low HCN concentrations as calculated from data from the International Critical Tables vol. III p. 365; it was estimated to be not more than 0.01 at 24° C. It follows that at most $8 \times 0.01 = 0.08$, or 8 % of the HCN originally present could be carried away with the aeration stream in the course of one hour, which results in a mean HCN concentration not more than 4 % lower than the original concentration.

Following the incubation with Vallisneria leaf strips the 25 ml portions of experimental solution were evaporated to dryness in order to remove cyanide completely; the residues were dissolved again in water and chloride was titrated with silver nitrate in the normal way. Traces of cyanide would, of course, disturb the chloride determination. The respiration-dependent absorption is already totally inhibited at a cyanide concentration (10^{-4} M) at which the light-dependent absorption is only partly inhibited. The inhibition of the respiration-dependent absorption is presumably caused by the inactivation of cytochrome oxidase, as in the carbon monoxide experiments.

The rate of chloride absorption at light saturation is lowered, but at low light intensities the light-dependent part of chloride absorption is not inhibited (Fig. 17). The same was found with photosynthesis, as described below. The cyanide-sensitive substance, which limits chloride absorption in the light, must be insensitive or nearly insensitive

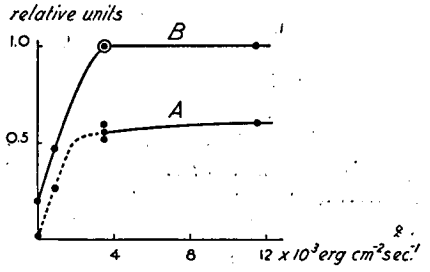


Fig. 17. Influence of light intensity on chloride absorption in the presence of hydrocyanic acid. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; sodium light. A. 10^{-4} M HCN; B. without HCN. Curve A is partly based on evidence from comparable experiments.

to carbon monoxide and is, therefore, presumably not an autoxidisable heavy-metal compound. It is not possible to say whether or not it is involved in the chloride absorption process proper, although the experiments indicate (Fig. 18) that the cyanide inhibition becomes less pronounced with decreasing chloride concentration.

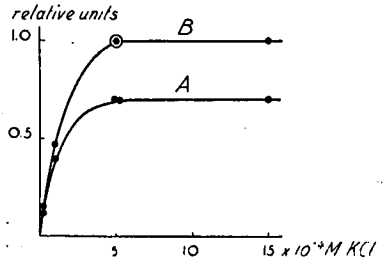


Fig. 18. Influence of chloride concentration on chloride absorption in the presence of hydrocyanic acid. Chloride solution with 2.5×10^{-4} M $CaSO_4$; 24° C.; 4500 ergs cm^{-2} sec^{-1} sodium light. A. 10^{-4} M HCN; B. without HCN.

The cyanide inhibition of chloride absorption is subject to long-term variations, as is also the case with the light intensity at which light saturation is reached (p. 553). The experiment shown in Fig. 19 was carried out three months later than the experiments described above: in the earlier experiments 10^{-4} M cyanide never caused more than 50 % inhibition in the light, but in this experiment the same concentration caused a nearly total inhibition. This result cannot be ascribed to experimental errors as the cyanide solution was always freshly prepared from the same stock of potassium cyanide. In photosynthesis such phenomena were not encountered.

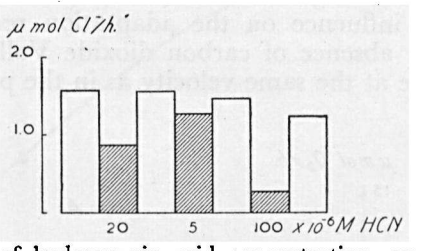


Fig. 19. Influence of hydrocyanic acid concentration on chloride absorption. 5×10^{-4} M KCl + 2.5×10^{-4} M $CaSO_4$; 24° C.; 7,000 ergs cm^{-2} sec^{-1} sodium light.

Photosynthesis in Vallisneria is also inhibited, which was to be expected according to literature. The literature of the inhibition of photosynthesis by cyanide is treated in detail by Rabinowitch in his monograph, to which may be referred here.

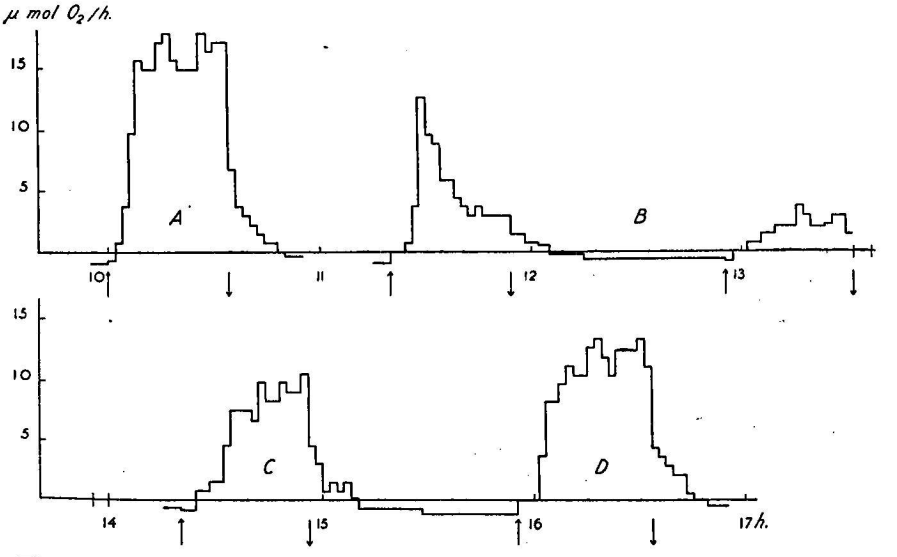


Fig. 20. Photosynthesis before adding hydrocyanic acid (A); in the presence of 3×10^{-5} M HCN (B); recovery after removal of HCN (C and D). 10^{-3} M CO_2 + 10^{-3} M $KHCO_3$ + 5×10^{-5} M $CaSO_4$, pH $\sim$ 6.4; 24° C.; 16,000 ergs cm^{-2} sec^{-1} sodium light (↑ light on; ↓ light off).

The experiments on the inhibition of photosynthesis by cyanide were carried out by means of the volumetric oxygen determination technique. They showed that the inhibition is not instantaneously maximal but increases gradually to its ultimate level (adaptation) (Fig. 20). The reversion of the inhibition (de-adaptation) is also gradual and it takes several hours before it is completed.

Surprisingly enough the adaptation reaction proceeds in the light only: even after prolonged incubation with cyanide in the dark photosynthesis starts with the peak shown in Fig. 20. Adaptation, once present, is not reversed again in the dark (Fig. 20B). Carbon dioxide reduction has no influence on the adaptation reaction, for, when illuminated in the absence of carbon dioxide Vallisneria leaves get adapted to cyanide at the same velocity as in the presence of carbon dioxide (Fig. 21).

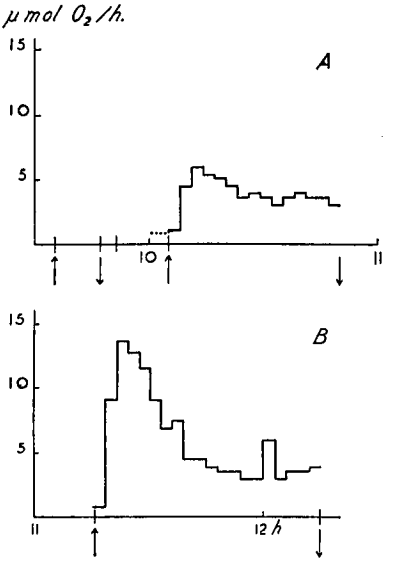


Fig. 21. Influence of carbon dioxide on the "adaptation" of photosynthesis to hydrocyanic acid. A. 12 minutes' pretreatment with 3×10^{-5} M HCN in the light in the absence of carbon dioxide; B. No pretreatment. Photosynthesis in a medium containing 10^{-3} M CO_2 + 10^{-3} M $KHCO_3$ + 5×10^{-5} M $CaSO_4$ + 3×10^{-5} M HCN, pH ∾ 6.4; 24° C.; 16,000 ergs cm^{-2} sec^{-1} sodium light (↑ light on; ↓ light off).

The substance reacting with cyanide is, therefore, probably not a constituent of the enzyme systems of carbon dioxide assimilation proper, nor of the liberation of oxygen from the primary oxidised product of water photolysis, because in both cases an influence of the presence of carbon dioxide on the adaptation reaction was to be expected; in the former case through carbon dioxide assimilation itself, in the latter case through the concomitant oxygen production.

At low light intensities photosynthesis after adaptation is not

inhibited by cyanide (Fig. 22), so cyanide does not inhibit the process in which light energy is transformed into the first stable form of chemical energy. The remaining possibility is that cyanide primarily inhibits photosynthetic phosphorylation. This possibility will be treated more fully in the Discussion (Chapter 10).

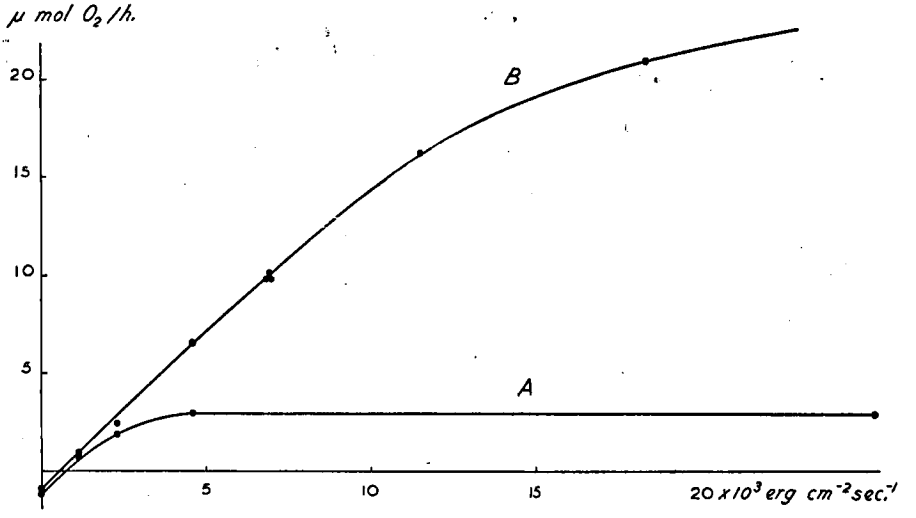


Fig. 22. Influence of light intensity on photosynthesis: A. in Vallisneria leaves fully adapted to hydrocyanic acid, B. in untreated leaves. 10^{-3} M CO_2 + 10^{-3} M $KHCO_3$ + 5 × 10^{-5} M $CaSO_4$, and either no inhibitor (B), or 3 × 10^{-5} M HCN (A); 24° C.; sodium light.

CHAPTER 9

PHOTOSYNTHETIC ASSIMILATION OF BICARBONATE

In Chapter 5 an experiment was described in which the influence of chloride on photosynthetic bicarbonate assimilation was examined. In view of the fact that in a previous paper I stated that Vallisneria leaves cannot utilise bicarbonate some explanation is needed here.

First it will be shown that oxygen is produced in a bicarbonate solution by Vallisneria leaves, which indicates that bicarbonate is utilised in photosynthesis. In this experiment the volumetric technique described on page 545-9 was used. The light intensity was 16.000 ergs cm^{-2} sec^{-1} sodium light. As a medium a 10^{-2} M potassium bicarbonate solution was given. Its pH was brought to 9.5 by adding potassium hydroxide. This was done in order to keep the carbon dioxide concentration too low to cause a measurable oxygen production: the carbon dioxide concentration was less than 10^{-5} M.

As shown in Fig. 23 the oxygen production was quite large, but it took about one hour to attain the steady-state. The rate of oxygen production finally attained was about one half the maximum rate

possible in carbon dioxide assimilation. It was found earlier that in the absence of carbon dioxide and bicarbonate there is no photosynthetic oxygen production in Vallisneria: VAN LOOKEREN CAMPAGNE (1955).

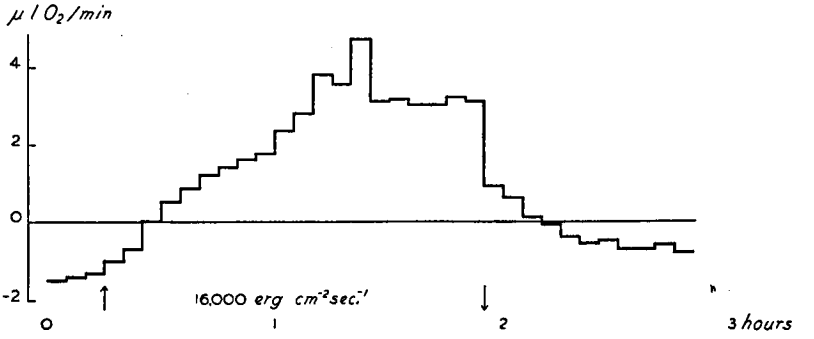


Fig. 23. Photosynthetic oxygen production in Vallisneria leaves from 10^{-2} M $KHCO_3$ + 5 × 10^{-5} M $CaSO_4$. Initial pH 9.5; final pH 9.7; 24° C.; 16,000 ergs cm^{-2} sec^{-1} sodium light (↑ light on; ↓ light off).

When the time course of oxygen production from bicarbonate and from carbon dioxide (Fig. 24) are compared a striking difference will be noticed. With carbon dioxide the oxygen production suddenly starts at full speed after some three minutes' time lag. This time lag cannot be explained by slow response of the volumeter to changes in the oxygen production rate, because the volume change per minute recorded is proportional to the oxygen concentration difference between the leaf tissue and the gas bubble in the vessel, and will, therefore, begin at about the same moment the oxygen production begins. If the oxygen production started immediately upon illumination

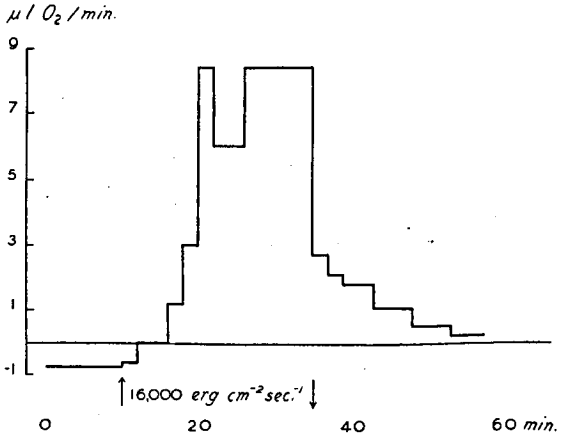


Fig. 24. Photosynthetic oxygen production in Vallisneria leaves from carbon dioxide. 10^{-3} M CO_2 + 10^{-3} M $KHCO_3$ + 5 × 10^{-5} M $CaSO_4$, pH ∾ 6.4; 24° C.; 16,000 ergs cm^{-2} sec^{-1} sodium light.

at full rate the volume changes recorded would increase exponentially until the maximum rate is approached. An S-shaped curve like the one in Fig. 24, therefore, indicates a real lag in the oxygen production.[1]) The duration of the lag period does not depend perceptibly on light intensity. (The apparent oxygen production after the light is turned off can be ascribed to the rather slow gas exchange between the intercellular gas spaces in the leaf strips and the surrounding medium (cf. Fig. 3).) With bicarbonate the oxygen production gradually increases for a considerable length of time (Fig. 23), before attaining the steady-state with some unexplained fluctuations. It seems; therefore, as if a considerable length of time is required before the leaves are adapted to the utilisation of bicarbonate, a phenomenon encountered in *Scenedesmus* by ÖSTERLIND (1951). Possibly, however, the very high pH was an interfering factor with Vallisneria, which is suggested by the fact that the oxygen consumption in the dark is always strongly enhanced in such alkaline media: it often amounts to some five times the normal respiration rate which is about 0.6 μmoles oxygen per hour.

The oxygen production from a certain bicarbonate concentration is quite variable, much more than is the case with carbon dioxide. The optimum lies in the range of 10^{-2} M bicarbonate.

For the explication of the divergent conclusion of the earlier experiments: VAN LOOKEREN CAMPAGNE (1955), it is important to realise how they were carried out. Experimental periods with only carbon dioxide in a low concentration alternated with periods in which 10^{-3} M potassium bicarbonate was given in addition. The oxygen production found was the same whether bicarbonate was present or not, a result repeatedly obtained. The crucial point is that the experimental periods during which the leaf strips were in the light were 20 minutes and that during the periods between, also about 20 minutes, the leaf strips were in the dark. The apparent absence of bicarbonate utilisation, then, can be explained when it is realised that owing to the short light period and the low bicarbonate concentration the bicarbonate utilisation had not the opportunity to attain a measurable rate.

It must be admitted that it was to be expected that Vallisneria leaves would be capable of utilising bicarbonate, because all submersed phanerogams investigated thus far can do it: ARENS (1933, 1936), RUTTNER (1947, 1948), STEEMANN NIELSEN (1947, 1951, 1952). Ruttner, indeed, concluded from his experiments that also Vallisneria can utilise bicarbonate in photosynthesis (1947), a conclusion no longer at variance with the results of this investigation.

[1]) The apparent lag might also be explained if the photosynthetic quotient largely exceeds minus unity during the first few minutes in the light.

CHAPTER 10

DISCUSSION

The subject of this paper is a phenomenon somewhat arbitrarily called chloride absorption. The mere reason for this is that the absorption of the chloride ions only was measured, and not that of cations. It is by no means certain that the anion especially is actively absorbed, and that the cation is passively carried along. The reverse is equally possible. More plausible in the author's opinion is that in any part of the absorption process both anion and cation, perhaps together, are transported by means of a mechanism utilising some kind of metabolic energy.

The results of the investigation described here indicate that the active chloride absorption in Vallisneria leaves consists of two parts differing in their source of energy. One part is a conventional respiration-dependent chloride absorption which proceeds in the dark as well as in the light, and which is inhibited by carbon monoxide, cyanide, and oxygen deficiency. From the carbon monoxide inhibition it was concluded that cytochrome oxidase is directly or indirectly involved in this part of the process. As cytochrome oxidase is localised in particles, presumably mitochondria only: MILLERD (1956), the activities of these particles must be taken into consideration when this respiration-dependent part of chloride absorption is discussed. Chloroplasts probably do not contain significant amounts of cytochrome oxidase: MCCLENDON (1953).

The other part of the chloride absorption is light-dependent, and is not inhibited by carbon monoxide. In this process chlorophyll is involved, so in the case of light-dependent chloride absorption the activities of the chloroplasts must be taken into consideration.

The question arises as to how, and where, light energy and respiratory energy respectively are utilised in the absorption process proper. First there is the possibility that the energy-consuming part of the absorption process occurs in the mitochondria as far as it is respiration-dependent, cf. ROBERTSON (1951), and in the chloroplasts for the part which is light-dependent. This gives rise to the problem as to how the ions reach these particles across the cell membrane, and how they are transferred from mitochondria and chloroplasts to the vacuoles without being dissipated again. This problem could be circumvented by the assumption that there is no impermeable cell membrane, and that the transfer takes place by means of an ion-carrier complex. It is fairly certain that the chloride ions absorbed are, at least partly, transferred into the vacuoles, because chloride absorption causes an increase in the osmotic value of the vacuole contents, as was demonstrated by Miss A. van Schreven and by Miss. A. G. van der Molen: ARISZ (1943, 1956). All this makes it improbable that all chloride absorbed should remain accumulated in the mitochondria and chloroplasts.

The second possibility is that mitochondria and chloroplasts merely

serve as generators of energy, the energy being utilised elsewhere in the cell in the energy-consuming reactions of ion absorption. This necessitates the assumption that the energy supply is the limiting factor in absorption as long as the absorption rate is below its maximum level, because otherwise the respiration-dependent and the light-dependent chloride absorption could not be quantitatively separated as was done in the carbon monoxide experiments.

Which of those two possibilities may be realised: whether the ions are fixed onto carriers at mitochondria and chloroplasts, or for instance at the outer cytoplasmic layers or at the tonoplast, in either case it is attractive to assume that the absorption process or processes derive their energy in one and the same chemical form both from respiratory and from light energy. To the extent this is in the form of an oxidation-reduction reaction sequence it would imply one of the three following possibilities: 1. Chloride is accumulated in chloroplasts and mitochondria before transfer into the vacuole is possible. This is improbable as discussed above. 2. Chloride is fixed to a carrier at chloroplasts and mitochondria, and released into the vacuole at the tonoplast. a. A carrier is reduced at chloroplasts and mitochondria in the act of ion fixation, and molecular oxygen is necessary in order to oxidise the ion-carrier compound again in ion liberation. This is, at least in the case of light-dependent chloride absorption unlikely in view of the experiments on the effect of oxygen deficiency and carbon monoxide, which rendered it probable that molecular oxygen is not required. b. Two substances are generated in chloroplasts and mitochondria, a reduced one and an oxidised one, one of them an ion-carrier complex. The two substances are then brought into reaction with each other at the tonoplast, thereby releasing the ion(s) from the complex. This possibility is related to the third. 3. The absorption process is localised entirely outside mitochondria and chloroplasts. Here again two substances should be generated, a reduced one and an oxidised one, one or perhaps both of them acting as carriers of cation and anion respectively. These two possibilities are not improbable. The production of reducing power, it is true, cannot be inhibited by carbon monoxide or anaerobiosis, because it is generated in fermentation if respiration is prevented, and because photosynthesis is not inhibited by carbon monoxide or anaerobiosis. The production of oxidising power in mitochondria, however, could conceivably be inhibited by carbon monoxide and anaerobiosis, thereby explaining the inhibition of respiration-dependent absorption alone. There remain, therefore, the possibilities 2b and 3, if an oxidation-reduction reaction sequence is involved in chloride absorption.

The effects of cyanide, however, and the induction lag phenomena (see below) suggest another possibility, *viz.* that energy-rich phosphates (e.g. adenosine triphosphate: ATP) supply the energy required. In this case the inhibition of the respiration-dependent absorption by carbon monoxide and anaerobiosis is readily explained, as phosphorylation in mitochondria is largely dependent on the proper functioning

of the cytochrome system and this is inactivated by carbon monoxide and anaerobiosis. The light-dependent absorption would then utilise energy-rich phosphates generated in "photosynthetic phosphorylation": ARNON (1956) in the chloroplasts. Photosynthetic phosphorylation proceeds optimally in the absence of oxygen, so Arnon supposes that the energy required is derived from the recombination of the products of water photolysis; see also WESSELS (1957). As free oxygen is not involved in this reaction it is conceivable that carbon monoxide has no influence on photosynthetic phosphorylation, and consequently on neither photosynthesis nor light-dependent chloride absorption. It is also in agreement with the finding that at low oxygen tension the light-dependent absorption proceeds unimpaired (Fig. 15).

As said in Chapter 8 the inhibition of photosynthesis by cyanide is probably not caused by the blocking of the mechanism which transforms light energy into the first stable form of chemical energy, nor that involved in carbon assimilation, nor that which brings about the conversion of the primary oxidised product of water photolysis into free oxygen. The only remaining possibility is that cyanide primarily inhibits photosynthetic phosphorylation in Vallisneria leaves. According to ARNON *et al.* photosynthetic phosphorylation in chloroplast suspensions is indeed inhibited by cyanide (1956). If it is true that energy-rich phosphates are the source of energy in chloride absorption in Vallisneria leaves, the possibility obviously exists that photosynthesis and light-dependent chloride absorption have some partial process reaction in common which is inhibited by cyanide. Experimental evidence is, however, insufficient to substantiate this hypothesis. Also the objection could be raised against it that the inhibition of chloride absorption by cyanide is rapidly reversed, whereas the capacity for photosynthesis is rather slowly restored. This difficulty could be avoided if one assumes that a low degree of inhibition of the common reaction does not yet lead to inhibition of the chloride absorption. This assumption is by no means unreasonable in view of the fact that chloride absorption is light-saturated at a light intensity much lower than required for photosynthesis (Fig. 7). For if photosynthesis proceeds at a rate of e.g. 5 μmoles oxygen per hour at the light intensity at which chloride absorption is just saturated, it is quite possible that (at higher light intensities) chloride absorption is no longer inhibited as soon as photosynthesis proceeds at a rate higher than 5 μmoles oxygen per hour. Such a partial reversion of cyanide inhibition presumably takes no more than some ten minutes.

In connection with the problem of the sites where energy is utilised in the absorption process the following remark on the question of the permeability of the outer cytoplasmic layer might be made. It has been suggested that the cytoplasm is, at least partially, freely accessible to ions, because in free-space experiments ions and uncharged molecules were found to penetrate into a space that could not be accounted for by cell walls and interstices only (for references vide EPSTEIN (1956).) This is often thought to indicate that the outer cytoplasmic layer is permeable to all small molecules and ions. A free accessibility

of the cytoplasm would imply that the internal ion concentrations, and consequently the pH would be directly dependent on the external ion concentrations and pH. But as many organisms like Vallisneria are not impaired in their activities even at a quite high pH in the medium (up to pH 10, or more), and other organisms can tolerate a very low pH, free accessibility of the cytoplasm to all ions, among which hydrogen and hydroxyl ions, would mean that the proteins (enzymes) of these organisms remain stable, and active, at quite extreme pH values. This, now, seems rather improbable, which constitutes an argument in favour of the theory that the cytoplasm has an outer layer which is relatively impermeable to ions; see also WALKER (1957).

The results of the free-space experiments mentioned above might be explained by assuming that the ion combines reversibly with a specific carrier, and that the ion-carrier complex is capable of crossing the membrane the free ion is not. If such a carrier exists for certain substances only and not for e.g. hydrogen and hydroxyl ions there is no incompatibility between the free accessibility found in the free-space experiments and the toleration of extreme pH values. Therefore, an outer cytoplasmic layer which is impermeable to free ions is not necessarily in contradiction with the results of the free-space experiments. On the ground of these considerations among other ones I should like to suppose that any ion to be absorbed or exchanged has to cross an outer cytoplasmic layer as an ion-carrier compound: cf. ARISZ (1954 II).

Now, with Vallisneria chloride ions are not given off to the medium when the leaves are healthy, so in this case there may be some advantage in supposing a somewhat different mechanism for the passage of chloride ions from the medium into the cytoplasm, *viz.* a mechanism as proposed by MITCHELL (1957). The essential feature of this mechanism is that an ion-carrier compound, once formed, cannot dissociate again, but that another ion of the same or a related species can replace the ion combined with the carrier, or that the combined ion can be transferred to another substance, or liberated in a process for which metabolic energy is required. In this case no net absorption or secretion (leakage) can occur if there is no energy available, but exchange remains always possible.

This mechanism offers the possibility that ions, after being bound to a membrane-bound carrier, are transferred to another substance with carrier function which can freely move about through the cytoplasm, and from cell to cell through the symplasm (parenchymatic long-distance transport): ARISZ and OUDMAN (1937), ARISZ and SCHREUDER (1956 I), ARISZ (1954 I, 1956). The ion then could be translocated without ever getting free in the cytoplasm and merging into a pool of free ions eventually present in the cytoplasm. At the tonoplast a comparable carrier mechanism could transfer the ions from the "cytoplasmatic carrier" across the tonoplast and liberate them into the vacuole.

The hypothesis could be very helpful in explaining how in photo-

synthetic bicarbonate assimilation bicarbonate ions could be transferred from the medium to the chloroplasts without rapidly dissociating into hydroxyl ions and carbon dioxide, which would be the case if the ions occurred in free state in the cytoplasm. If they did dissociate, the resulting carbon dioxide molecules could not be prevented from diffusing out again, and there would be no possibility that bicarbonate could enhance photosynthesis. But if, according to the hypothesis outlined above, the bicarbonate ions travel through the cytoplasm as an ion-carrier compound in which the bicarbonate ions are stabilised, dissociation into carbon dioxide and hydroxyl ion is prevented, and bicarbonate can be assimilated at a high rate even at very low carbon dioxide concentration. There is good reason to suppose that chloride ions and bicarbonate ions are translocated by means of separate mechanisms, for it was found that chloride absorption is inhibited by bicarbonate, whereas bicarbonate assimilation is not inhibited by chloride (Chapter 5). Apparently the chloride-specific mechanism can be blocked by bicarbonate ions; the bicarbonate-specific mechanism, which may be a carboxylating mechanism, is insensitive to chloride ions.

Next some comment should be given on the connection between the experiments reported in this paper and the investigations of Arisz *et al.* on chloride absorption in Vallisneria during 24 hours' periods. ARISZ (1953, 1956) found that cyanide inhibits specifically the uptake of chloride from the medium into the symplasm of Vallisneria leaves, but not the transfer from the symplasm into the vacuoles or the transport along the leaves.

ARISZ explains these facts by assuming that cyanide inhibits a process in which chloride is transferred through the outer layers of the cytoplasm. In terms of Mitchell's theory this might be interpreted as an inhibition by cyanide of the transfer of the ions by a carrier, possibly by cutting off the supply of the energy required in the process, which might mean that it is not a carbohydrate derivative.

From their experiments on the influence of pre-illumination ARISZ and SOL (1956) conclude that in the light a substance is formed in Vallisneria leaves which promotes chloride absorption. This substance is probably another than is involved in the effect of sucrose. The substance is transportable, as was shown in experiments in which the leaf strips were only partly immersed in the chloride solution. When the "free" parts (immersed in distilled water) were in the light the "absorbing" parts (immersed in chloride solution) absorbed more chloride than when the free parts were in the dark. ARISZ and SOL tentatively ascribe a carrier function to the substance. The present author would suggest the identity of the substance with a carrier to which the ions are fixed, and in combination with which they travel about through the symplasm.

In the present investigation no indication was obtained that a substance was formed in the light. This result was certainly due to the short duration of the experiments and their design which differed

greatly from the way the experiments by Arisz and Sol were carried out.

The enhancing effect of sucrose on chloride absorption: ARISZ and SOL (1956) might be ascribed to a stimulation of the respiration-dependent production of energy-rich phosphate. This then causes an increased respiration-dependent absorption of chloride and possibly also promotes the transport of an ion-carrier complex.

With 2,4-dinitrophenol ARISZ (1953) could inhibit specifically the passage of chloride into the vacuole, whereas the uptake from the medium and the transport along the leaf strips was less impaired.

Other inhibitors as azide yielded comparable results, which were, however, better reproducible (ARISZ, unpublished results). The results with dinitrophenol might be interpreted as an indication that energy-rich phosphates are needed for the secretion of the ions into the vacuoles, but not for the preceding uptake into the symplasm or the fixation to a carrier, as the case may be. The present author, however, found in short-duration experiments that the inhibition of chloride absorption caused by dinitrophenol begins slowly, increases slowly in the course of hours and continues to increase even when the inhibitor is no longer present in the medium. In his opinion, therefore, the inhibition by dinitrophenol seems to be indirect and not necessarily an indication that energy-rich phosphates are required in the part of the process which is inhibited by dinitrophenol. The inhibition by azide likewise was neither constant nor completely reversible.

A phenomenon remaining to be discussed is the induction lag shown in photosynthesis (Fig. 24). It might be supposed that photosynthetic phosphorylation does not start immediately after the light is turned on, thereby delaying the onset of carbon assimilation. The supposition is based on the fact that the adaptation reaction to cyanide (Chapter 8, p. 563) proceeds in the light only.

If, as made likely above, cyanide primarily inhibits photosynthetic phosphorylation, it follows that the enzyme system involved in this reaction changes its reactivity to cyanide upon illumination. This change in reactivity to cyanide indicates a chemical transformation which then is supposed to bring about the activation of the enzyme system. It is not too far-fetched to suppose that this chemical transformation will take some minutes' time, thereby delaying the onset of photosynthetic phosphorylation for a same period of time.

This possibility to explain induction effects was not yet considered by RABINOWITCH in his monograph (II^2 1956). In the author's opinion it is not at a variance with data from the literature on induction effects. WASSINK and KATZ (1939) for instance found with Chorella that in cyanide-poisoned cells fluorescence does not decrease again after a maximum rate is reached within the first minute in the light, as it does in uninhibited cells. If one assumes that strong fluorescence can be caused by the inactivity of the enzyme system of photosynthetic phosphorylation: cf. STREHLER (1952), and that cyanide inhibits photosynthetic phosphorylation in Chlorella as it is supposed to do in Vallisneria it is plausible to ascribe the induction lag period in Chlorella as well as in Vallisneria primarily to a

temporary inactivity of the mechanism of photosynthetic phosphorylation, which then is prolonged *ad infinitum* after adaptation to cyanide.

Tentatively it is suggested that the chemical transformation (activation) of the enzyme system of photosynthetic phosphorylation might consist in an oxidation of a heavy-metal compound (cytochrome) because especially oxidised heavy-metal compounds react with cyanide. Alternatively an oxidation of an alcoholic group (e.g. in ascorbic acid) to a carbonyl compound might be suggested, the =CO-group also reacting reversibly with cyanide: JAMES (1953 I, 1953 II).

Light-induced chloride absorption shows, when the light is turned on, a comparable time lag as photosynthesis in Vallisneria (Chapter 3, Fig. 6). This suggests that a common process is limiting during the induction phase. If one or more stages of the chloride absorption process require energy-rich phosphates as the source of energy this common process is presumably photosynthetic phosphorylation. If, on the other hand, chloride absorption is brought about by one or more oxidation-reduction reactions exclusively, it might be supposed that the generation of the reduced or of the oxidised substances or both requires energy-rich phosphates. (It has been suggested that the reduction of pyridine nucleotide in chloroplasts requires the energy of ATP; STREHLER (1952). This is, however, not supported by recent results obtained by ARNON *et al.* (1957). It cannot, of course, be excluded that the induction lag in chloride absorption has nothing to do with the lag in photosynthesis.

The resulting picture of the chloride absorption process in Vallisneria leaves is the following: chloride ions and accompanying cations diffuse passively through the cell wall; subsequently the ions are bound to a carrier with the help of metabolic energy, presumably at the outer cytoplasmic boundary, in order to cross an outer layer which is impermeable to ions; perhaps the ions remain bound in the form of some kind of ion-carrier compound which is carried about from cell to cell in the symplasm; and finally at the tonoplasts the ions are somehow transferred into the vacuoles with or without the utilisation of metabolic energy. This picture possibly holds also for ion absorption in plant roots and other ion transport processes, but it is beyond the scope of this paper to extend the hypotheses thus far.

SUMMARY

The light-dependent chloride absorption in Vallisneria leaves was studied in its relation to the respiration-dependent chloride absorption, and to photosynthesis.

Light-dependent chloride absorption begins at full rate some five minutes after the light is turned on; it ends within two minutes after the light is turned off (Fig. 6). Light saturation is reached at a much lower light intensity than with photosynthesis (Fig. 7).

The action spectra of chloride absorption and photosynthesis are identical (Fig. 8), so chlorophyll is involved in light-dependent chloride absorption.

Carbon dioxide has no instantaneous influence on chloride absorption in the light, which proves the absence of a direct relationship between chloride absorption and carbon assimilation. Bicarbonate ions inhibit chloride absorption in the light

as well as in the dark, but chloride ions do not inhibit photosynthetic bicarbonate assimilation.

Carbon monoxide inhibits the respiration-dependent chloride absorption only; it has no influence on light-dependent chloride absorption or photosynthesis (Chapter 6). The same probably holds for oxygen withdrawal (Chapter 7).

Cyanide inhibits chloride absorption instantaneously and fully reversibly. The inhibition of photosynthesis increases gradually when the leaf tissue is in the light until a stationary level is reached; in the dark the reaction with cyanide which causes the inhibition does not take place at all (Fig. 20). The inhibition is gradually reversible.

In Chapter 9 the photosynthetic assimilation of carbon dioxide and bicarbonate has been treated; a correction of an earlier paper on photosynthesis in Vallisneria has been included there.

The experimental data are discussed in Chapter 10.

ACKNOWLEDGEMENTS

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APPENDIX

DERIVATION OF THE VOLUMETER EQUATION

The volumeter is essentially a vessel partly filled with a watery solution in equilibrium with a gas phase which is kept at constant pressure and temperature. The water phase contains a certain amount of the gases in solution, in the case of air essentially nitrogen, oxygen and a little carbon dioxide. If, e.g. by photosynthesis, a small amount of oxygen is produced within the volumeter and a proportional amount of carbon dioxide is simultaneously removed, the vessel being shaken, a new equilibrium will be established. Part of the oxygen produced will pass into the gas phase and will therefore cause an increase in the oxygen partial pressure. Carbon dioxide is much more soluble than oxygen, therefore the carbon dioxide removed will for a relatively greater part be derived from dissolved carbon dioxide. Consequently the decrease in carbon dioxide in the gas phase and the concomitant decrease in its partial pressure will be smaller than the increase in oxygen in the gas phase and its partial pressure. As the total gas pressure is kept constant the increase in oxygen partial pressure (minus decrease in carbon dioxide partial pressure) will cause a decrease in the nitrogen partial pressure, and this in its turn will cause nitrogen to pass from the liquid to the gas phase. From this reasoning it will be clear that the gas exchanges occurring are quite complicated and that, especially when the liquid volume is large compared to the volume of the gas phase, the influence of changes in partial pressure

and amount dissolved of any of the gases present, even nitrogen, cannot be neglected.

Therefore an equation was derived in the following way:

If V_G = initial volume of the gas phase
V'_G = final volume of the gas phase
V_L = initial volume of the liquid phase
V'_L = final volume of the liquid phase ($V'_L = V_L$)
ΔV = increase in volume ($\Delta V = V'_G - V_G$)
p_{O_2}; p_{CO_2}; p_{N_2}; p_{CO}; p_{H_2O} = initial partial pressures of the gases
p'_{O_2}; p'_{CO_2}; p'_{N_2}; p'_{CO}; p'_{H_2O} = final partial pressures
Δp_{O_2}; Δp_{CO_2}; Δp_{N_2}; Δp_{CO} ($\Delta p_{H_2O} = 0$) = changes in partial pressure
p_S = total gas pressure minus water vapour pressure

s_{O_2}; s_{CO_2}; s_{N_2} s_{CO}; = solubility factors of the gases (expressed as volume units of gas at prevailing temperature which are dissolved in one volume unit of water in equilibrium with a gas phase consisting of the gas concerned only). The solubility factors are assumed to be independent of the composition of both gas- and liquid phases.

ΔO_2 = increase in oxygen (volume units at prevailing temperature and atmospheric pressure minus water vapour pressure)

ΔCO_2 = increase in carbon dioxide (same units) ($\Delta CO_2 = -Q \times \Delta O_2$, in which Q = photosynthetic quotient)

then:

1. Initial total quantity of oxygen = $(V_G + s_{O_2}V_L)p_{O_2}$ (energy units)

1a. Final total quantity of oxygen = $(V'_G + s_{O_2}V'_L)p'_{O_2} =$
$(V_G + \Delta V + s_{O_2}V_L)(p_{O_2} + \Delta p_{O_2})$

1b. but also = $(V_G + s_{O_2}V_L)p_{O_2} + \Delta O_2 p_S$.

From equations 1a and 1b Δp_{O_2} can be obtained explicitly:

2. $$\Delta p_{O_2} = \frac{-\Delta V p_{O_2} + \Delta O_2 p_S}{V_G + \Delta V + s_{O_2} V_L}$$

2a. In the same way $\Delta p_{CO_2} =$

$$\frac{-\Delta V p_{CO_2} - \Delta CO_2 p_S}{V_G + \Delta V + s_{CO_2} V_L} = \frac{-\Delta V p_{CO_2} + Q \Delta O_2 p_S}{V_G + \Delta V + s_{CO_2} V_L}$$

2b. and $$\Delta p_{N_2} = \frac{-\Delta V p_{N_2}}{V_G + \Delta V + s_{N_2} V_L}.$$

The total gas pressure is kept constant; p_{H_2O} is constant, because the temperature is constant; therefore p_S is constant,

3. so $\Delta p_{O_2} + \Delta p_{CO_2} + \Delta p_{N_2} = 0$

In this equation Δp_{O_2}, Δp_{CO_2}, Δp_{N_2} can be eliminated by means of 2, 2a, 2b and then the equation can be solved for $\Delta O_2/\Delta V$:

$$4.\quad \frac{\Delta O_2}{\Delta V} = \frac{1}{p_s} \times \frac{\begin{array}{l}(V_G+\Delta V)^2 p_s + (V_G+\Delta V) V_L \{(s_{CO_2}+s_{N_2})\, p_{O_2} + (s_{O_2}+s_{N_2})\, p_{CO_2} + \\ +(s_{O_2}+s_{CO_2})\, p_{N_2}\} + V_L{}^2 (s_{CO_2} s_{N_2} p_{O_2} + s_{O_2} s_{N_2} p_{CO_2} + s_{O_2} s_{CO_2} p_{N_2})\end{array}}{\begin{array}{l}(1+Q)\,(V_G+\Delta V)^2 + (V_G+\Delta V)\, V_L \{s_{CO_2} + Q s_{O_2} + (1+Q) s_{N_2}\} + \\ +V_L{}^2 (s_{CO_2} s_{N_2} + Q s_{O_2} s_{N_2})\end{array}}$$

In this equation the value of $\Delta O_2/\Delta V$ is dependent on the value of ΔV. Theoretically, therefore, $\Delta O_2/\Delta V$ should be calculated for each single value of ΔV. Under certain experimental conditions, however, the influence of ΔV on $\Delta O_2/\Delta V$ can be neglected, as may be seen from the following calculation:

With the apparatus used here (Fig. 2) 0.2 ml is the maximal value ΔV can attain; the minimal value is, of course, zero. Consequently the largest possible change in $(V_G + \Delta V)$ is 0.2 ml. Now, in Fig. 25

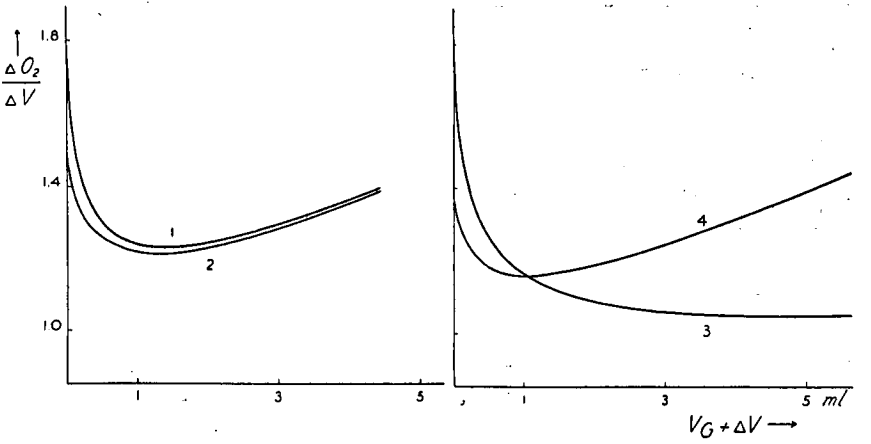

Fig. 25. Curves showing $\Delta O_2/\Delta V$ (oxygen produced in photosynthesis versus volume change recorded with the volumeter) plotted as a function of $(V_G + \Delta V)$ (final volume of the gas phase in the volumeter. Curve 1. gas phase 20 % O_2, 2 % CO_2, 78 % N_2; curve 2. gas phase 49 % O_2, 2 % CO_2, 49 % N_2; curve 3. gas phase 20 % O_2, 80 % N_2, liquid phase bicarbonate solution at pH 9 or more; curve 4. gas phase 1.7 % O_2, 2 % CO_2, 3.3 % N_2, 93 % CO. Liquid phase assumed to be 15 ml watery solution in all curves.

curve 1 $\Delta O_2/\Delta V$ is plotted as a function of $(V_G + \Delta V)$, the gas phase assumed to consist of air with 2 % carbon dioxide and the liquid phase to be 15 ml. If under these conditions V_G is at least 0.9 ml it may be seen from Fig. 25 curve 1 that the change in $\Delta O_2/\Delta V$ corresponding to a 0.2 ml change in $(V_G + \Delta V)$ is not more than 0.01, or about 1 percent of the total value of $\Delta O_2/\Delta V$; 1 percent is well within the experimental error. In the experiments described here V_G was 1 ml or more, which makes the error in $\Delta O_2/\Delta V$ even

smaller. If, however, V_G is e.g. 0.3 ml the change in $\Delta O_2/\Delta V$ corresponding to a 0.2 ml change in $(V_G + \Delta V)$ is about 0.14, or more than 10 %.

A similar calculation was applied for the other experimental conditions occurring in the various experiments.

In practice apart from ΔV many of the terms in the numerator and denominator of the right-hand side of equation 4 can be neglected:

$$4a.\quad \frac{\Delta O_2}{\Delta V} = \frac{1}{p_s} \times \frac{V_G^2 p_s + V_G V_L (s_{CO_2} p_{O_2} + s_{CO_2} p_{N_2}) + V_L^2 s_{CO_2} s_{N_2} p_{O_2} + s_{O_2} s_{CO_2} p_{N_2})}{(1+Q) V_G^2 + V_G V_L s_{CO_2} + V_L^2 s_{CO_2} s_{N_2}}$$

If $Q = -1$ the term $(1 + Q)\, V_G$ becomes zero (Fig. 25, curves 1, 2). In the experiments gas phase air with 2 % CO_2; $V_G = 2$ ml): $\Delta O_2/\Delta V = 1.20$.

With a bicarbonate solution at a pH higher than 9 there is no molecular carbon dioxide present in the vessel, therefore Q is virtually zero and all CO_2 parameters in equation 4 have to be omitted. The result is:

$$4b.\quad \frac{\Delta O_2}{\Delta V} = \frac{1}{p_s} \times \frac{V_G p_s + V_L (s_{N_2} p_{O_2} + s_{O_2} p_{N_2})}{V_G + V_L s_{N_2}} \quad \text{(Fig. 25, curve 3)}$$

In the experiments (gas phase air; $V_G = 1$ ml): $\Delta O_2/\Delta V = 1.13$

With a gas phase consisting mainly of carbon monoxide the terms containing p_{O_2} and p_{CO_2} as factors can be neglected; all nitrogen parameters in the equation are replaced by the corresponding carbon monoxide parameters; $p_S = p_{CO}$.

The resulting equation is:

$$4c.\quad \frac{\Delta O_2}{\Delta V} = \frac{V_G^2 + V_G V_L s_{CO_2} + V_L^2 s_{O_2} s_{CO_2}}{(1+Q) V_G^2 + V_G V_L s_{CO_2} + V_L^2 s_{CO_2} s_{CO}} \quad \text{(Fig. 25, curve 4)}$$

In the experiments (gas phase 1.7 % O_2, 3.3 % N_2, 93 % CO, 2 % CO_2; $V_G = 4.5$ ml): $\Delta O_2/\Delta V = 1.3^5$.

With the numerical values the parameters in the equations 4, 4a, 4b, 4c have under experimental conditions, it was discovered that $\Delta O_2/\Delta V$ is surprisingly insensitive to relatively large variations in these values, as can be shown by the substitution of slightly different numerical values for the parameters in the equation.

From the relative positions of curves 1 (20 % O_2, 80 % N_2, 2 % CO_2) and 2 (50 % O_2, 50 % N_2, 2 % CO_2), for instance, it is clear that a considerable change in the composition of the gas phase has no significant influence on $\Delta O_2/\Delta V$, if V_G is larger than 1 ml.

By means of equation 4a, 4b or 4c, according to the experimental conditions, the oxygen production ΔO_2 can be calculated from the recorded volume change ΔV. From ΔO_2, which is expressed in volume units at prevailing temperature and atmospheric pressure minus water vapour pressure, the oxygen production was calculated in molar units according to well-known principles.

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