ACTA BOTANICA NEERLANDICA, Vol. 3 (1), 1954

GAS-EXCHANGE IN THE VESICLES (AIRBLADDERS) OF ASCOPHYLLUM NODOSUM

BY

P. M. L. TAMMES
Zoological Station, Den Helder

(Received July 23th, 1953)

CONTENTS

INTRODUCTION 114
MATERIAL AND METHODS 116
EXPERIMENTS: 117
a. permeability of the walls 117
b. exchange of gases 119
c. entry of seawater 120
DISCUSSION 120
SUMMARY 122
REFERENCES 122

INTRODUCTION

A study was made of the exchange of gases through the vesicle-walls of the brown alga *Ascophyllum nodosum* under laboratory conditions.

The gas filled air-bladders or vesicles of *Fucaceae* and *Laminariaceae* have been studied by a number of botanists. The development of the vesicles was described by REINKE (1876), who observed that in *Fucaceae* a vesicle originates in a predisposed place on the thallus, e.g. in the form of a flat disc. The anatomy of the young vesicle is the same as in the normal thallus. On the outside a number of layers of closely packed assimilating parenchyma cells (cortex) are observed. On the inside a pith of hyphae is found, to which a re-enforcing or translocating function is ascribed. Between the hyphae, intercellular cavities are found, filled with mucus. The cavities are particularly spacious in the places where the vesicles will be formed and gasbubbles are secreted in these cavities. The bubbles enlarge and unite and thus form the vesicles. The vesicle, then, has the same outer layers of closely packed parenchyma cells, while on the inside there are the living remains of the hyphae, which are fibrous and loose. The vesicle should thus be considered as a closed intercellular space.

A question arises as to why the gas is secreted in the intercellular cavities and not in the interior of the cells. According to Henry's law, the solubility of gases in fluids is proportional to the pressure. In the

interior of a cell under osmotic turgor much more gas can be kept in solution before saturation takes place and a bubble will be formed. On the outside of the cells, under normal pressure, saturation will take place at lower concentrations. When gases can diffuse through the walls of the cells, bubbles will form in intercellular cavities or on the surface of the plants and not in the interior of the cells. This fully agrees with observations made on submersed plants forming oxygen at photosynthesis.

The composition of the gas in the vesicles was studied by WILLE (1889), ZELLER and NEIKIRK (1915), LANGDON (1917), LANGDON and GAILEY (1920), COLLA (1930–1935), DAMANT (1936), VALENCE AND COULT (1951). Oxygen and nitrogen, though found in varying concentrations, approach the composition of normal air. Carbon dioxide is present only in very small amount, the concentration being somewhat higher during the night. An interesting observation was made by LANGDON (1917) and by LANGDON AND GAILEY (1920), who found the occurrence of carbon-monoxide in the large vesicles of *Nereocystis*. Though there are indications that carbon-monoxide may also occur in other species, its presence has not been adequately proved. According to LANGDON and GAILEY (1920) the carbon-monoxide in *Nereocystis* is also formed during darkness and is thus a product of metabolism. The inside of the vesicles is sterile, as was found by RIGG and HENRY (1935), and the carbon-monoxide cannot, therefore, be formed by bacterial action. In addition to these gases there is a small amount of water vapour.

It is commonly held that oxygen is formed in the vesicles by photosynthesis and that an interchange of this gas and the gases of the surrounding medium takes place. The normal composition of the air is thus approached. VALENCE and COULT (1951) observed that the oxygen content rarely decreases much below 20 %. Carbon dioxide from which the oxygen is formed is present in the seawater or is formed by respiration. The solubility of carbon dioxide is, however, so much higher than that of oxygen and nitrogen that it is only present in very small amounts in the gas of the vesicles. The carbon dioxide that may be formed during the night will either quickly dissolve in the water of the tissues and diffuse to the outside or it will disappear at photosynthesis.

MORAVEK (1929) applied various dilutions of seawater to both sides of the vesicle wall of *Nereocystis* and observed that chlorides were translocated in either direction along the concentration gradient.

In the present investigation vesicles of *Ascophyllum nodosum* were placed under different conditions of light and darkness and in gas-free or aerated seawater. The change in volume and composition was then observed and an explanation was given of the phenomena that occur under natural conditions. Further the permeability of the wall for gases, water and other substances was studied.

The experiments were carried out at the Laboratory of the Zoological Station at Den Helder. The author is indebted to Prof. Dr W. H. ARISZ for his criticism and to Mr H. POSTMA for his help with the analysis.

Material and Methods.

Ascophyllum nodosum was collected at low tide on the stony slopes of Nieuwediep harbour near Den Helder. The plants keep fresh for a few days in a moist and cool place in a basket. Storage for longer periods was possible in an aquarium building with a glass roof.

The experiments were carried out at room temperature or in a refrigerator. A suitable gasfree medium was prepared by bottling seawater when still boiling.

The approximate decrease of the gas in the vesicle can be judged from the occurrence of dimples or deflation. Old vesicles, however, have thick walls that are not flexible and often may not display any dimples or deflation when gas is lost. Therefore young vesicles were used in the experiments.

The concentration of oxygen was determined with the so called Jordan gas pipette (1927). The following solution was used as an oxygen adsorbing agent.

NaOH or KOH 4 molar.	11,2 cc
Distilled water	40 cc

To this is added 10 gr $Na_2S_2O_4$ and 1 gr of Natrium-antrachinon-betasulfonate. The solution was kept under paraffin-oil. For the adsorption of carbon dioxide a one molar solution of NaOH was used.

The permeability of the vesicle walls for other substances as gases was studied by filling the vesicles with solutions. This was done by means of a calibrated syringe. The hollow needle was introduced through the flat part of the thallus (Fig. 1) and afterwards the hole was closed with a wooden clamp (Fig. 2) The other end was

Fig. 1

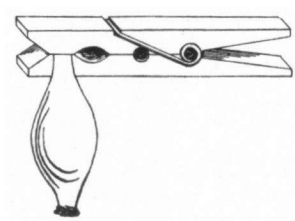

Fig. 2

dipped in paraffin and the end with the clamp was in the experiments always kept above the level of the fluid. Changes in the volume of the solution were measured with the syringe, with a correction for water adhering to the inner wall of the vesicle. In experiments where a

gain of volume is expected, the vesicles were only filled for a small part.

In control experiments dead vesicles were used. These were killed by immersion in seawater of about 80° C.

Experiments

A. Permeability of the walls

The permeability of the walls for gases can be demonstrated by keeping the vesicles in gasfree seawater. The vesicles then form dimples and become deflated after some days in darkness and subsequently seawater will enter by suction. This process occurs at about the same rate at low temperature, so it is for the greater part not due to respiration of the oxygen; it also occurs in dead vesicles. The results of the experiments are given in the following table:

Vesicles in gasfree seawater. Each batch 5 vesicles.

Condition	Temperature ° C	Treatment	Dimples after:	Deflation after:	Remarks
alive	16	darkness	1 day	2 days	some water inside
alive	3	„	1 „	2 „	
dead	16	„	1 „	2 „	much water inside
dead	3	„	1 „	2 „	
Controls in aerated seawater. Each batch 5 vesicles.					
alive	16	darkness	no dimples or deflation after 2 days.		no water inside
alive	3	„			
dead	16	„			
dead	3	„			

The loss of gas and the influx of water in the first series of these experiments in gasfree seawater may go so far that after 4–5 days some of the vesicles no longer float but sink to the bottom. It also follows from these experiments that water may enter in the vesicles, after a loss of gas. The walls of the vesicles must therefore be permeable for water.

This permeability for water was also demonstrated by filling the vesicles with a solution that differs from the outside solution. Water is then translocated through the walls and can be estimated by measuring the change in volume. In using tapwater against seawater the following results were obtained.

Vesicles with different concentrations at both sides.
Each batch of 3 large vesicles. Temperature 16° C.

Inside	Outside	cc used	cc after 24 hrs	Remarks
tapwater	seawater	1,0	0,6-0,7-0,8	
tapwater	tapwater	1,0	1,1-1,1-1,0	control
seawater	tapwater	0,5	0,8-0,9-1,0	
seawater	seawater	0,5	0,5-0,5-0,5	control

Various substances can diffuse through the walls. A number of vesicles was filled with a solution of 1 gram of ammoniumphosphate

per Liter of seawater. The ammonia was then ascertained in the surrounding solution after 2 days at 3° C. MORAVEK (1929) observed for *Nereocystis* that chlorides were translocated through the walls of the vesicles along a concentration gradient. He used diluted seawater in different concentrations.

When applying a solution of 1 gram of sodium nitrate per liter of seawater inside the vesicles, nearly all nitrate was found in the surrounding solution after 2 days The determinations were made by a method worked out by FOYN (1951) for seawater.

Eosine, which is known not to penetrate well into protoplasm, was found to diffuse from the inside of the vesicles into the surrounding medium and after 2 days a red colour was observed. The vesicles were filled with a 0,2 % solution of eosine in seawater.

The pathway of the diffusion could be followed by using potassium-ferrocyanate. A 1 % solution of this salt was used inside the vesicles in a mixture of equal parts of tapwater and seawater. The vesicles were then left for 2 days in seawater and then sections were made from the wall. These sections were immediately fixed with alcohol, to which a few drops of ferrichloride had been added. A precipitate of prussian blue was then formed in those places of the sections where the cyanide was present. The sections were then cleared with chloralhydrate in order to give more prominence to the stained parts. The internal layers displayed a dark blue colour, but more on the outside a light shade of blue could only be observed in the walls of the cells and not in their interior. So it is obvious that the diffusion went through the connecting walls and not through the protoplasm.

Sugars also diffuse through the vesicle walls. This can be demonstrated by applying equimolar solutions of sucrose and glucose on both sides. Concentrations were used which had about the same osmotic value as seawater. That is 20 % sucrose or 10,5 % glucose. After the experiment dry weight and volume were determined. All experiments were performed at 3° C in order to prevent the loss of sugar by respiration or bacterial decomposition.

Glucose molecules pass about 3 times as fast through the walls as sucrose molecules. This causes a difference in osmotic concentration between both sides. This difference will be leveled by translocation of water.

When glucose is put inside the vesicles and sucrose on the outside, up to two thirds of the volume of the solution in the vesicles disappears. In the end the solution inside the vesicles has the same percentage of dry weight as the sucrose solution on the outside. When sucrose is put inside, and the vesicles are only partly filled, the amount increases up to 2,5 times the original volume and the percentage of the dry weight in the end is about the same as in the surrounding glucose solution. Controls with the same solution on both sides did not show large changes in volume or dry weight percentage.

B. Exchange of gases

It was shown before that in the dark, vesicles in gasfree seawater give off part of their gas, so that they become dimpled or deflated. This phenomenon also occurs at low temperature or in dead vesicles and the loss of gas must therefore, in the first place, be due to a removal of the gas as a result of the low pressure of gas in the outside solution.

If, under conditions of darkness, the vesicles are brought into aerated instead of gasfree seawater, the results are different. At room temperature a slow decrease is observed, this decrease is very slow at low temperature and it is not observed in dead vesicles. The decrease in volume is connected with the use of oxygen by respiration, as can be seen from the following tables.

Loss of volume in aerated seawater in darkness
Each batch five vesicles

Condition	Temperature ° C	Vesicles with dimples after days 1 2 3 4 5 6 7	Remarks
alive	16	– – 1 2 3 3 5	after 7 days water inside
dead	16	– – – – – – –	after 7 days no water inside
alive	3	– – – – – – –	
dead	3	– – – – – – –	

Loss of oxygen in aerated seawater. Each batch 3 vesicles.
Trace of CO_2 present. All vesicles in darkness and alive.

Time	Temperature ° C	Oxygen %	Temperature ° C	Oxygen %
at start	—	22-22-23		22-22-23
after 2 days . . .	20	15-10- 8	5	15-16-16
after 4 days . . .	20	4- 4- 7	5	16-13-15

If vesicles are kept in gasfree seawater in sunlight, their gas diffuses out as a result of the low pressure outside, but new oxygen is produced by photosynthesis and the percentage of oxygen increases considerably.

The results are given in the following table. A trace of sodium bicarbonate was added together with the vesicles to procure enough carbondioxide for photosynthesis.

Vesicles in sunlight and in gasfree seawater.
Each batch 3 determinations. Temp. ± 16° C. No carbon dioxide found.

Condition	Days	Oxygen %	Average oxygen %	Remarks
alive	0	21 22 21	21	full
alive	2	51 52 49	51	full
alive	3	60 68 71	69	full
dead (control)	3	— — —	—	all with dimples or deflated

The table shows that at ± 16° C the oxygen percentage in the living vesicles in sunlight may be about 70 % oxygen after 3 days in gasfree seawater. The dead control vesicles lose their gas, which is not replaced by new oxygen. As a result they will become dimpled or deflated and seawater will enter.

C. Entry of seawater

In full vesicles with a positive pressure, seawater is never found inside. This occurs only when, due to some reason or other, the inside pressure becomes negative. When much gas is lost this is due to the resilience of the elastic walls. This condition of negative pressure can be obtained under laboratory conditions, by keeping the vesicles in the dark in gasfree seawater, or even when exposed to aerated seawater in the dark for several days and at room temperature. All dimpled or deflated vesicles will then get some water inside in the end. In the beginning the water is barely perceptible, but after some days under these conditions, more and more water enters and in the end the vesicles are for the larger part filled with seawater. Then gradually the dimples will disappear and the vesicles look apparently normal, but the inside is filled with water and hardly any gas is found.

In the month of December it was observed that on newly gathered algae some of the vesicles contained considerable amounts of water. This seems to agree with our general view on this subject.

Discussion

Valence and Coult (1951), who analysed the changes in the composition of gas in the vesicles of *Fucus vesiculosis*, found the oxygen percentage may be markedly enhanced by photosynthesis. Damant (1936) observed a correlation between the pressure in the vesicles of *Ascophyllum* and the oxygen percentage. He gives values from no pressure at all with 20 % oxygen to 4 lbs per square inch with 35 % of oxygen in the gas of the vesicles. The pressure was highest in April and lowest in November. He also observed that the oxygen percentage was highest in the upper vesicles, which got most of the sunlight. In the present investigation it was found that the oxygen content of vesicles may, in gasfree seawater, amount to 70 % after several days exposure to sunlight. From these observations it can be concluded that the gas in the vesicles is conditioned by the formation of oxygen during periods of photosynthesis.

Gases diffuse through the walls of the vesicles. This can be demonstrated by keeping vesicles for a few days in the dark in gasfree seawater. The gas disappears and the vesicles become deflated and then have the shape of the hollow bowl of a spoon. The same is observed in dead vesicles or in living vesicles at low temperature, which demonstrates that loss of gas is for the major part due to diffusion.

Diffusion of gases through the wall was also demonstrated in an experiment by Damant (1936). *Ascophyllum* strands were tied to a weight and a rope and thrown in deep water. After a few hours, when the plants were again brought to the surface, it was observed that most

of the gas had disappeared and that the vesicles were deflated. This can be explained as follows. More gas can dissolve under high pressure. In the sea, however, the saturation can only take place at the surface. When such water circulates to the depth a large saturation deficit of gases will occur and all gases present will rapidly go into solution. This is according to Henry's law that the solubility of gases is proportional to the pressure. The gas from vesicles, when brought into deep water will, therefore, rapidly diffuse and dissolve in the seawater. As a consequence of this, loss of gas from vesicles in deep water will, within a few hours, be as large as the loss of gas during days in gasfree seawater in the dark.

Gases may diffuse from both sides. The composition of the gas in the vesicles will therefore always strive towards the composition of air in equilibrium with the water around the vesicles. Differences in partial pressure will thus be leveled out.

VALENCE and COULT (1951) observed that, even after prolonged periods in the dark, the oxygen content rarely fell below 20 %. In the authors experience, however, a considerable loss of oxygen was found under laboratory conditions and especially at high temperatures. When oxygen is used by respiration of the tissues the volume of the vesicles decreases gradually, because nitrogen goes out due to the fact that the partial pressure of the nitrogen is too high when oxygen is used. A similar phenomenon was described by EGE (1918) on the function of airstores carried by some aquatic insects. From the air, oxygen is used by the insect and the partial pressure of this gas is lowered. Then new oxygen diffuses but at the same time nitrogen goes out. The bubble, though taking in oxygen, becomes gradually smaller untill the insect has to go to the surface to replenish his supply. Carbon dioxide that is set free immediately dissolves in the water. At low temperature the store will last much longer because less oxygen is used. This was also found in the vesicles. When kept at a low temperature in the dark in aerated seawater, the time required for dimples or deflation was much longer that at room temperature. The walls of the vesicles offer some resistance and make the processes of exchange slower than in ordinary gas bubbles. However, the principle of the exchange remains the same.

Variation exists in the thickness of the walls; the older vesicles on the lower parts of the plants have, in general, thicker walls. Diffusion through these walls is slower and it takes a longer time before such vesicles become dimpled or deflated under experimental conditions. DAMANT (1936) claims that this may be an adaptation to places where a large difference exists between the tides. At high water too much gas will diffuse from the vesicles and this can only be counterbalanced by thick walls or by intense photosynthesis.

The walls were not only permeable for gases but also for water, salts and sugars. Water could be forced through the walls when a difference in osmotic concentration was given between both sides. When pressure in the vesicles becomes negative seawater will be sucked in. Salts diffuse through the walls, as was also observed for chlorides by MORAVEK

(1939) for *Nereocystis*. With potassium ferrocyanide the pathway of the diffusion could be followed through the walls between the cells and not through the protoplasm.

The pressure and composition of the gas in the vesicles, will under natural conditions, depend on the intensity of photosynthesis. This can however be counteracted by a loss of gases. Under certain conditions the loss of gases may prevail over the photosynthesis and the pressure will then become negative and seawater will be sucked in through the walls. This was observed on some vesicles in the month of December.

SUMMARY

1. The gas in the vesicles (airbladders) of the brown alga *Ascophyllum nodosum* is conditioned by the formation of oxygen during periods of photosynthesis.

2. The walls of the vesicles were found permeable for gases, water, salts and sugar. Seawater will, however, only enter the vesicle when the pressure becomes negative.

3. An exchange of gases takes place through the permeable walls. Differences in partial pressures of gases between both sides of the vesicle wall are leveled.

4. Under natural conditions the pressure of the gas and the oxygen content depend on the intensity of photosynthesis. This is, however, counteracted by other factors.

a. loss of gases by diffusion when the partial pressure of the oxygen in the vesicles is higher than in the surrounding water.

b. loss of gases due to a saturation deficit in deeper water, e.g. at high tides. The gases then disappear into the surrounding water. DAMANT (1936).

c. loss of oxygen by respiration. The nitrogen then also leaves the vesicle. The formed carbon dioxide is highly soluble and thus disappears.

When these factors prevail over photosynthesis, a negative pressure may develop and seawater will be sucked in through the walls. This was observed on some vesicles in the month of December. It can also be induced under laboratory conditions.

REFERENCES

COLLA, S., 1930, Variasioni del contenuto in O, nelle vesicle idrostatice di alcune alghe brune. Atti R. Ac. Naz. Lincei. Cl. Fis. Mat. Nat. 11. Ricerce sul contenuto gassoto di alcune alghe.

COLLA, S., 1931, Il Raporti ha i gas ei tessuti in Fucus virsoides.

COLLA, S., 1932, Ricerce sulla secresione di gas di Fucus virsoides. Atti R. Ac. Naz. Lincei. Cl. Fis. Mat. Nat. 12, 13.

COLLA, S., 1935, Ricerce sui gas nei rei vegetali. Nuovo giornale Botanico Italiano 42.

DAMANT, G., 1936, Storage of oxygen in the bladders of the seaweed Ascophyllum nodosum and their adaptation to hydrostatic pressure. Jnl. Exp. Biol. 14.

EGE, R., 1918, On the respiratory function of the air stores carried by some aquatic insects. Zeitschr. f. allgem. Physiologie.

FØYN, E., 1951, Nitrogen determinations in sea water. Rep. Norw. Fish. Mar. Invest. 14.

JORDAN, H. G., 1927, Uebungen aus der Vergleichenden Physiologie. Berlin.

LANGDON, S. C., 1917, Carbon monoxide, occurrence free in kelp. Jnl. Am. Chem. Soc. 39.

LANGDON, S. C. and W. R. GAILEY, 1920, Carbon monoxide, a respiration product of Nereocystis luetkeana. Bot. Gazette.

MORAVEK, V., 1929, Osmotic studies on Nereocystis. Protoplasma, Bd. 7.

REINKE, J., 1876, Beitrage zur Kenntniss der Tange. Pringsh. Jahrb. f. Wiss. Bot. 10.

RIGG, G. B. and B. S. HENRY, 1935, On the origin of gases in the float of bladder-kelp. Am. Jnl. of Botany, 22.

VALENCE, K. B. and D. A. COULT, 1951, The composition of the gas contained in the vesicles of Fucus vesiculosis. Ann. of Botany, 15. N. S.

WILLE, N., 1889, Ueber die blasen der Fucaceen. Biologiske Förening Forhandlingar Stockholm, I, p. 63.

ZELLER, S. M., and A. NEIKIRK, 1915, Gas exchange in the Pneumatocyst of Nereocystis luetkeana. Publ. Pudget Sound Biol. Station. Vol. I, p. 25.