On the Effect of Nitrates on the Carbon-Assimilation of Marine Algae.

BY

E. A. NEWELL ARBER, B.A.,

Trinity College, Cambridge; University Demonstrator in Palaeobotany.

IN a former paper[1], some account was given of experiments on the effect of salts on the assimilation of carbon dioxide in *Ulva latissima*, L. The object of that work was to obtain some qualitative idea of the relative value of the principal salts of sea water in regard to the maintenance of carbon-assimilation. The salts, then made use of, were the chlorides and sulphates occurring in sea water. In the present communication will be found an account of experiments with nitrates, and other salts, on the same Alga by a similar method, and of attempts to extend the work to other marine Chlorophyceae.

Nitrates.

Molisch[2] and Kossowitsch[3] have shown that the higher Algae obtain their nitrogen entirely in the combined form. Very little is known of the influence of nitrates on marine Algae. With fresh-water Algae, the effect of the presence of different nitrogenous compounds in the medium seems to vary

[1] Arber ('01), p. 39. [2] Molisch ('95).
[3] Kossowitsch ('94).

greatly with the nature of the salt, and with different Algae. Loew and Bokorny[1] found that *Spirogyra* flourished better in sodium nitrate ($NaNO_3$) than in potassium nitrate (KNO_3). The presence of KNO_3 induced an abnormal amount of starch-formation, but quickly resulted in the death of the Alga. Ammonium salts were found to be directly injurious to *Spirogyra*; an addition of 0·1 per cent. NH_4Cl quickly caused death. Stange[2] says that *Spirogyra*, *Cladophora*, and *Zygnema* will never tolerate more than 1 per cent. KNO_3, and *Oscillaria* 1·5 per cent.; while species of *Pleurococcus* have been observed to thrive in 12 per cent. KNO_3. In the higher plants, Stange also found that a solution of KNO_3 gave disastrous results in many cases, and Halophytes were generally quickly killed by a solution containing 0·10 gram-molecules per litre KNO_3. Quite recently Jacobi[3] has shown that potassium nitrate decreases the assimilation of submerged plants, such as *Elodea* and *Myriophyllum*.

The nitrate which occurs in sea water is magnesium nitrate, and this is undoubtedly the sole source of nitrogen to such Algae as *Ulva*. The amount is exceedingly small, about ·002 per cent. During the course of this work I made a considerable number of experiments with four nitrates, viz. those of potassium, sodium, magnesium, and ammonium, each being used separately. Two series of experiments were made in each case, with the exception of ammonium nitrate. In one, a certain percentage of the nitrate alone was dissolved in *distilled* water, in the other, in *sea* water.

(A) *Potassium Nitrate.*

The following preliminary experiments show very well the disastrous effect of the presence of potassium nitrate on the power of carbon-assimilation. In Experiment I, the distilled water contained 1 per cent. NaCl; in Experiment II, 1 per cent. KNO_3 in addition to 1 per cent. NaCl.

[1] Loew and Bokorny ('87). [2] Stange ('92). [3] Jacobi ('99).

Experiment I. January 12, 1900. 1 per cent. NaCl in distilled water.

Date.	*Days.*	*Amount of Starch.*
Jan. 13	1	little[1]
Jan. 17	5	moderate

Experiment II. January 13, 1900. 1 per cent. NaCl + 1 per cent. KNO_3 in distilled water.

Date.	*Days.*	*Amount of Starch.*
Jan. 16	3	none
Jan. 18	5	the slightest trace

Experiments with different percentages of KNO_3 in distilled water gave the following results. For sake of comparison, a similar series (except that 1·5 per cent. was used instead of 1 per cent.) of percentages of NaCl, made a week later, are also given.

Experiment III. April 13, 1900. *Ulva* in distilled water containing

Date.	*Days.*	0·1 % KNO_3.	0·5 % KNO_3.	1 % KNO_3.
Apr. 14	1	a trace	a trace	a trace
Apr. 16	3	a trace	a trace	the slightest trace
Apr. 19	6	a trace	little	the slightest trace

Experiment IV. April 20, 1900. *Ulva* in distilled water containing

Date.	*Days.*	0·1 % *NaCl.*	0·5 % *NaCl.*	1·5 % *NaCl.*
Apr. 21	1	a little	a trace	a little
Apr. 23	3	moderate	moderate	moderate
Apr. 26	6	large	moderate	large

[1] The scale of starch-accumulation used will be found fully explained in my former paper.

There was also a marked difference in the condition of the Alga. After six days in the KNO_3 solutions, the seaweed was not far from the point of death, while the Algae in the NaCl solutions were quite healthy. The object of these experiments with distilled water, as in the case of the other nitrates, was to see whether *Ulva* could assimilate in solutions of this salt alone, as it was found to do in the case of sodium chloride. I concluded that this was not the case. The results of Experiments I and II show that the absence of assimilation was not due so much to the absence of certain necessary salts, as to a detrimental effect caused by the presence of the nitrate. With a view to testing this point, I made experiments (as also with other nitrates) in which certain percentages of potassium nitrate were *added to sea water*, on the assumption that if this salt was not harmful to the plant the carbon-assimilation would be normal. A series of solutions were made consisting of sea water to which ·5 per cent., 1 per cent., and 2 per cent. KNO_3 was added; and in these *Ulva* was exposed to light. I never obtained from Algae in solutions containing ·5 per cent. KNO_3 more than a 'little' starch, from those in 1 per cent. KNO_3 more than a 'trace,' and in 2 per cent. KNO_3 solutions no starch whatever was found. I therefore concluded that the presence of an appreciable amount of KNO_3 in the sea water caused an inhibition of the CO_2-assimilation.

(B) *Sodium Nitrate.*

As with other nitrates, the proportions of sodium nitrate used were those equivalent to 0·1, 0·5, 1, 2, &c. per cent. KNO_3, the latter being taken as a standard in all cases. 1 gram KNO_3 is equivalent to ·8415 gram $NaNO_3$. The following experiment is typical of the effect of this nitrate in sea water:—

Experiment V. June 7, 1900. Sea water + $NaNO_3$.

Date.	*Days.*	*Equivalent to 1% KNO_3.*	*Equivalent to 0·5% KNO_3.*	*Control sea-water alone.*
June 12	5	moderate	not quite moderate	moderate
June 14	7	moderate	moderate	maximum

Similar experiments with distilled water gave results slightly more favourable than those in Experiment III. In all cases in Experiment V, the amount of starch obtained was less than in the control in sea water alone, although after five days in 1 per cent., it was nearly the same. I never obtained a 'large' amount of starch, but on the other hand the Alga continued in good condition for some time, in marked contrast to those in KNO_3 solutions. I concluded that, so far as these experiments went, sodium nitrate was not able to take the place of sodium chloride in regard to CO_2-assimilation, and that the presence of sodium nitrate in sea water somewhat inhibited the power of CO_2-assimilation, but not in such a marked degree as in the case of potassium nitrate.

(C) *Magnesium Nitrate.*

This is the nitrate which occurs in very small proportions in sea water: 1 gram KNO_3 is equivalent to 1·465 grams $Mg(NO_3)_2$.

Experiment VI. June 7, 1900. Sea water + $Mg(NO_3)_2$.

Date.	*Days.*	*Equivalent to 1% KNO_3.*	*Equivalent to 0·5% KNO_3.*	*Control sea-water alone.*
June 12	5	little	little	moderate
June 14	7	moderate	large	maximum

Experiments in which magnesium nitrate was added to distilled water gave results similar to those with sodium nitrate. I was not able to make as many experiments with this salt as I could have wished, but the results with sea water were, on the whole, very similar to those with $NaNO_3$, perhaps a little more favourable, but never quite equal to the control.

(D) *Ammonium Nitrate.*

With this salt experiments were only made with sea water: 1 gram KNO_3 is equivalent to 0·792 gram NH_4NO_3. In experiments with quantities equivalent to 1 per cent. and

·5 per cent. KNO_3, no starch whatever was obtained even in a week, or at the most the slightest 'trace.' The control was the same as in experiments with KNO_3. The Alga became unhealthy, and died in a very short time.

Such conclusions as may, I think, be drawn from these experiments, but which, in some cases, can only be regarded as provisional, are as follows. No solution, containing a moderate percentage of these nitrates *alone*, is at all comparable to one of an equivalent percentage of NaCl, as a medium for normal CO_2-assimilation. The effect of the presence of 1 per cent. KNO_3, or its equivalent of the other nitrates experimented with, is in all cases to lower the amount of CO_2-assimilation as judged by the amount of starch found in the thallus. With magnesium nitrate this is least marked, and next sodium nitrate. Potassium nitrate has a very injurious effect, while ammonium nitrate is absolutely fatal. These results are remarkably in accordance with those of Loew and Bokorny in regard to *Spirogyra*, and also with the conclusions of Stange as to the prejudicial effect of KNO_3 on many fresh-water Algae. In the case of *Ulva*, $NaNO_3$ was also found to be less injurious than KNO_3, and the ammonium salt equally fatal, but in the former case there was no sign of any abnormal amount of starch being formed.

These results were, in the main, confirmed at the Bradford meeting of the British Association, where I gave a brief sketch [1] of the chief conclusions to which I had attained. In a paper which followed, by Messrs. Letts and Hawthorne [2], on the relation of this Alga to the pollution of sea water by sewage, it was stated that no carbohydrates beyond cellulose could be found, on analysis, in specimens growing in the polluted sea water. Dr. Letts said at the time that he was much struck by the absence of all traces of carbohydrates such as starch. These authors have made out a very good case to show that *Ulva* can, under some circumstances, derive its entire nourishment from organic materials, especially from substances rich in combined nitrogen, and without carbon-

[1] Arber ('00), p. 934.

[2] Letts and Hawthorne ('00), p. 935.

assimilation. There is little doubt, however, that this faculty is only attained by a process of gradual and natural accommodation, the absence of which in the experiments recorded here, I think, fully explains why the Algae did not flourish.

As to the exact cause of the lowering of the CO_2-assimilation by the presence of these nitrates, it is impossible to offer more than suggestions. It is, however, certain that the result here is not due in any way to plasmolysis. Taking the amount of common salt in sea water as 2·5 per cent., the amount of KNO_3, which is the isotonic equivalent of 2·5 per cent. NaCl, is about 4·3 per cent. KNO_3, a larger percentage than was used, and which even then would not have caused plasmolysis. Since plants absorb all substances, essential or injurious, presented to them in a soluble form, it follows that, seeing there is no plasmolysis, the nitrate, with other salts absorbed from sea water, penetrates within the protoplast. This being so, it seems to me that there can be either a physical, or a chemical cause for the effect produced by these nitrates. It is known that many soluble crystalline substances cannot diffuse through certain plasmatic membranes. If this is the case here, the diosmosis of such salts as NaCl, which in *Ulva* would seem to have a peculiar value as absorption-products, would be checked, and consequently an inhibition of the CO_2-assimilation might take place. On the other hand, this inhibition may be due to a chemical change, which takes place within the plant on the absorption of a considerable quantity of these nitrates, and which may be directly injurious to the protoplast, unless a process of long accommodation has taken place.

With regard to the first of these alternatives, there is no evidence, and I am inclined to favour the chemical theory, to which certain facts would seem to point. Pfeffer[1] says that 'potassium nitrate, when absorbed, is gradually converted into a salt of an organic acid, the traces of nitric acid set free being immediately absorbed by the protoplast.' It is possible that, when a considerable quantity of a nitrate is

[1] Pfeffer ('00), p. 131.

absorbed, a larger amount of free acid would be set free by the decomposition. As such Algae as *Ulva* are well known to be extremely sensitive to acid solutions, which are generally injurious to such plants, the inhibition of the CO_2-assimilation may be due to a greater amount of acid than usual being set free. The varied degree of inhibition, which different nitrates were found to cause, may conceivably be explained by the metal of certain nitrates having a greater affinity for the organic acid radical. The potassium and ammonium of KNO_3, and NH_4NO_3, may be more eager to unite with the oxalic or other organic acid than the sodium or magnesium of $NaNO_3$, and $Mg(NO_3)_2$, and thus cause a greater inhibition. It was pointed out in a former paper that certain sulphates, especially calcium sulphate, were found to be similarly injurious. Possibly this effect may be explained in the same way, for Schimper[1] has shown that the fate of both nitrates and sulphates is closely analogous, in that they are both reduced. The type of reaction in these cases is—

$$\underset{\text{Sugar}}{C_6H_{12}O_6} + 2HNO_3 = \underset{\text{Asparagin}}{C_4H_8N_2O_3} + \underset{\text{Oxalic acid}}{C_2H_2O_4} + 2H_2O + 3O$$

Schimper has also pointed out that, while potassium oxalate is exceedingly common in plants as the first stage in the formation of calcium oxalate, the presence of sodium oxalate is exceedingly rare. This fact may possibly have some bearing on the assumption suggested here, that the difference in effects caused by potassium and sodium nitrates, is due to a difference of chemical affinity on the part of the metallic radical.

Potassium Phosphate.

With a view to supporting the explanation here suggested to account for the effect of nitrates on *Ulva,* some similar experiments were made with potassium phosphate. The phosphates which could be used for this purpose are very

[1] Schimper ('90).

few, owing to their insolubility or acidity; or because some (sodium phosphate) precipitate in sea water. Four experiments were made in which 1 per cent. potassium-phosphate was added to sea water, and in these starch-free *Ulva* was exposed to light, with a control in sea water under the same conditions. After six days the amount of starch obtained was, in three experiments, 'a trace,' while, in the fourth experiment, a 'little' was obtained. The Alga became unhealthy in a short time. The control continued in good condition, and gave a 'moderate' amount of starch in the same time.

The conclusion was that the presence of an appreciable quantity of potassium phosphate in sea water inhibited the CO_2-assimilation. Schimper[1] has shown that the fate of phosphates is altogether different from that of nitrates and sulphates. But in the formation of calcium oxalate, which according to Schimper takes place as follows,—potassium phosphate in the presence of oxalic acid forms potassium oxalate, and then potassium oxalate in the presence of an inorganic calcium salt forms calcium oxalate,—it is clear that, in the first step at least, phosphoric acid is set free. Hence from the standpoint adopted here, the amount of free acid, the result with phosphates should be similar to that with nitrates and sulphates, as indeed it was found to be.

Phosphorus, Iodine, Iron.

I have now concluded experiments on what I have termed the *principal salts* of sea water, using that term only in the sense of forming the greater percentage of that substance. Other elements, such as iron and phosphorus, are equally essential to the plant, but are remarkable as occurring only in the minutest traces in sea water. Although no experiments, with the exception of those on potassium phosphate, were made with these bodies, it may be of interest briefly to point out a fact too generally overlooked, namely, how minute the traces of these elements in sea water really are.

[1] Schimper ('90).

	Percentage in sea-water.	Percentage in total ash of various Fucaceae[1].
Iron	0·0005	0·29–0·34
Combined nitrogen	0·0002	
Iodine, less than .	0·00001	0·31–1·13
Phosphorus . . .	——	1·36–4·4

With regard to phosphorus, no chemical analysis of sea water contains any estimation whatever of that substance; the trace being infinitely small[2]. Noll[3] has drawn special attention to this in a paper on the culture of marine Algae. The same author[4] regards iodine, which occurs in an almost equally small proportion, as not indispensable to Algae. The necessity of iron was first pointed out by Gris[5] in 1843, and his are probably among the earliest observations, which showed that an inhibition of the carbon-assimilation in a plant can be caused by the absence of an essential inorganic salt. Molisch[6] has found, and it is generally admitted, that the molecule of chlorophyll does not contain iron, and it is therefore highly probable that iron is essential to the metabolism of the chloroplast. It would seem, therefore, that in sea water we have certain essential elements, in what is probably the smallest degree of concentration ever made use of in nature by plants. A better illustration of the selective capacity of the plant, and its power of absorbing substances in direct proportion to the amount necessary for metabolism, and in altogether disproportionate amount to the ratio of the substances in a given volume of the medium, would be hard to find.

THE STARCH-ACCUMULATION IN MARINE ALGAE.

One point, which has been very prominent throughout these experiments, is the large accumulation of starch which occurs in these Algae, and the very slow rate of translocation.

[1] Goedechens, vide Pfeffer ('00), p. 128.
[2] Voelcker ('50), pp. 346–7.
[3] Noll ('92), pp. 282–3.
[4] Noll ('92), p. 285.
[5] Gris ('44).
[6] Molisch ('92).

This is marked even in *Ulva*, and as I showed in a previous paper, formed one of the chief difficulties of the work. The Alga had to be darkened for several weeks before becoming starch-free. At the conclusion of the work on *Ulva*, I was very anxious to make similar observations on other marine Chlorophyceae. Unfortunately very few members of this group are at all suitable for such a purpose, and in none is the form of the thallus so favourable for observation as in *Ulva*. *Enteromorpha intestinalis*, Link, was tried, but abandoned as I was unable, even after weeks or months of darkening, to get all the starch out. Not having the time at my disposal to attempt a direct estimation of the amount of carbon assimilated,—a problem seemingly of great difficulty when the plant is immersed in a liquid,—I determined to try *Cladophora rupestris*, Kg. Material, which was very kindly sent me by Professor Phillips, was completely darkened in sea water in the laboratory, but after two months the Alga still contained starch and was becoming so unhealthy that, although another and equally unsuccessful attempt was made in very diffuse light, the work had to be abandoned. It had occurred to me that want of oxygen, or insufficiency of salts in a limited quantity of sea-water, might account for this. Pennington [1], in regard to similar difficulties with the destarching of *Spirogyra*, found that the former played a very important part in the process. I therefore arranged, through the courtesy of the Director, for the destarching to be carried out at the Marine Biological Laboratory at Plymouth. A large tank there was almost completely darkened, and through it a supply of sea-water was pumped continuously, the inflow impinging sharply on the surface of the water in the tank, and thus ensuring sufficient saturation with air. The tank was stocked early in January with *Cladophora* which I obtained from the neighbouring coast. After five months' darkening, while the Alga continued healthy, and in good condition, it still contained starch. In a few experiments which I made with the partially destarched Alga, by exposing it to light in sea water, I found

[1] Pennington ('97).

that the rate of accumulation was correspondingly long. It was also found to be by no means so easy as in *Ulva* to judge of the amount of starch in the thallus, even roughly, and for these reasons the work was finally abandoned. *Cladophora rupestris* is undoubtedly not an obligate Halophyte, and it is possible that in such Algae the necessity for sodium chloride, which I found in the case of *Ulva* to be so important for the maintenance of carbon-assimilation, is not so marked, and that the plant can continue to make use of other salts, more especially those of fresh water, for such a purpose. This would seem to be the real distinction between an obligate and nonobligate Halophyte, rather than any insufficient selective power on the part of the former. Unfortunately, however, for the reasons just described, I am not able to add anything for or against this supposition.

In conclusion I have to again express my thanks to Mr. Darwin for the interest he has taken in the work and the help which he has at all times placed at my disposal. I have also to express my indebtedness to the Director, and to Mr. Garstang, of the Marine Biological Laboratory at Plymouth, for the courtesy with which every facility was afforded for the work which I carried on there.

Conclusions.

(1) The addition of a nitrate to sea water causes an inhibition of the carbon-assimilation.

(2) With ammonium nitrate, the inhibition is very marked, and the presence of this salt is quickly fatal.

(3) Potassium nitrate causes a more marked inhibition than sodium nitrate.

(4) Magnesium nitrate, the nitrate occurring in sea water, causes the least marked inhibition.

(5) Potassium phosphate added to sea water in an appreciable percentage, causes a considerable inhibition.

(6) In the thallus of *Ulva*, *Enteromorpha* and *Cladophora*, there is a marked storage of starch, and a very slow rate of translocation.

Bibliography.

Arber, A. E. N. ('00): Brit. Assoc. Rep. Bradford, 1900.
——————— ('01): Ann. of Bot., vol. xv, 1901.
Gris, E. ('44): Compt. Rend., 1844-7.
Jacobi, B. ('99): Bot. Zeit., 1899.
Kossowitsch, P. ('94): Bot. Zeit., 1894.
Letts and Hawthorne ('00): Brit. Assoc. Rep. Bradford, 1900.
Loew and Bokorny ('87): Journ. f. prak. Chem., N.F., Bd. xxxvi, 1887.
Molisch, H. ('92): 'Die Pflanze in ihr. Bez. z. Eisen,' 1892.
——————— ('95): Sitz. der k. Acad. Wien, Bd. civ, Abth. I, 1895; Bd. cv, Abth. I, 1896.
Noll, F. ('92): Flora, 1892.
Pennington, M. E. ('97): Contrib. Bot. Lab. Univ. Pennsylvania, 1897.
Pfeffer, W. ('00): 'Physiology of Plants' (trans. by Ewart), 1900.
Schimper, A. F. W. ('90): Flora, 1890.
Stange, B. ('92): Bot. Zeit., 1892.
Voelcker, A. ('50): Chem. Gaz., vol. viii, 1850.