cultures might have been the source of considerable error. However, under the conditions of the experiment the influence of the presence of the stock culture agar on the plates is believed to have had relatively little influence on the observed results.

In general, the results obtained from both the Petri dish cultures and the sawdust cultures corresponded rather closely. This similarity may suggest a possible explanation for not only the extensive growth of mycelium in the sawdust cultures containing dextrose and asparagine, but also their small loss in weight.

The addition to the sawdust cultures of dextrose, either alone or in combination with asparagine, greatly increased the amount of mycelium. In the dextrose series inoculated with *Lentinus lepideus*, for example, the amount increased with an increase of dextrose. For this reason the cultures to which 5.28 per cent dextrose had been added would have been given first place and the controls last place, if an estimate of the expected loss in weight had been based on the volume of mycelium produced. Actually, the 5.28 per cent dextrose cultures lost considerably less weight than the controls. The amount of fungal growth in the *Lentinus lepideus* cultures to which dextrose or dextrose and asparagine was added is shown in figure 2.

This observed relationship between the volume of mycelium produced and the presence of dextrose or dextrose and asparagine, may justify the following tentative hypothesis: that the test fungi, *Lenzites trabea* and *Lentinus lepideus*, differ markedly with respect to their ability to attack Norway pine sapwood sawdust when more available nutrients, such as dextrose alone or both dextrose and asparagine, are present. *Lenzites trabea* does not appear to exhibit selective feeding, the wood substance appears to be destroyed at the same or somewhat increased rates when the more available nutrients are present. *Lentinus lepideus*, on the other hand, appears to utilize the more available nutrients and produces large quantities of vegetative mycelium but does not attack the wood substance as rapidly in the presence of nutrients as it does when these are absent. The stimulation of mycelial growth of *Lentinus lepideus* by concentrations of dextrose and dextrose and asparagine that reduced its ability to decay wood would appear to largely exclude the possibility that the observed retardation may have been due to osmotic effects.

SUMMARY

A study of the effect of the addition to wood of dextrose and dextrose and asparagine on its rate of decay by wood-destroying fungi indicates that the rate of decay of wood caused by some fungi, *Lenzites trabea* for example, may be increased by the addition of dextrose or dextrose and asparagine if the amounts added are not too great. In the case of other fungi, *Lentinus lepideus* for example, it appears that the rate of decay of wood it causes is decreased if dextrose or dextrose and asparagine is added to the culture. In such cases the fungus may for a time at least develop largely at the expense of the sugar and asparagine present and only to a limited degree at the expense of the wood substance.

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THE AVAILABILITY OF DIFFERENT FORMS OF NITROGEN TO A GREEN ALGA [1]

C. A. Ludwig

CONSIDERABLE WORK has been done on the utilization of different compounds as sources of nitrogen for green plants. Much of this was with higher plants, sometimes under conditions which assured neither sterility nor even a low plane of microbiological activity in the medium surrounding the roots. Under such conditions there could be no certainty when assimilation occurred that the nitrogenous compounds supplied were the ones actually absorbed by the plants. Some work has also been done with algae. Part of it is subject to the same criticism, but much has been under strict biological control, which is easier with microscopic plants. In practically all cases the criterion of nitrogen absorption was the qualitative one of the appearance of visible growth. The work reported here, conducted in connection with investigations in this laboratory on biological nitrogen fixation, amplifies the previous work on algae with quantitative data for the assimilation of nitrogen from several compounds by a unicellular organism in pure culture.

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The writer is indebted to Ellen K. Rist, of this laboratory, for most of the nitrogen determinations made during this study.

The literature on the subject of nitrogen absorption by higher plants has been reviewed (3, 19, 42, 77a, 79). While this has not been done recently for the algae, it is not attempted here except with regard to the particular compounds tested. No papers reporting assimilation are cited except where it seems fairly certain that the cultures were genuine pure cultures and that the substance under study was the only source of nitrogen available to the organism. (But in general the possibility of nitrogenous impurities in the reagents or of changes during heat sterilization is not considered.) On the other hand, in instances where non-assimilation or toxicity was reported, it has been considered that moderate contaminations with other organisms, or the presence of other nitrogen sources in the medium, did not contribute much doubt as to the significance of the results; and a number of such studies are cited.

METHODS.—The organism used was a species of *Chlorella* (Dr. F. B. Wann's No. 11, obtained from Dr. Franklin E. Allison, of this laboratory). The advantage of a unicellular plant for work of this type lies in the fact that cultures can be grown by the usual bacteriological methods, thus simplifying the problem of sterility.

The solution for the control cultures contained nitrogen as potassium nitrate and had approximately the following composition: $MgSO_4$, 0.01 M.; KNO_3, 0.0125 M.; KH_2PO_4, 0.009 M.; $Fe_2(SO_4)_3$, 0.0000106 M.

For each experimental culture, except as noted, the potassium nitrate was replaced with the equivalent amount (to supply approximately 175 mg. nitrogen per liter) of the nitrogenous compound to be tested. The chemicals were usually ordinary "C.P." materials, while the magnesium sulphate was a therapeutic preparation of Epsom salts. The ammonium acetate, citrate, tartrate, formate, succinate, and lactate were prepared by neutralizing the acids with excess of strong ammonia and boiling off the excess. In a few cases the salt was concentrated under reduced pressure, crystallized, and washed; but more often it was used directly without purification. The stock solutions were analyzed for ammonia by distillation with sodium hydroxide and were found to contain approximately the expected amounts. Tap water was used except in a few experiments where parallel cultures were grown in media made up with distilled water. These cultures showed no evident advantages for the distilled water media, and tap water was then used exclusively on account of the greater certainty of including sufficient traces of those necessary elements which were not added specifically. Cultures in which no nitrogen was added were always prepared. They always grew for a few days, but soon became yellow, and on analysis showed not more than a trace of nitrogen, the value of which was used as a control correction.

The pH was measured colorimetrically, but was not controlled closely. It most often had some value between 5 and 7 after sterilization of the medium and before inoculation. The pH at this time is probably of little importance, however, unless it is fairly acid, because determinations at the close of an experiment, when taken promptly, showed a preponderance of values in the neighborhood of 5.5 or lower. This fact suggests that the carbon dioxide (5 per cent) in the aeration stream had fixed the reaction approximately at pH 5.5 in most of the cultures which were not for some reason more acid. This reaction is one at which the organism [2] has been shown by the work of Hopkins and Wann (41b, 41c, 101a) to grow well and at which the amount of available iron is not likely to be limiting. In flasks where calcium carbonate ("precipitated chalk" 1 gm. or ground marble 2 gm. per liter) was used, the pH was probably higher directly around the cells, since they always settled to the bottom in close contact with the material. The growth results render it doubtful, however, that there was any significant removal of iron from the solution by the calcium carbonate such as the authors just cited (41b, 41c, 41d, 101a) observed when calcium phosphate was precipitated in the solution.

Media were sterilized in the autoclave except as indicated otherwise. No tests were made to determine if any decomposition of the nitrogenous materials occurred during this treatment. However, most of the compounds are fairly stable at the temperature concerned, and with the exception of the non-assimilable materials and of urea, the amount of nitrogen absorbed by the organisms was greater than that which it seems at all likely would be transformed to some other form during sterilization. Inoculations with equal volumes of a well shaken suspension of the organism were made with a sterile pipette. Contaminations were rare. Where they occurred, the cultures were either rejected or the presence of the foreign organism was considered in interpreting the results.

The culture vessels were pyrex Erlenmeyer flasks closed with 2-hole rubber stoppers fitted with glass tubes for aeration. In the first experiment one liter, in the second 100 ml., and in all later ones 200 ml. of solution per culture was used. All cultures were run in duplicate in each experiment, and the results given are means of the two cultures unless otherwise indicated. In general duplicates agreed well, although a twofold variation was not very unusual, and a greater occurred occasionally.

The aeration stream consisted of air enriched with approximately 5 per cent of carbon dioxide. It was bubbled through strong sulphuric acid, mercuric chloride solution, and then three to five (usually four) of the culture flasks in series at a rate which allowed apparently undiminished growth in the last flask.

Light was furnished by mazda lamps of clear glass with porcelain reflectors. The intensity varied in the different experiments but was around 300–500 foot candles (3200–5400 lumens). Illumination was

[2] While the organism used by these authors is designated merely as *Chlorella* sp., Dr. Hopkins stated in a personal communication to the writer that the one used in all their work together was *Chlorella* No. 11.

continuous except in the last experiment, where the lighted and unlighted periods were 16 and 8 hours, respectively. In the second experiment part and in later experiments all of the culture flasks were placed in a white tray containing a layer of water about as deep as that of the culture solution.

At the close of an experiment the cells in each culture were collected on filter paper, washed with distilled water, dried, and the nitrogen determined by the Kjeldahl method, using copper sulphate as the catalyst in the earlier work and elemental selenium in the later.

RESULTS.—The results are summarized in table 1. The mean uptake of nitrogen per culture is given for each material, both as mg. per culture and as a percentage of the amount absorbed by the corresponding (potassium nitrate) control cultures. Theoretically it should be possible, by comparing the means of the percentages, to list all the different nitrogen sources in the order of their utilization. However, some of the means are from only a few determinations where the variability was large, thus making large differences necessary for significance. And even if this were not true, it would hardly be justifiable to carry the comparison so far. A number of investigators (26, 29, 81, 95) have called attention to the fact that many plants absorb nitrate nitrogen best at a more acid reaction than that at which they use ammonium nitrogen best. Urhan (100) has shown that some species of *Chlorella* and *Scenedesmus* take up nitrogen from nitrate and nitrite best in a moderately acid medium and from ammonium in a more alkaline one. Tiedjens (95) has further made the point that where the nutritive values of different nitrogen carriers are being compared, the conditions for each should be optimum for its assimilation. Since it was impractical to determine and employ such conditions for each nitrogen source considered, small differences must be considered as without significance. In many cases, however, differences are great enough to be fully significant or at least highly suggestive; and in practically all cases the results are ample for answering the question primarily under investigation—Is the nitrogen in the compound assimilable?

It will be seen from table 1 that the organism absorbed nitrogen from a very considerable number of different nitrogen sources, both inorganic and organic, but did not do so from others, some of which might have been expected to be suitable sources of the element.

DISCUSSION.—*Potassium nitrate.*—The growth, as shown both by the appearance of the cultures and by the amount of nitrogen assimilated, was good. This was to be expected, since almost all reported tests of algae with nitrate in the past have shown its utilization. But most pure cultures have been isolated with nitrate-containing media, and it is possible that the use of a different source of nitrogen in the isolation medium might give organisms of a different character. Since the general situation is so well known, and the number of studies which could be mentioned is very large, none will be cited in this connection. Nitrate is not assimilable by all algae, however. Some of the flagellated forms, especially those of the genus *Euglena*,[3] are notable as being unable to use this material or to use it only with difficulty (31, 32, 34, 35, 66). However, some other algae seem also to be lacking in this ability (13, 25, 51, 88), although it is possible that most of them would show the ability if the proper experimental conditions were provided. Thus, doubt as to the reported result for *Scenedesmus acutus* Meyen (13) has been expressed by Klebs (32, p. 183) and Senn (89, p. 71); and Grintzesco (39) has reported a definitely contrary finding.

Potassium nitrite.—The nitrite was assimilable in the low concentrations used, but even these concentrations seemed slightly toxic when added to a nitrate medium. Possibly a more alkaline medium would have given better results with nitrite. The result agrees with that of most other work with algae on this point, for while there is abundant evidence (8, 9, 12, 14, 17, 18, 27, 47, 72, 77, 83, 98, 100, 102) that nitrite is assimilable under proper conditions, it is also common experience that it is non-assimilable or toxic in too high concentration (10, 17, 72, 75). In view of the general acceptance of the idea that nitrite is an intermediate stage in the reduction of nitrate by higher green plants, it is interesting to note that Beckwith (11, 12), Hall (41a), ZoBell (102), and Sommer (90) have recently produced evidence that the same is true for unicellular green algae. Warburg and Negelein (101b) have shown that *Chlorella pyrenoidea* Chick may produce nitrite from nitrate, but the result was observed only at high acidity in the dark and probably means little where the normal metabolism of the organism is concerned.

Inorganic ammonium salts.—It is convenient to discuss together the assimilation of nitrogen from ammonium sulphate, ammonium chloride, ammonium phosphate, and ammonium carbonate since the amounts of nitrogen taken up from these sources were nearly the same. These amounts were somewhat greater than those secured from the control solutions, and this fact suggests a superiority of ammonium over nitrate as a source of nitrogen under the conditions of the experiments. Moreover reduction to about 3.5 in the pH of the sulphate and chloride media during growth, a greater nitrogen absorption and smaller pH change where nitrate and ammonium were used in combination, and the result from adding calcium carbonate to the phosphate medium suggest in addition that with better buffering the superiority might have been even greater.[4] Results with these nutrients by other in-

[3] It is realized that the Euglenae and similar organisms are probably animals rather than plants, but many of them contain chlorophyll and exhibit a metabolism essentially like that of green plants. Their nitrogen nutrition is therefore interesting in connection with this study and citations concerning it are included.

[4] These remarks refer to nitrogen uptake, only. Dry matter production might give a somewhat different result, since assimilated nitrate nitrogen is often more

vestigators have usually been essentially the same as reported here. Thus ammonium sulphate (2, 4, 7, 9, 20, 21, 22, 28, 31, 34, 35, 38, 50, 60, 66, 71, 72, 73, 74, 75, 76, 77, 83, 84, 88, 94, 99, 101, 102), ammonium chloride (17, 18, 23, 25, 36, 47, 54, 55, 60, 85a, 88, 102), and ammonium phosphate (31, 34, 35, 38, 56, 66, 71, 72, 82, 83, 84, 94, 102) have all been found to be suitable sources of nitrogen to various species of algae under the proper conditions. On the other hand, to occasional species some of these inorganic ammonium salts have occasionally proved to be unavailable or even toxic (10, 13, 25, 28, 32, 35, 51, 62, 88, 92, 96). In general it appears that algae which cannot use inorganic sources of nitrogen, or use them with difficulty, usually occur in nature in habitats such as sewage, contaminated water, heavily manured soils, etc., which contain a great deal of soluble organic nitrogen.

Because of the volatility of $(NH_4)_2CO_3$, a carbonate solution was sterilized separately and analyzed for ammonia, after which the proper amount was added aseptically to the culture solution. The pH was usually considerably above 7.0 before inoculation (but see above). Considerable ammonium carbonate probably was present also in the ammonium oxalate plus calcium carbonate medium because of the very low solubility of calcium oxalate, and in the urea medium because of conversion of the urea during sterilization. Nitrogen was well utilized from all these solutions. In the few previous studies found (12, 49, 82, 94, 102) ammonium carbonate was reported to support growth of algae. To these should perhaps be added those reporting assimilation of urea, for which see below. It is likewise possible that the non-availability or deleterious effects sometimes reported for urea should be credited to this compound instead.

Organic ammonium salts.—It will be convenient to discuss these compounds in two groups, the first to include the lactate, oxalate, succinate, tartrate, and citrate and the second the salts of the first three members of the fatty acid series — formate, acetate, and propionate. Reference to table 1 shows that the organism assimilated more nitrogen from each of the compounds of the first group than from nitrate or from any of the inorganic ammonium salts used. This may be due to secondary rather than to direct effects of the anions, for a medium with an organic ammonium salt will develop less acidity for a given uptake of nitrogen than will one with a salt of an inorganic and therefore stronger acid. Also there is the possibility that the organic acid radical can be used as a supplementary source of carbon or that it increases the availability of the iron. The good results with the oxalate are especially interesting in view of the fact that oxalate is very toxic to many plants. Since oxalate toxicity is apparently due in most cases to the precipitation of calcium, its absence in this instance is to be considered as verification of Hopkins and Wann's finding (41b) that the organism requires little or none of this element.

The results show also that under the conditions involved, ammonium ion is more readily absorbed than nitrate. In this connection it is interesting to note that Pearsall and Loose (80) have recently reported that *Chlorella vulgaris* removed ammonia more rapidly than nitrate from a medium containing NH_4NO_3, while Braarud and Føyn (18) reported that a species of Chlamydomonas removed nitrate and nitrite more rapidly than ammonia from a medium containing $NaNO_3$, $NaNO_2$, and NH_4Cl.

Not much previous work has been done on the assimilation of nitrogen from these salts by algae. Ammonium lactate (99), citrate (99, 52), and tartrate (6, 9, 25, 50, 88) have been reported to be utilizable by algae. However, the tartrate (48, 88) and the lactate, succinate, and citrate (35) have also been reported to be unavailable to some organisms. In fact, the addition of a citrate to a peptone medium (35) prevented the development of *Euglena deses* Ehrenb. Ammonium oxalate (53) in an otherwise suitable medium prevented the development of *Porphyridium cruentum* Naeg., but calcium oxalate was very favorable to it.

For the ammonium salts of the lower fatty acids the results show a lower assimilation of nitrogen from the formate than from the control, still less from the acetate, and none at all from the propionate. The acetate was not toxic when used in half the usual concentration in a medium containing available nitrogen from another source but was unavailable or practically so at that and higher concentrations when used alone. The half concentration of propionate prevented utilization of nitrogen which was otherwise available. It does not seem likely that the failure of the acetate or propionate to support growth was due to impurities in the preparations. It cannot have been due to the ammonia, for in the first acetate experiment the ammonia was taken from the same bottle as used for other preparations which were well utilized. In later ones the salt was crystallized and washed before being used. This later preparation supported growth in low concentration and did not prevent growth on other nitrogen sources in half concentration. Also, medium for part of the cultures with this preparation was sterilized by filtration through porous porcelain. The medium sterilized by this means did not support growth at the usual concentration, thus indicating that the manner of sterilization did not alter the results obtained. The ammonium propionate was prepared from freshly distilled acid and was crystallized, washed, and dried before use.

It is interesting to note that some previous workers have found salts of the lower fatty acids to be harmful to the organisms they used, while others have found them harmless or even valuable as a source of carbon. Kufferath (53) found that both sodium formate and calcium acetate prevented the growth of *Porphyridium cruentum* Naeg. on an agar medium, Dusi (35) found that ammonium acetate would not

efficient than ammonia nitrogen in the synthesis of plant substance, but the lack of dry weights renders such a comparison impossible here.

support growth of *Euglena anabaena* var. *minor* Mainx, and Jacobsen (45) reported a similar effect of these salts on *Haematococcus pluvialis* at a concentration of one per cent in a medium containing NH_4NO_3 as a nitrogen source. On the other hand, Pringsheim (85) found that *Chlorogonium euchlorum* could be grown in the dark with ammonium acetate, and has called attention to the fact that, for continued thriving in the dark on a medium containing highly hydrolyzed muscle protein as a nitrogen source, *E. gracilis* requires in addition a fatty acid—acetic, butyric, or caproic, preferably—as a carbon source. Acids much higher in the series, other organic acids, and sugars are not suitable. Other investigators (9, 30, 37, 58, 99) have reported work showing that some algae, often other than the Chlamydomonadaceae, can endure and often utilize the acetate ion. In some of these cases (9, 99) the organism involved grew with ammonium acetate as the sole source of nitrogen.

The progressive unavailability to the present organism of the nitrogen in these fatty acid salts is puzzling in view of its great availability in the other organic acid salts studied. The results are somewhat suggestive of a progressive toxicity of the anions but unfortunately no positive statement can be made, as it was impossible to continue the work till this point could be determined. The use of calcium chloride, magnesium chloride, and aluminum chloride in one experiment was to test the possibility that the effect is capable of prevention by an application of the principle of the antagonism of ions.[5] The results indicate that neither calcium, magnesium, nor aluminum ions antagonize those of either of the three acids under the conditions involved. In fact, in the only case (formate) in which growth occurred at the usual concentration, it was either unaffected or poorer in the presence of the extra salt.

Acetamide.—Acetamide was found to be a good source of nitrogen for the organism. This result is in line with some previous reports (9, 25, 65, 83, 85a) but apparently contrary to some others, in one of which (83) it is claimed that certain blue-green algae cannot assimilate acetamide, and in another of which (59) two species of flagellates are said to be unable to assimilate acetamide, propionamide, butyramide, or valeramide.

Urea.—The urea medium was found to contain a ready source of nitrogen. Other investigators (8, 11, 12, 17, 18, 23, 65) have also reported similar results. It is possible, however, with all these results that enough conversion of the urea to ammonium carbonate had taken place during sterilization to render the results questionable. Thus Chick (23) found that her urea-containing control liquid yielded about 25 per cent of its nitrogen as free ammonia. In still other experiments the material (or its breakdown products) has been found unavailable or harmful (52, 62). The value of this substance needs further study under conditions which will insure that it alone is presented to the plant as a nitrogen source.

Uric acid.—This compound proved to be an excellent nitrogen source in spite of considerable acidity of the medium, this result being in agreement with most other findings (23, 84, 94). In one published result (8), however, the material was found to be almost, and perhaps absolutely, unavailable.

Guanidine carbonate.—This compound was tested in several concentrations and was found to be an excellent source of nitrogen in all of them. Loew and Bokorny (62) reported guanidine toxic to species of *Spirogyra* but thought the toxicity might be due to the alkalinity of the solution.

Hydroxylamine and *glucosamine.*—Hydroxylamine and glucosamine were used as hydrochlorides. The failure to grow in the first of the two experiments could have been due to the high acidity, pH 3 to 4; but in the second experiment the reaction was corrected by adding calcium carbonate. Few workers have reported on these compounds, but Jacobsen (44) claimed that *Chlorogonium euchlorum* Ehrb. utilized glucosamine, while Lutz (64) and Loew (61) have both reported hydroxylamine as toxic. Usami (100a) reported M/1000 hydroxylamine hydrochloride as toxic to *Fontinalis antipyretica* L., a bryophyte.

Amino acids.—The compounds to be considered under this heading are glycine, alanine, valine, leucine, phenylalanine, tyrosine, l-cystine, aspartic acid, glutamic acid, and for convenience, asparagine. With the exception of aspartic and glutamic acids used alone, when they produced a pH around 3 in the media, these substances all supported growth to some degree, and some, including glycine, alanine, asparagine, and aspartic and glutamic acids when neutralized with calcium carbonate, supported heavy growth and permitted a large accumulation of nitrogen in the cells. No previous work seems to have been done to determine the availability to algae of cystine, but varying results have been reported for the others. Thus a good many workers, using altogether a considerable number of different species of algae, have found that glycine (7, 9, 16, 18, 25, 27, 28, 33, 34, 35, 43, 47, 57, 66, 77, 83, 85a, 94, 96, 101), alanine (9, 16, 18, 25, 33, 34, 35, 94, 96), valine (33, 34, 35, 57), leucine (6, 9, 16, 25, 33, 34, 35, 44, 57, 84, 88, 94, 96), phenylalanine (33, 34, 35, 57), tyrosine (16, 17, 25, 33, 57), aspartic acid (9, 23, 33, 34, 35, 84, 94), glutamic acid (33, 34, 35, 85a), and asparagine [4, 6, 7, 8, 13, 17, 18, 22, 23, 25, 27, 28, 32, 33, 34, 35, 44, 45, 50, 56, 57, 65, 66, 67, 68, 69, 70, 71, 77, 78 (not seen; cited by Oltmanns on p. 157 of "Morphologie und Biologie der Algen"), 82, 83, 85a, 88, 94, 96, 101] will all satisfy more or less satisfactorily the nitrogen requirement of one to many species. Occasionally, however, one or more of these compounds has proved to be non-assimilable or even toxic to some organisms. Two investigators (33, 35, 66) have reported organisms to which all of the amino acids mentioned above (except cystine, which was not tested) were unavailable, while others (11, 12, 16, 28, 32, 34, 43, 48, 54,

[5] For the suggestion of this possibility the writer is indebted to Dr. Oran Raber, who has done work on this subject.

TABLE 1. *Nitrogen absorbed from various nitrogenous compounds by Chorella sp. (F. B. Wann's No. 11). (% in terms of KNO_3 = 100.)*

Sources of nitrogen: other modifications of the culture solution	1st exper.		2nd exper.		3rd exper.		4th exper.		5th exper.		6th exper.		N-absorbed mean %
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	
Potassium nitrate													
Usual conc.	27.5 [a]	100	10.2	100	10.7	100	3.7 [a]	100	4.9	100	5.6	100	100
½ conc.							3.4 [b]	92					92
With ppt. chalk					15.6	146	3.8	102					124
Potassium nitrite													
1/10 conc.					3.1 [b]	29							29
1/50 conc.					1.0 [b]	10							10
1/10 conc. + 9/10 KNO_3					7.6 [b]	71							71
1/50 conc. + 49/50 KNO_3					10.1 [b]	95							95
Ammonium sulphate													
Usual conc.	35.1	128					4.8	129	7.1	143			134
½ conc.							2.5 [b]	67					67
½ conc. + ½ KNO_3									8.5	173			173
Ammonium chloride													
Usual conc.	35.8	131					2.9 [b]	77	8.1	164			124
½ conc.							2.9 [b]	78					78
½ conc. + ½ KNO_3									8.2	166			166
Diammonium phosphate													
Usual conc.	14.5 [b]	53							10.3	208	7.7	137	133
With ground marble											10.4	184	184
Ammonium carbonate													
Usual conc.	49.3 [b]	179					3.1	84					132
½ conc.							5.9	157					157
Ammonium formate													
Usual conc.	26.9	98							4.3	87	3.2	58	81
⅕ conc.											3.8	67	67
With ground marble											12.4	221	221
With 0.0125N $CaCl_2$											0.3 [b]	4	4
With 0.0025N $CaCl_2$											3.1 [b]	55	55
With 0.0125N $MgCl_2$											3.2 [b]	56	56
With 0.0025N $MgCl_2$											3.0 [b]	53	53
With 0.0125N $AlCl_3$											0.7 [b]	13	13
With 0.00125N $AlCl_3$											1.5 [b]	27	27
Ammonium acetate													
Usual conc.	0.0 [b]	0					13.7 [b, c]		—0.1	—2	No growth		0
½ conc.							4.9 [b, c]						
⅕ conc.											8.0	143	143
1/10 conc.											4.0	71	71
½ conc. + ½ KNO_3									6.1	124			124
½ conc. + ½ NH_4-oxalate									10.6	216			216
½ conc. + ½ NH_4-citrate.									8.2	167			167
With ground marble, etc.[d]													0
Ammonium propionate [e]													0
Ammonium lactate											13.8	244	244
Ammonium oxalate													
Usual conc.	51.6 [b]	188					10.2	274	10.8	220	9.1	162	211
½ conc.							10.6 [b]	283					283
½ conc. + ½ KNO_3									8.9	180			180
With ground marble											12.0	214	214
Ammonium succinate									12.4	251	17.6 [f]	312	203
Usual conc.	12.2	45							9.0	183			183
½ conc. + ½ KNO_3											15.5 [f]	277	277
Ammonium tartrate													
Usual conc.	47.1 [b]	171							5.5 [b]	112	8.9	158	147
With ground marble											16.6	295	295
Ammonium citrate													
Usual conc.									12.2	248	12.4	221	234
⅕ conc. + ½ KNO_3									10.6	216			216
With ground marble											18.7	332	332
Acetamide					22.5	210					5.6	100	155
Urea	26.5	97											97

TABLE 1 (*Continued*)

Sources of nitrogen: other modifications of the culture solution	1st exper.		2nd exper.		3rd exper.		4th exper.		5th exper.		6th exper.		N-absorbed mean %
	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	mg.	%	
Guanidine carbonate													
Usual conc.											12.5	222	222
Double conc.					18.3 [b]	172							172
⅓ conc.					6.6 [b]	62							62
1/37 conc.					0.8 [b]	8							8
Double conc. + KNO_3					21.4 [b]	201							201
⅓ conc. + KNO_3					12.6 [b]	118							118
1/37 conc. + KNO_3					11.6 [b]	108							108
Uric acid			Growth [g]								11.7	209	209
Hydroxylamine hydrochloride													
Usual conc.					No growth								0
With ground marble											No growth		0
Glucosamine hydrochloride													
Usual conc.					No growth								0
With ground marble											No growth		0
Glycine	35.2	128									16.2	289	209
Alanine			Growth [h]								17.5	311	311
Valine			1.4	14 [i]							3.4	61	38
Leucine			3.8	39 [i]							6.2	111	75
Phenylalanine			5.4	55									55
Tyrosine			Growth [g]								Growth [g]		Growth
l-cystine			Little or no growth								Growth [g]		Growth
Aspartic acid.													
Usual conc.			No growth										0
With ground marble											9.5	170	170
Asparagine	24.3 [i]	89									14.4	256	173
Glutamic acid													
Usual conc.			No growth										0
With ground marble											9.1	161	161
Peptone (Fairchild's)													
1.5 gm. per liter	84.2 [b]	307											307
Egg albumen													
1.5 gm. per liter	Slight growth												Growth
Diphenylamine													
Usual conc.	No growth												0
1/100 conc. + KNO_3							No growth						0
p-aminoazobenzene			No growth										0
Azobenzene													
Usual conc.	No growth												0
1/100 conc. + KNO_3							No growth						0
Diazoaminoazobenzene			No growth										0
No nitrogen added	1.7 [b]		0.5		—0.1		0.0		0.2		0.4		

[a] Mean of four cultures. [b] One culture only. [c] Culture ran much longer than most of the others. The analytical result is out of line with the qualitative observations and is doubtless too high. Not included in final average. [d] Duplicate cultures with ground marble and single cultures with $CaCl_2$, $MgCl_2$, and $AlCl_3$ as used with the formate, and at pH values at the close of the experiment ranging from 5.1 to 5.6 gave no growth in any case. [e] Two experiments, each in duplicate, at the usual concentration, a single culture each at 1/5 and 1/10 concentration, duplicate cultures with half propionate and half KNO_3, and cultures with ground marble, $CaCl_2$, $MgCl_2$, and $AlCl_3$ as used with the formate and acetate at pH values ranging from 5.3 to 6.0 gave no growth in any case. [f] There is some doubt about these high results, owing to the possibility of a slight fungous infection of the stock solution of succinate and owing to the failure to agree with the first experiment. [g] Not analyzed because of a precipitate which may have contained nitrogen. [h] Lost in analysis. [i] Value probably too low, owing to accident in analysis.

62, 74, 75, 83, 85a, 99) have made similar reports for one or more of them. Not many investigators have worked with known mixtures of amino acids. Such evidence as has been presented (16, 43, 66, 75) seems to indicate that mixtures are little, if any, better than the best component of the mixture used alone.

Peptone. — Fairchild's peptone was used. It supported an abundant growth and allowed a heavy absorption of nitrogen. This result is in harmony with that secured in most of the numerous previous experiments (4, 5, 6, 7, 8, 9, 13, 14, 15, 16, 17, 22, 23, 24, 25, 30, 31, 32, 34, 35, 38, 39, 41, 47, 50, 52, 54, 56, 59, 66, 67, 68, 69, 70, 71, 77, 82, 83, 84, 85, 85a, 87, 88, 94). However, as with most other nitrogen sources, there are organisms and circumstances in which it is non-assimilable (17, 25, 32, 35) or also toxic (1, 25, 40, 45, 54, 86, 93).

Egg albumen.—Only a very small amount of growth took place on egg albumen, and since the cells could not be separated from the coagulum, no quantitative figure can be given for the nitrogen assimilated. There was some turbidity, possibly caused by bacteria; and possibly also the bacteria made available to the algal cells the small amount of nitrogen they absorbed. In any case, egg albumen seems to be but a poor source of nitrogen for this organism. This result is in agreement with that of Bialosuknia (16) with *Diplosphaera Chodati* Bial., which did not grow on egg white.

Miscellaneous aromatic compounds. — The compounds to be considered in this group are diphenylamine, p-aminoazobenzene, azobenzene, and diazoaminobenzene. Of these the first and third proved to be definitely very toxic. The other two did not support growth in the usual concentration of the nitrogen carrier and it is very likely that they are also very toxic.

General considerations. — When the results of this study and of the previous ones cited are considered, it becomes clear that most algae can use a considerable number of compounds as sources of nitrogen. It is also clear that most of the materials considered, even though very favorable nitrogen sources for some species, are poorly available, unavailable, or even toxic to others. Likewise, a material available to a given organism under one set of conditions may be unavailable under another set. These results would be still more evident if the published information on the availability to algae of still other nitrogenous compounds were also considered. It is true that most of the species studied were unicellular Chlorophyceae, since these are the easiest ones to isolate in pure culture by the usual methods, and that studies are needed with other types of algae, but it does not appear likely that such an extension of the range of our information would change, except in detail, the statements just made.

In this connection it may be remarked that the results given above are confirmatory of the idea that in nature green plants do not need to depend on nitrification to make organic nitrogen assimilable. On the contrary, many or most of them are able to utilize it as soon as ammonification has occurred, or even sooner.

SUMMARY

Various nitrogenous substances were studied in an otherwise complete mineral medium for the assimilability of the nitrogen by a species of *Chlorella* (Wann's No. 11) in artificial light. The criterion used was the amount of nitrogen accumulated in the cells. Subject to a small uncertainty due to possible slight decomposition of some materials in autoclaving, the results seem to justify the following conclusions:

The nitrogen in potassium nitrate is utilizable by the organism.

The nitrogen in many ammonium salts, both inorganic and organic, is also available, appearing in general to be more so than that in potassium nitrate. Some of the organic compounds are particularly favorable sources of the element, appearing much better than potassium nitrate in this regard.

The ammonium salts of the fatty acids, however, seem to form an exception. The formate is approximately as good a source of nitrogen as potassium nitrate, the acetate is much poorer, and the propionate cannot be used at all. No higher member of the series was investigated. The cause of the lack of availability is not clear. Neither calcium, magnesium, nor aluminum chloride increased the availability.

Acetamide, urea(?), guanidine carbonate, uric acid, several amino acids, potassium nitrite in dilute solution, and peptone are suitable as sources of nitrogen.

Hydroxylamine and glucosamine hydrochlorides, even at suitable acidities, do not furnish available nitrogen.

Diphenylamine, p-aminoazobenzene, azobenzene, and diazoaminobenzene are non-assimilable; the first and third are definitely toxic; and the other two are probably so.

While calcium appears to be unnecessary to the organism calcium carbonate is sometimes helpful in ammonium salt and some other cultures. The benefit is probably due chiefly to a more favorable reaction of the medium rather than to a specific effect of the calcium.

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THE EFFECTS OF SODIUM CYANIDE AND METHYLENE BLUE ON OXYGEN CONSUMPTION BY NITELLA CLAVATA[1]

Edward Ross

PRELIMINARY EXPERIMENTS by the author (Ross, 1934) dealing with the action of cyanide and methylene blue on *Nitella* cells have been continued. Oxygen consumption was measured by the Warburg manometric technique. Current theories have been discussed in the light of the respiratory mechanism of *Nitella*. A mathematical analysis of the quantitative data obtained has been applied to the questions of residual respiration in the presence of cyanide and methylene blue-cyanide antagonism.

Work by Warburg (1919), Emerson (1927), and Genevois (1927) indicates that the substrate is instrumental in determining the type of effect produced by cyanide on *Chlorella*. Under autotrophic conditions, it accelerated oxygen consumption; in 1 per cent glucose solution, inhibition of respiration was reported. However, Genevois demonstrated typical inhibition for *Scenedesmus basiliensis* both in Ringer's solution and in glucose, but oxygen consumption was completely inhibited by cyanide only when the alga was under autotrophic conditions.

The literature dealing with the effects of methylene blue on green plant cells is relatively meager, while there is little concerned with the dye-cyanide antagonism. Watanabe (1932) found the autotrophic respiration of *Chlorella* to be accelerated more than 100 per cent by 5.0×10^{-4} M methylene blue, but he states that this was not proportionately additive to the cyanide increase. In this study of *Nitella*, experiments with various concentration combinations of cyanide and methylene blue were performed.

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