NITRITE AND FORMALDEHYDE FORMATION IN CERTAIN ALGAE

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(WITH FOUR FIGURES)

I. Photochemical reduction of nitrate to nitrite within the algal cell

For a number of years there has been disagreement on the question as to whether or not light plays an important part in the reduction of nitrate to nitrite by the green plant. Some investigators, including NIGHTINGALE (**12, 13, 14**), THOMAS (**16**), ECKERSON (**7, 8, 9, 10**), MUENSCHER (**11**), and ANDERSON (**4**), have presented evidence that some reduction may take place without light. In much of the work the presence of nitrate-reducing micro-organisms was not ruled out. Moreover, in some of the experiments the reducing action was studied by testing for nitrite formed by adding nitrate to the expressed juice of plants. This procedure brings about contact between nitrates and other compounds that might not occur in the intact plant. A method which will detect the presence of, and an increase in, nitrite in a short period of time and without destroying the plant would give more conclusive evidence. It is believed that such a method was used in the work presented in this paper.

MATERIALS AND METHODS

The ALLISON (**1, 2, 3**) magneto-opic apparatus was employed in these experiments, since it was shown by the writer and co-workers (**15**) that by this means formaldehyde could be detected and the amount estimated within small and sufficiently transparent algal cells. Because the method is relatively new and not in general use, a brief explanation of it will be given.

The ALLISON magneto-optic apparatus detects liquids or substances in solution without change in these substances. It is only necessary that sufficient light be transmitted through the tube containing the liquid or solution to make the necessary observations. BISHOP, DOLLINS, and OTTO (**6**) have shown that when a calibrated circle is attached to the analyzing nicol quantitative determinations may be made. The circle readings vary directly with the concentration.

Preliminary experiments showed that the algal suspensions must be dilute or only a few readings could be made before the concentration of the nitrite formed was greater than could be represented by angle readings. It was also found necessary to use light of very low intensity, usually about 0.1 international foot candles. When conditions for nitrite formation by the algae were poor and the light intensity high, higher readings were sometimes obtained for solutions from which the algae had been filtered than for solutions containing algae.[1] Readings were never as high for the filtered as for the unfiltered solutions when these conditions were good, but the differences were always smaller at higher than at low light intensities. The procedure finally adopted for these experiments was as follows: A dilute algal suspension (mixed culture in a solution containing nitrate) was placed in the observing tube and the highest angle at which one of the minima of magnesium nitrite[2] (11.03) could be seen was determined to within 0.5°. A similar reading was obtained on the blank, a solution from which the algae had been filtered. The tubes containing the algal suspensions and the blank were then exposed to the same source of light at equal distance and for the same length of time. Readings were made at intervals, usually of 10 minutes, on the tube containing the algae. Only initial and final readings were made on the blank. The circle readings used in the graphs represent relative amounts. For absolute values, curves must be calibrated using known amounts of the substance to be determined. Since relative values were all that were necessary in the present study this time-consuming procedure was omitted.

Experimentation

Many determinations were made but only two representative curves are presented (figs. 1, 2). Figures 1 and 2 are taken from experiments in which the readings for both formaldehyde and magnesium nitrite were made. These figures show that the reduction of nitrate to nitrite is photochemical. Where the light intensity was sufficiently low and the cultural conditions for the algae favorable, the difference between the solution containing the algae and that from which the algae had been filtered was very great. It is shown in figure 2 that at low light intensity and with the addition of phosphate, the difference in angle readings between the tube containing the algae and that containing the solution from which the algae had been filtered, was 30° at the end of the experiment. On the following day, after the tube containing the algae had been in the dark for 20 hours, it was exposed to light of higher intensity, 1.71 instead of 0.109 foot candles, along with an unexposed aliquot of the filtered solution used the day before. After the third 10-minute exposure, the reading of the blank was 54.5 as compared with a reading of 26.5 after the sixth exposure the day before. The corresponding readings for the

[1] After the preparation of this paper, checks were made by another observer, Mr. Thomas E. Booth. Since the sensitivty of his eyes was much less than that of the writer, much more concentrated algal suspensions could be used, and with more concentrated suspensions the amount of nitrite formed was always greater than that of the filtered aliquots.

[2] The minima for magnesium nitrite were used because the first preliminary work was done with algae which had grown in a magnesium sulphate solution in which the other ions were present only as impurities. The minimum or minima of any other salt of nitrous acid could be used provided the cation was always present in amounts equivalent to or greater than the nitrite.

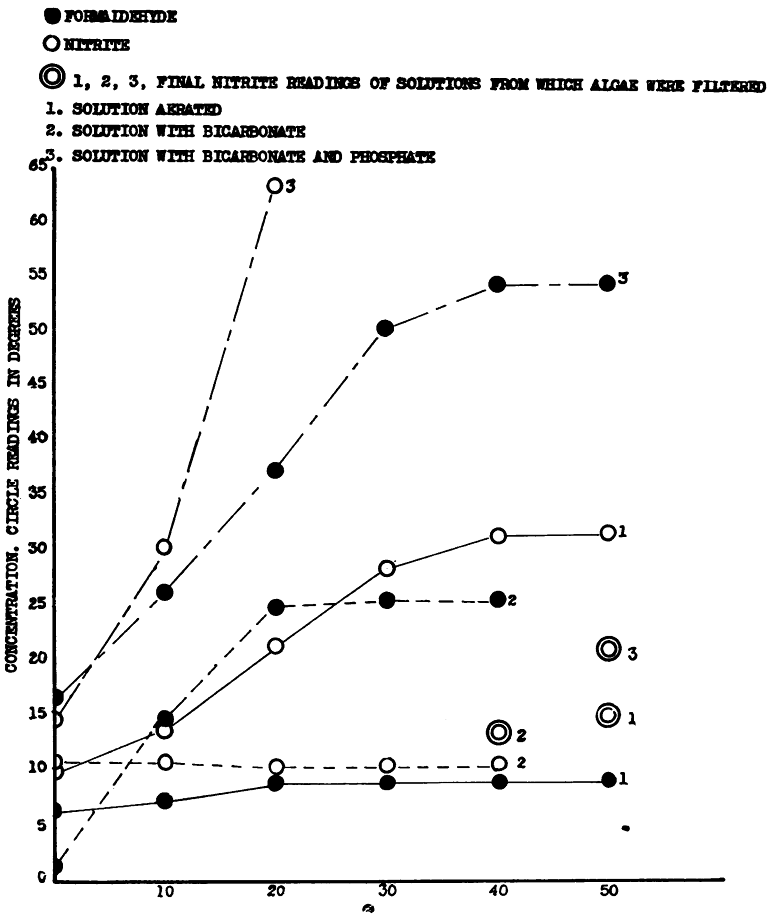

FIG. 1. Formation of nitrite and formaldehyde by algal suspensions with aeration, with bicarbonate, and with bicarbonate and phosphate.

algae were 75° (readings not taken above 75°) and 56.5°. The difference between the amount of nitrite formed by the algae and that in the solution outside of the algae is not accurately shown by the difference in angle readings because, as is shown later in the paper, there is probably a reaction taking place between the nitrite and the formaldehyde. The reading for the filtered solution indicated the total nitrite formed, while that in the tube containing the algae probably indicated only that which had not yet combined with the formaldehyde.

II. Effect of phosphate on the formation of nitrite and formaldehyde

The procedure used in studying the effect of phosphate on the formation of nitrite and formaldehyde was similar to that described in the work on

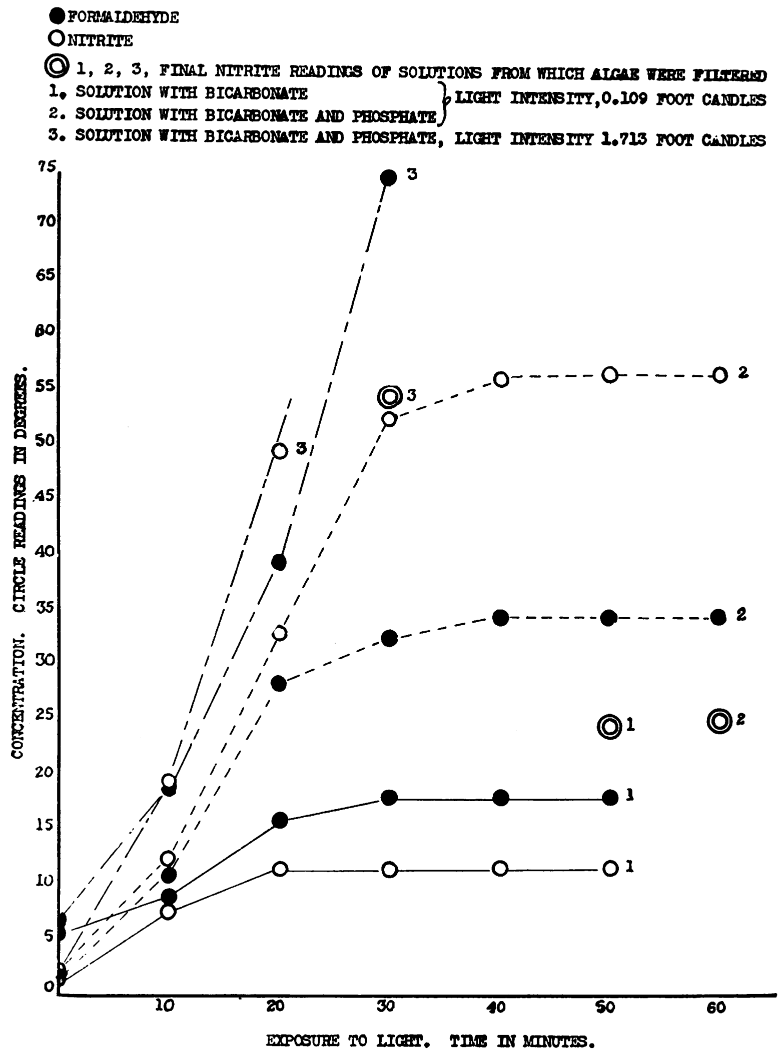

FIG. 2. Formation of nitrite and formaldehyde by algal suspensions, under various conditions as indicated.

nitrate reduction in the first part of this paper. The solution used for much of the work was as follows:

Salt	Per liter
KNO_3	5 mg.
$MgSO_4 \cdot 7H_2O$	10 mg.
$CaSO_4$	$\frac{1}{2}$ cc. saturated solution

The only phosphate present in the solution in which the algal suspensions were made was that present as impurities in the salts and gave an angle reading of 2° which would represent an amount less than one part in 10^{11}. Two p.p.m. of acid potassium phosphate were added to study the effect of the phosphate. An amount of potassium chloride sufficient to furnish the amount of potassium added with the phosphate was added in some cases to an aliquot without phosphate, but the results were the same as when it was not added. In some cases aliquots of the same suspensions with and without phosphate were exposed at the same time, while in others a suspension was exposed the first day without phosphate and the phosphate was added after the algal suspension had been in the dark overnight and readings were again made. The results for cultures exposed the first day without phosphate and

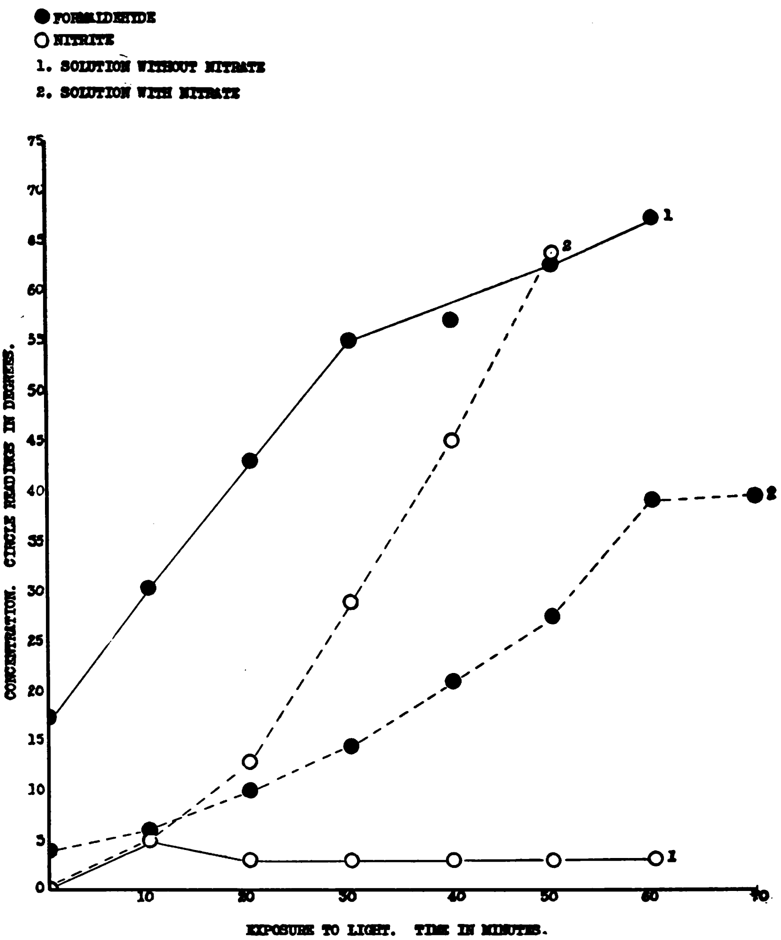

FIG. 3. Formation of nitrite and formaldehyde by algal suspensions in solutions with and without nitrate.

the following day with phosphate are shown in figures 1 and 2. It may be noted that the addition of phosphate increased the rate of formation of both nitrite and formaldehyde but that the increase was greater for the formation of the nitrite. No increase was found in the rate of nitrite formation where phosphate was added to solutions without algae.

III. Relationship between nitrite and formaldehyde concentrations within the cell

A few experiments were performed to test the theory of BALY, HEILBRON, and HUDSON (5) as to the combination of formaldehyde and nitrite in the formation of proteins. According to the theory of these investigators, activated formaldehyde combines with nitrite in the first step of protein synthesis. They say that this reaction takes place before the condensation of formaldehyde to sugars and that it is not until the amount of formaldehyde is in excess of that of the nitrite that sugars are formed.

The results obtained from experiments performed by the writer soon showed that the second part of the theory (5) was probably wrong since the amounts of nitrite indicated were lower than those of the formaldehyde only when conditions for nitrite formation were poor; that is, if no sugars were formed until the rate of formation of formaldehyde exceeded that of the nitrites, these algae could not form sugars. Furthermore, readings for dextrose (fig. 4) increased on illumination at the same time that the nitrite readings were increasing.

Experiments to show the combination of formaldehyde with the nitrites indicate that this part of the theory is correct. Figure 1 shows the results of an experiment in which the algal suspension was first aerated with carbon dioxide-free air for 5 hours in the dark and then the procedure as indicated in the first part of the paper followed. Curves 1, figure 1, show a large amount of nitrite as compared with the amount of formaldehyde. The tube was then placed in the dark until the following day. Before the initial readings for the second day, 2 p.p.m. sodium bicarbonate were added. For further evidence, algal suspensions were made in solutions in which the only nitrate present was the small amount present in the salts as an impurity. In some cases one aliquot of the suspension, as it was made up, and another to which nitrate had been added were exposed to light and observations made as described above. In other experiments the solutions were exposed to light, and readings made on the first day. After being in the dark overnight nitrate was added and light exposures were made again on the second day. Figure 3 represents the results of an experiment according to the latter method. It may be noted that when the conditions are unfavorable to formaldehyde formation, the amount of nitrite was greater than when more formaldehyde was present. On the other hand, when nitrate was absent or

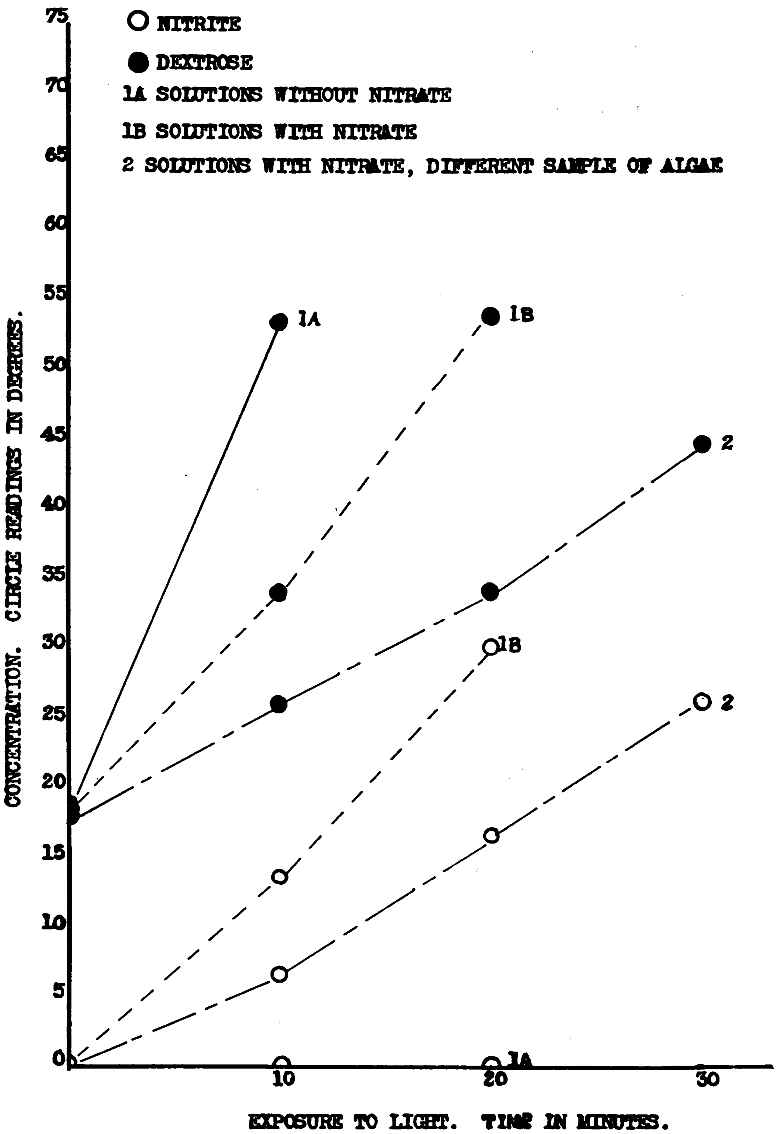

FIG. 4. Formation of nitrite and dextrose by algal suspensions in solutions with and without nitrate.

nearly absent from the solution, the formaldehyde reached higher values than when nitrate was present.

The results of these experiments are in agreement with the theory of BALY, HEILBRON, and HUDSON (5) concerning the combination of formaldehyde and nitrite as the first step in protein synthesis. Moreover, no trace of the ammonium ion could be detected when it was looked for in several of these studies. Considering the great sensitivity of the apparatus, about three parts in 10^{12} for most eyes and a considerably greater sensitivity for the writer, it seems unlikely that nitrite is reduced to the ammonium ion before combining with formaldehyde or some other carbon compound in protein synthesis.

Summary

1. Light plays an important part in the reduction of nitrate to nitrite within the algal cell.

2. The addition of phosphate to the nutrient medium greatly accelerates the reduction of nitrate to nitrite and the formation of formaldehyde by certain algae.

3. Nitrites probably combine with formaldehyde or some early condensation product of formaldehyde in the first step of protein synthesis.

4. Protein synthesis and the condensation of formaldehyde to sugars may proceed simultaneously.

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LITERATURE CITED

1. Allison, F. Magneto-optic method of analysis as a new research tool. Ind. & Eng. Chem. Anal. Ed. **4**: 9–12. 1932.
2. ————. The Magneto-optic method of analysis. Jour. Chem. Educ. **10**: 71–78. 1933.
3. ————, and Murphy, E. J. A magneto-optic method of chemical analysis. Jour. Amer. Chem. Soc. **52**: 3796–3806. 1930.
4. Anderson, V. L. Some observations on the nitrate-reducing properties of plants. Ann. Bot. **38**: 699–706. 1924.
5. Baly, E. C. C., Heilbron, I. M., and Hudson, D. P. Photocatalysis, pt. 2. The photosynthesis of nitrogen compounds from nitrites and carbon dioxide. Jour. Chem. Soc. London **121**: 1078–1088. 1922.
6. Bishop, E. R., Dollins, C. B., and Otto, I. G. Magneto-optic nicol rotation method for quantitative analysis of calcium. Jour. Amer. Chem. Soc. **55**: 4365–4370. 1933.
7. Eckerson, S. H. Protein synthesis by plants. I. Nitrate reduction. Bot. Gaz. **77**: 377–390. 1924.
8. ————. Influence of phosphorus deficiency on metabolism of the tomato (*Lycopersicum esculentum* Mill.). Contrib. Boyce Thompson Inst. **3**: 197–217. 1931.

9. ———————. Seasonal distribution of reducase in various organs of an apple tree. Contrib. Boyce Thompson Inst. **3**: 405–412. 1931.

10. ———————. Conditions affecting nitrate reduction by plants. Contrib. Boyce Thompson Inst. **4**: 119–130. 1932.

11. MUENSCHER, W. C. Protein synthesis in *Chlorella*. Bot. Gaz. **75**: 249–267. 1923.

12. NIGHTINGALE, G. T., and SCHERMERHORN, L. G. Nitrate utilization by asparagus in the absence of light. Science n. s. **64**: 282. 1926.

13. ———————, and ———————. Nitrogen assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.

14. ———————, and ROBBINS, W. R. Some phases of nitrogen metabolism of polyanthus narcissus (*Narcissus tazetta*). New Jersey Agr. Exp. Sta. Bull. 472. 1928.

15. SOMMER, A. L., BISHOP, E. R., and OTTO, I. G. Detection and estimation of formaldehyde within the cell of a green plant by the Allison apparatus. Plant Physiol. **8**: 564–567. 1933.

16. THOMAS, W. The seat of formation of amino acids in *Pyrus malus* L. Science n. s. **66**: 115–116. 1927.