ON THE ABSORPTION OF NITRATE BY MAIZE IN WATER CULTURE

BY

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INTRODUCTION

If we study the influence of environmental factors on the rate of a physiological process, certain rules may become apparent which give us some understanding of the mechanism of the process involved. In this way BLACKMAN's (1905) statement of the rule of limiting factors in photosynthesis led to the conception of a catenary process, consisting of a diffusion, a photochemical and a dark chemical process, the rate of which was dominated by the 'slowest' partial process of the chain (e.g. WARBURG, 1921, VAN DEN HONERT, 1930, and others).

In the same way VAN DEN HONERT (1933, 1936) investigated the influence of concentration, temperature and pH on the rate of absorption of phosphate by sugar cane roots in continuously flowing water cultures. The asymptotical shape of the curve representing the rate of phosphate absorption plotted against the phosphate concentration could be explained by the assumption of a phosphate carrier with such a great affinity to phosphate that it was practically saturated at a phosphate concentration as low as 1 p.p.m. As is well known, the conception that the entrance of a solute into a living cell is accompanied by a binding or adsorption to some protoplasmic constituent is very common among plant physiologists (e.g. ARISZ (1945), LUNDEGÅRDH (1950) and many others).

The mechanism of this carrier system was considered comparable to that of a revolving belt, which is loaded with ions at the outside surface and carries them inward to deposit them somewhere inside the cell and comes back to the surface empty to be loaded again. The rate of ion absorption was assumed to be proportional to the degree of loading of the belt and therefore, in an asymptotical way, dependent on the ion concentration in the medium. Furthermore, the rate of intake was assumed to be proportional to the revolving speed of the endless belt, which was considered to be greatly dependent on temperature.

From the influence of the pH on the absorption rate it could be inferred that only monovalent H_2PO_4-ions were absorbed and that divalent HPO_4-ions were not. Therefore the pH influence could be almost quantitatively explained by a concentration effect upon the

monovalent ions in the solution. A very similar conclusion was arrived at by OLSEN many years later (1953a, l.c.p. 842).

From the high temperature coefficient in the range of the lowest phosphate concentrations the conclusion was drawn that even under these conditions phosphate absorption is not governed by a diffusion process. Therefore, in contrast to carbon dioxide intake in photosynthesis, there seems to be no measurable diffusion resistance inserted between the environment and the carrier system. Evidently only the protoplasm at the very surface of the roots 'looking through its cell wall windows towards the outside world,' participates in active absorption.

Water absorption was shown to have a pronounced influence on ion intake in peas (HYLMÖ, 1953) and broad bean (BROUWER, 1954). As this complicating effect proved to be negligeable in sugar cane and also in maize, at least at low salt concentrations (VAN DEN HONERT, HOOYMANS and VOLKERS, 1955), this last object was considered suitable to study the influence of concentration, temperature and pH on the rate of active nitrate absorption in water cultures.

MATERIAL AND METHODS

The methods used were essentially the same as described by VAN DEN HONERT, HOOYMANS and VOLKERS (1955). Again maize seedlings of a single cross hybrid D × 9 were reared in pots with garden soil and subsequently transferred to water culture jars with a WOODFORD and GREGORY (1948) solution, which was renewed twice a week. After 2–6 weeks, depending on the time of the year, the root systems had developed sufficiently to be used in absorption experiments.

Before the absorption experiments were made, the plants were allowed to adapt themselves to the desired experimental conditions of concentration, pH and temperature during at least 20 minutes and subsequently transferred into the solution from which the absorption was determined. This was a modified Woodford and Gregory solution with nitrates replaced by sulfates and different nitrate concentrations supplied as KNO_3, as described in our previous publication (nitrate series, l.c.p. 147). The duration of the absorption period varied between 10 and 60 minutes. The nitrate concentration at the beginning and at the end of this period was determined colorimetrically by means of the phenol disulfonic acid method.

All rates of intake of nitrate determined were calculated in terms of the absorption rate at arbitrarily chosen standard conditions. These were: a temperature of 20° C, pH 6 and a nitrate concentration not too far from 10 p.p.m. In the graphs the data obtained under these conditions are given by encircled dots. The initial and final concentration in the absorption experiments are indicated by the length of the horizontal lines through the symbols.

The nutrient solutions were stirred by an air stream throughout. The pH was determined colorimetrically and kept at the desired value by adding a small amount of CO_2 to the air stream or by addition of a few drops of 0.33 N. Na_2CO_3 to the culture solution.

For further details may be referred to the above mentioned publication.

EXPERIMENTAL RESULTS AND DISCUSSION

Influence of pH.

One of the first questions investigated was the influence of the pH on the rate of nitrate absorption. As mentioned before, the influence of pH on phosphate absorption in sugar cane roots was shown to be a consequence of a shift in the equilibrium between monovalent and divalent phosphate ions. As nothing of the kind will occur with nitrate, the absorption rate of this ion might well be expected to be independent of the pH.

There is no complete agreement between the data found in literature concerning this question. HOAGLAND and DAVIS (1923) found that nitrate is taken up by *Nitella* much more rapidly from acid (pH 5) than from alkaline solutions. HOAGLAND and BROYER (1940), using excised barley roots, found nitrate absorption to be independent of the pH between 6.0 and 7.8, but a slight decrease towards pH 3.6. ARNON, FRATZKE and JOHNSON (1942) found little influence of the pH between 4 and 9 on the nitrate absorption in tomato, lettuce and Bermuda grass. The same result had WEISSMAN (1950) with wheat seedlings in the pH range between 4.3 and 6.3, at least in absence of NH_4.

On the other hand OLSEN (1953b) found a pronounced decrease in nitrate absorption with increasing pH between 5.5. and 7.9. This decrease was ascribed to the competition of bicarbonate ions because it was found to be much less pronounced if a five-fold nitrate concentration was used. However, the pH influence disappeared completely in the presence of a higher bicarbonate concentration, which makes Olsen's explanation less probable.

As Fig. 1 shows, also the present authors found a pronounced influence of the pH on the velocity of nitrate absorption determined
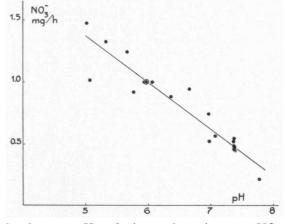

Fig. 1.   Relation between pH and nitrate absorption rate. NO_3 concentration about 10 p.p.m., temperature 20° centigrade.

at 20° C. Especially between pH 6 and 8 very consistent results were obtained, and the absorption rate at pH 7.4 always amounted to about 50 per cent of that determined at 'standard conditions' at pH 6. Between pH 6 and 4.5 the results were variable, sometimes indicating a considerable increase of absorption towards lower pH values, sometimes a decrease. An explanation of this phenomenon has not yet been found.

A decreased nitrate absorption at higher pH values might invalidate the explanation given in the case of phosphate uptake, at least if there would be evidence that the mechanism of pH influence was the same in both cases. This, however, is not the case, as may follow from the following experiments.

Combined influence of pH *and nitrate concentration*

The results of the experiments on the combined influence of pH and nitrate concentration are graphically represented in Fig. 2.
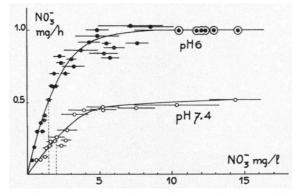

Fig. 2.   Relation between concentration and absorption rate of nitrate at pH 6.0 (dots) and 7.4 (circles) at 20°. Vertical dotted lines indicate half values.

It is clear that an increase of the pH from 6.0 to 7.4 causes a decrease in the rate of uptake of about 50 per cent over the whole concentration range investigated. This was shown to be the case even at a nitrate concentration as high as 50 p.p.m.

In the case of phosphate absorption, however, where a higher pH results in a decrease of the relative monovalent ion concentration, the effect can be compensated by an increase in total phosphate concentration. In other words, at higher pH values the absorption curve is shifted towards higher concentrations, but reaches asymptotically the same maximum absorption value (Fig. 3).

Evidently the situation is different in the case of nitrate absorption. Expressed in terms of the carrier hypothesis the most probable explanation seems to be that the capacity of the carrier itself is affected by the pH of the medium. In other words, the 'loading capacity' for nitrate ions of our 'revolving belt' is reduced to half by a pH shift from 6.0 to 7.4. However, the affinity of the carrier to NO_3 ions is
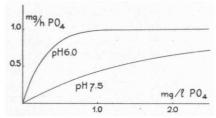
Fig. 3. Diagram illustrating the relation between the rate of phosphate absorption (ordinate) and phosphate concentration (abscissa) at different pH values. After VAN DEN HONERT (1933).

hardly affected, as the 'half value' (the concentration at which 50 per cent of the maximum absorption rate is found, a value comparable to the Michaelis—Menten constant in enzyme reactions) is found to be nearly the same at pH 6.0 (1.4 p.p.m.) and 7.4 (2.0 p.p.m.). It may be questioned whether the difference found actually falls outside the experimental errors.

At first sight, the pH effect described might also be explained as an influence on the revolving speed of our endless belt. Later on, from the combined influence of pH and temperature, it will be evident that this explanation would be less elegant.

A rate of intake half the maximal is probably sufficient for the nitrate supply of the plant. In this respect GOEDEWAAGEN's (1933) results are of interest, who found a maximum development of wheat in water culture already at nitrate concentrations between 1 and 2 p.p.m.

Combined influence of nitrate and bicarbonate concentration

As described in a preceding paragraph OLSEN (1953b) explained the depressing influence of higher pH values on the rate of nitrate intake to the presence of bicarbonate ions competing with the nitrate ions at the adsorption sites. If this competition would be the actual cause of the phenomenon it would be most prominent at low nitrate concentrations.
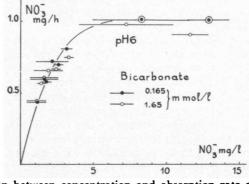

Fig. 4. Relation between concentration and absorption rate of nitrate at pH 6 and two different concentrations of bicarbonate.

This was investigated by measuring the rate of nitrate absorption at pH 6 over a range of concentrations in the presence of 0.162 resp. 1.62 M. bicarbonate. The pH was kept at the desired value by regulating the CO_2 supply to the aerating air stream. Of course, a higher CO_2 pressure is necessary to keep the pH at 6 in the presence of a higher bicarbonate concentration.

As Fig. 4 shows, no influence of the addition of bicarbonate on the position of the absorption curve is found. The 'half value' remains at about 1.4 p.p.m. = 0.021 mM, independent of the 8 resp. 80 fold molar concentration of bicarbonate ions. On the one hand this indicates that, at least at pH 6, bicarbonate ions do not compete with nitrate ions, on the other hand it is evident that the first binding process of nitrate ions is something much more specific than a mere ion absorption by virtue of electrical charges.

Combined influence of temperature and concentration

In Fig. 5 the results of experiments are represented in which the influence of the nitrate concentration was investigated at pH 6 at three different temperatures. As before, the rate of uptake determined at standard conditions (20° C, $NO_3 \pm 10$ p.p.m.) was taken as a unit and all other values expressed in terms of this unit.

It is evident that, within the experimental errors, the temperature influence is the same over the whole range of concentrations investigated, e.g. at the 'half value' of 1.4 p.p.m. which, consequently, remains valid also at 30° and 7° C. As in the case of phosphate absorption in sugar cane roots (VAN DEN HONERT, 1936) where strikingly similar curves were obtained, these relations can be explained by the
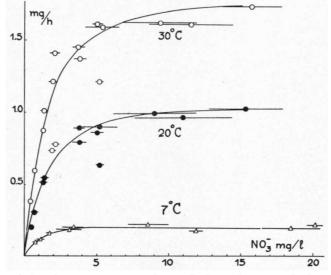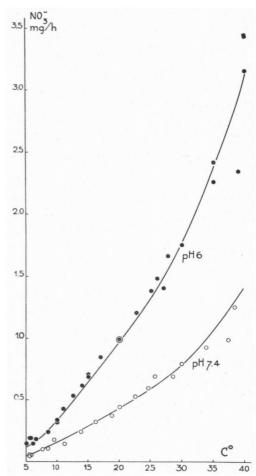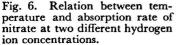

Fig. 5.   Relation between concentration and absorption rate of nitrate at three different temperatures.

assumption that the adsorption or binding equilibrium between carrier and nitrate ions is hardly influenced by temperature, but that the 'revolving speed' is greatly dependent on it.

The above mentioned set of curves differs from the well-known Blackman-curves for the rate of CO_2 intake in photosynthesis in that in this last instance the temperature influence is small at low CO_2 concentrations. Indeed Blackman would never have pronounced his rule of limiting factors the way he did, had he not studied carbon dioxyde absorption but phosphate or nitrate uptake. For here at low concentrations, two factors (concentration and temperature) are limiting at the same time.

If CO_2 is a limiting factor photosynthesis behaves as a diffusion process with a low Q_{10}, evidently because a measurable diffusion resistance is inserted between the external medium and the first binding site of CO_2 in the cells (VAN DEN HONERT, 1930). As in the case

Fig. 6. Relation between temperature and absorption rate of nitrate at two different hydrogen ion concentrations.

of phosphate absorption (VAN DEN HONERT, 1936) no measurable diffusion resistance between external medium and nitrate carrier system seems to be present here, so that the site of first binding of the nitrate ion must be found at the protoplasmic surface of the very outside of the epidermal root cells.

Combined influence of temperature and pH

As shown in Fig. 6, the influence of the temperature on the rate of nitrate intake was studied between 5° and 40° centigrade and at pH 6.0 and 7.4. Again the rule of limiting factors does not apply here, as temperature and pH limit the absorption velocity together at the same time.

If we plot the logarithm of the absorption rate against the temperature (Fig. 7) a constant temperature coefficient will be indicated by a
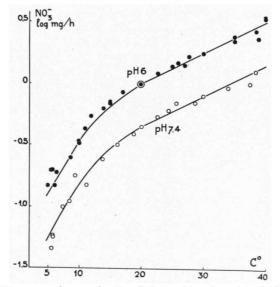

Fig. 7. The same data as in Fig. 6, but with a logarithmical ordinate.

rectilinear curve. Within the experimental errors such a constant Q_{10} of 1.7 became apparent between 20° and 40°. However, between 5° and 10° a Q_{10} as high as 7 was found, with a transition range between 10° and 20° C.

It may be questioned what the cause may be of the extremely high temperature coefficients between 5° and 10°. Dr H. L. Booy offered us an attractive hypothesis to explain this phenomenon. The influence of temperature on the viscosity of fatty substances shows very high temperature coefficient near their melting points. It might well be that, at low temperature, the speed of entrance of ions (or the mobility of the ion carrier) is inhibited by a 'solidification' of the lipoid membrane surrounding the protoplasm.

As the temperature seems to influence a mobility factor ('revolving speed' of our carrier system) in the same way at pH 6.0 and 7.4, but very differently at high and low temperature, whereas the pH influence is constant over the whole temperature range, it seems probable that the mechanism of the pH influence differs from that of the influence by temperature. For that reason, in a previous paragraph, the pH influence was explained as decreasing the loading capacity of the carrier system. One may visualise a decrease of the number of momentary available NO_3 binding sites at the protoplasmic surface.

SUMMARY

The combined influence of several environmental factors (nitrate concentration, pH, bicarbonate concentration and temperature) on the rate of nitrate absorption was investigated in intact maize roots in water culture. By means of a carrier hypothesis (picture of a revolving belt) the results may be described as follows.

No measurable diffusion resistance is present between the carrier system and the environment. The carrier, therefore, acts at the very surface of the epidermal root protoplasm; it has a highly specific affinity to nitrate ions and its loading capacity is influenced by pH between 6 and 8. A 'solidification' of lipoid membranes is supposed to inhibit nitrate absorption at temperatures below 10° centigrade.

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