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Studies on the mechanism of urea uptake by plant roots*)

II. The effect of some external factors on the uptake

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In the previous paper (Olszańska 1968), the kinetics of urea uptake and the effect of its concentration and of inhibitors on this process were discussed. The experimental results indicate rather a passive character of the urea uptake. The proportionality of urea uptake to its concentration and the lack of KCN, NaN_3 and DNP effect seem to be evidence of the absence of an active component in the urea uptake.

In the present paper experiments on the effect of temperature, pH, ions and organic substances are reported.

METHOD

The experiments were carried out with isolated young (4—5 days old) maize roots, variety "Sterling". The samples of roots (1g) cut into segments of about 1.5 cm of length, were incubated with 5 ml of urea solution labelled with ^{14}C. The urea uptake was estimated as the difference in solution activity before and after incubation with roots. The details of the method are given in the previous paper. The results are means of 3 replications.

RESULTS

1) Effect of temperature

The kinetic curves of urea uptake from 0.0017 M solution were examined at room temperatures (21° and 24°C) and at the temperatures near 0° (0°, 1° and 3°C). From the curves (fig. 1) the temperature coefficient Q_{10} was calculated according to the formula:

$$Q_{10}=1+10\frac{(m_2-m_1)}{m_1(t_2-t_1)}, \qquad (1)$$

*) The work is based on a part of the author's Ph. D. thesis, and was performed at the Department of Applied Atomic Physics and Radiochemistry of the Timiriazev Agricultural Academy, Moscow.

where m_1, m_2 — the amounts of the urea absorbed at temperatures t_1 and t_2 respectively, and $t_2 > t_1$.

In the initial period of absorption the effect of temperature on the urea uptake was not observed (fig 1). As the time of absorption was prolonged the difference between urea uptake at low and room temperature increased. The intensity of urea uptake was greater at 21° and 24° than at temperatures near 0°.

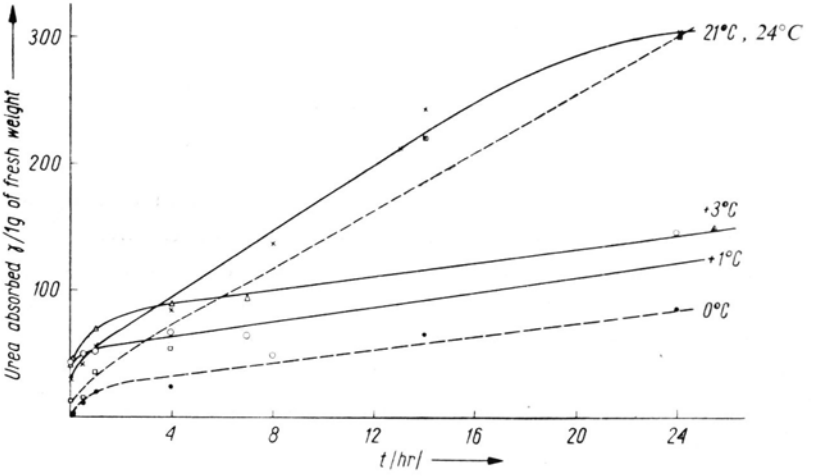

Fig. 1. Kinetics of urea uptake at various temperatures.
Lines — — — — and ———: parallel experiments

The calculation of Q_{10} for the various absorption periods, on the basis of absolute amounts of urea taken up in a given time, shows that the value of Q_{10} changes in dependence on the duration of urea absorption.

Table 1 lists the amounts of urea taken up, calculated on the basis of the curves in fig. 1.

Table 1

Amounts of urea absorbed at 0° and 24°C
(mg of urea/1g of fresh weight)

Time of absorption (hr)	Amounts of urea absorbed at:	
	0°C	24°C
4	0.0300	0.0670
8	0.0410	0.1190
12	0.0520	0.1710
16	0.0630	0.2230
20	0.0740	0.2750

Below the examples of calculation of Q_{10} after 4, 8 and 20 hrs of absorption are given for the temperatures 0° and 24°C.

1. After 4 hrs of absorption:

$$Q_{10}=1+10\frac{0.0670-0.0300}{0.0300\times 24}=1.51;$$

2. After 8 hrs of absorption:

$$Q_{10}=1+10\frac{0.1190-0.0410}{0.0410\times 24}=1.79;$$

3. After 20 hrs of absorption:

$$Q_{10}=1+10\frac{0.2750-0.0740}{0.0740\times 24}=2.13;$$

Thus the Q_{10} values changed from 1.51 after 4 hrs of absorption to 2.13 after 20 hrs.

In table 1 it can be seen that the increase in the amount of urea taken up is constant and equals 0.0110 mg at 0°C and 0.0520 mg at 24°C during 4 hrs of absorption. Recalculated to 1 hr it makes 0.00275 and 0.0130 mg, respectively. These values represent the rate of urea uptake, and if we use them in formula 1 instead of the absolute values, we obtain:

$$Q_{10}=1+10\frac{0.01300-0.00275}{0.00275\times 24}=2.55;$$

The Q_{10} value obtained this way is constant for all linear part of the curve and it characterises the relation of urea uptake to temperature in the given period of absorption.

The amounts of urea absorbed at 1°, 3° and 21°C are given in table 2.

Table 2

Amounts of urea absorbed at 1°, 3° and 21°C
(mg of urea/1 g of fresh weight)

Temp.	Amounts of urea absorbed after		Increase
	4 hrs	8 hrs	
1°	0.0630	0.0740	0.0110
3°	0.0890	0.1000	0.0110
21°	0.0950	0.1470	0.0520

Although the absolute amounts of urea taken up are different from those given in the tabl. 1, but the rate of urea uptake is the same*) and so is Q_{10}.

For the initial period of urea uptake Q_{10} was not calculated since the results could be burdened with a large error (as the amounts of urea taken up were very small). Nevertheless, the calculations of Q_{10} on the basis of the absolute amounts of absorbed urea show that Q_{10} became smaller as the time of absorption was shorter: $t_{hr}\to 0$ then $Q_{10}\to 1$. It means that for the initial period of absorption $Q_{10}\approx 1$.

*) The identity of both figures is probably accidental.

3. Effect of pH

In all previous experiments the pH of the urea solutions was not controlled regularly. The few determinations gave values from 6,36 to 7.05. The difference in pH of the solution was probably caused by the different amounts of carbonate and ammonium ions in the water used for preparing the solutions. For this reason it was necessery to check if the difference of pH could affect the urea uptake. The experiments of Cook (1952) and Volk (Volk a. Mc Aucliffe 1954) showed

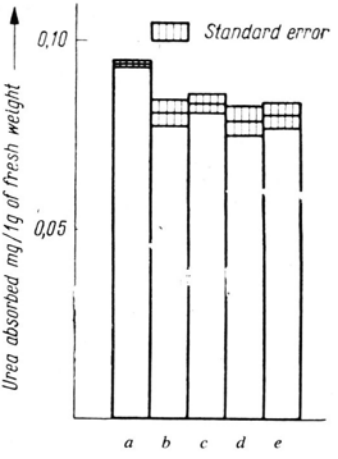

Fig. 2. Amounts of urea taken up from solution of different pH.
a — control pH 6.36; *b* — pH 5.29; *c* — pH 7.22; *d* — pH 8.10; *e* — pH 9.18

a certain effect of pH on the urea uptake but the authors admitted that it was difficult to differentiate the true effect of pH from that of the buffer compounds.

Our experiments were carried out in the pH range 5—9, time of absorption was 4 hrs, the treatments were as follows:

1) control — 0.0017 M urea-^{14}C, pH 6.36.
2) 0.0017 M urea-^{14}C+0.2 M boric acid, pH 5.29.
3) 0.0017 M urea-^{14}C+buffer, pH 7.22.
4) 0.0017 M urea-^{14}C+buffer, pH 8.1.
5) 0.0017 M urea-^{14}C+0.05 M borax, pH 9.18.

The buffer solutions were prepared with 0.2 M boric acid and 0.05 M borax. The solutions of boric acid and borax were prepared with 0.0017 M solution of urea-^{14}C. In this way, independently of the proportion of H_3BO_3 and $Na_2B_4O_7$ solutions in the buffer mixture, the ambient solution had always the same radioactivity and concentration. The results of the experiments are presented in table 3 and fig. 2.

The columns 3, 4 and 5 show the pH of the solutions after 4 hrs of absorption in 3 test tubes (3 replications). It can be seen that the pH of the solutions did not change during the experiment, except in treatment 2, where it increased by 0.6.

Table 3

Effect of pH on urea uptake

Initial pH	Amounts of absorbed urea (mg)	pH after incubation			Average pH (mean of I, II, III)
		I	II	III	
6.36	0.0941±0.0009	5.95	6.57	6.25	6.26
5.29	0.0801±0.0036	5.91	5.80	5.96	5.89
7.22	0.0837±0.0025	7.17	7.09	7.07	7.11
8.10	0.0793±0.0039	7.99	8.07	8.05	8.03
9.18	0.0807±0.0034	9.14	9.13	9.11	9.13

It was observed that roots placed in the basic solutions (pH 8.1 and 9.18) became yellowish.

The results show that pH exerted no effect on urea uptake. It is visible, however, that the addition of boric acid or borax caused some decrease of urea uptake as compared with the control treatment (fig. 2). As the changes of pH between 5 and 9 did not affect the urea uptake, it is supposed that the slight inhibition of the uptake was caused by addition of BO_3^{3-} and $B_4O_7^{2-}$ ions.

4. Effect of some ions

This series of experiments was carried out in order to investigate the effect of ions on urea uptake. The salts: NaCl, NH_4Cl, $CaCl_2$ and $NaNO_3$, Na_2CO_3, Na_3PO_4 were added to the solution of 0.0017 M urea-^{14}C. The concentrations of the salts were: 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}N. As control pure urea-^{14}C solution was used, time of absorption was 4 hrs. The results of the experiments are given in fig. 3 *A, B, C*.

The presence of Na^+ and NH_4^+ (as Cl^- salts) had no significant effect on the urea uptake, except for the 10^{-4}N NH_4Cl treatment. In the case of Ca^{2+} ions two parallel experiments were carried out in order to verify the corectness of the results obtained (fig. 3*B*). Both experiments were done at different times but with the same solution, and in both, despite the different absolute amounts of uptaken urea, higher absorption was observed in presence of Ca^{2+} ions. Between the first and the second experiment the urea solution with 10^{-3}N $CaCl_2$ was partly decomposed, probably as a result of activity of the microflora. The radioactivity of the solution decreased from 2489 counts/100sec. to 2057 counts/100sec, this indicating that some part of the urea was decomposed and radioactive CO_2 evolved from the solution. The actual urea concentration, calculated on the basis of the difference in radioactivity of the solutions, was 0.0014 M, while the initial one was 0.0017 M. Thus, the result of urea uptake reported in fig. 3*B* (for 10^{-3}N $CaCl_2$ treatment in the experiment II) represents the amount of urea taken up from 0.0014 M solution and not from 0.0017 M as in other treatments. Nevertheless, as the urea uptake is proportional to urea concentration (Olszańska 1968), it was possible to recalculate the value of urea uptake from 0.0014 M solution for the value of urea uptake from 0.0017 M solution.

The figure obtained (0.0810 mg) — marked with the dotted line in fig 3*B* — is similar to those obtained in other treatments.

In the presence of CO_3^{2-}, NO_3^-, PO_4^{3-} ions (as Na-salts) the amounts of urea taken up are different from those in the controls. As the concentration of NO_3^- increased, the urea uptake showed also a tendency to increase, but only at the highest NO_3^- concentration (10^{-1}N) the increase became significant (fig. 3*C*).

In the case of PO_4^{3-} the urea uptake changed very irregularly. It is possible

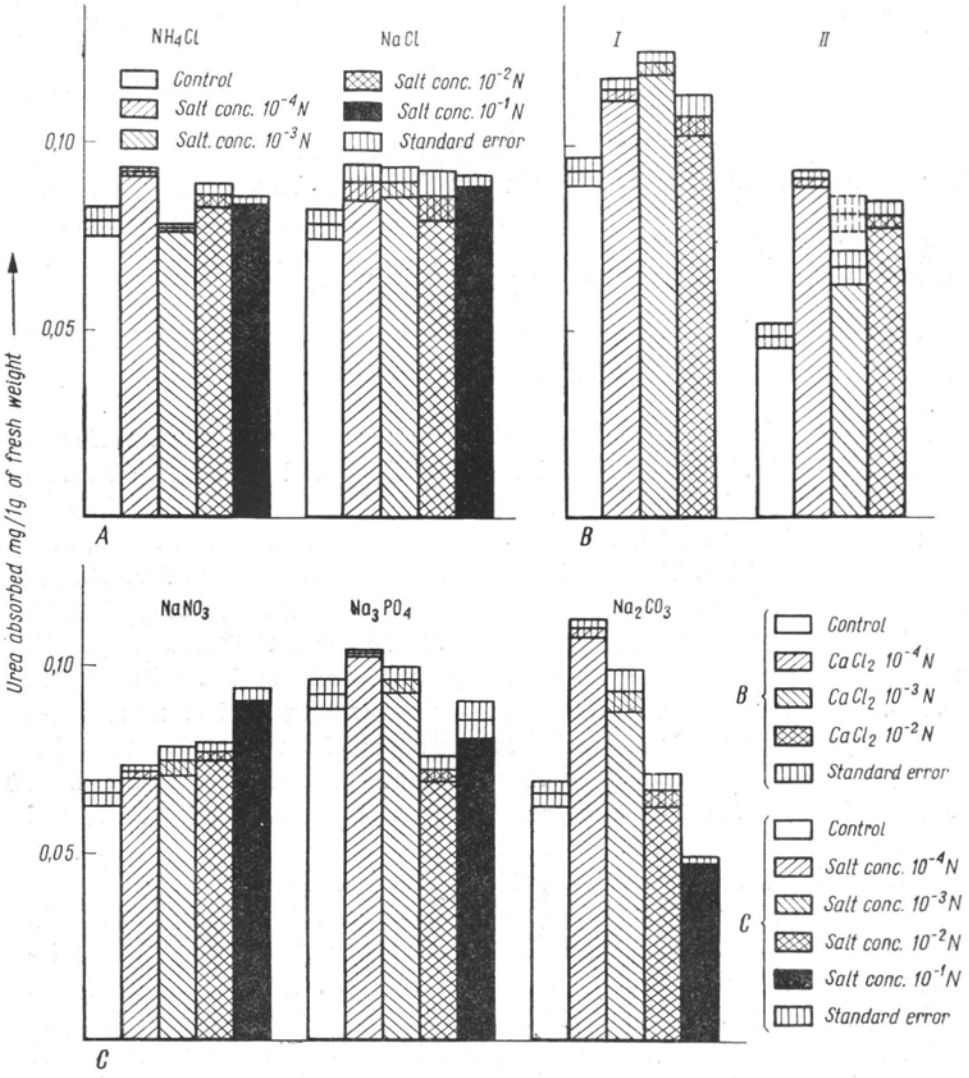

Fig. 3. Amounts of urea taken up:

A — in the presence of NH_4Cl and NaCl; B — in the presence of $CaCl_2$ (experiments I and II); C — in the presence of $NaNO_3$, Na_3PO_4, Na_2CO_3

that in 10^{-1} and 10^{-2}N solutions the urea uptake was disturbed by the high pH (within the range 10—12).

Very characteristic changes in urea uptake occured in the presence of CO_3^{2-} ions. In the 10^{-4} N solution there was a distinct stimulation of urea uptake, but the increase of the salt concentration caused a gradual inhibition of the uptake (fig. 3*C*).

This fact may be partly explained by the specificity of the method. Namely, the urea uptake was estimated as the difference in the radioactivity of solutions before and after incubation with roots. The absorbed urea could undergo decomposition in the plant tissue and ^{14}C in the form of CO_2 or HCO_3^-, CO_3^{2-} ions could go out, back into ambient solution. This process would increase the radioactivity of the ambient solution and, in this connection, decreased the measured values of urea uptake.

Consequently, any factor decreasing ^{14}C excretion from the tissue into the solution results in an increase of the determined values of urea uptake. Undoubtedly, the presence of carbonate ions in the solution may be such a factor. If this holds true, we should expect an apparent stimulation of urea uptake as the concentration of carbonate increases. But the experimental results show opposite — after the stimulation of urea uptake at 10^{-4}N Na_2CO_3 there was the gradual inhibition at higher salt concentrations. This points to a direct effect of carbonate ions on the urea uptake. It is possible that the higher concentration of carbonate caused an inhibition of urea hydrolysis in the plant tissue, decreasing in this way its uptake from the solution. Some effect of carbonate on plasma permeability is also possible (Glinka 1962).

5. Effect of sugars and amino acids

The literature gives some data concerning the protective effect of sugars, mainly sucrose, against damage induced by application of urea on the foliage (Cook 1952; Kuykendall 1954; Montelaro 1952; Norton 1954; Ozaki 1954). It was supposed that the addition of sucrose results in a decrease of urea absorption (Cook 1952; Kuykendall 1954).

The present experiments were carried out in order to establish the possible stimulatory or inhibitory effect of some organic substances on urea uptake. The treatments were as follows:

1) control — 0.0017 M urea-^{14}C.
2) 0.0017 M urea-^{14}C+0.00017 M sugar (or amino acid).
3) 0.0017 M urea-^{14}C+0.010 M sugar (or amino acid).
4) 0.0017 M urea-^{14}C+0.0100 M sugar (or amino acid).

The time of absorption was 4 hrs.

As sugars glucose and sucrose were added, as amino acids — glycine, alanine,

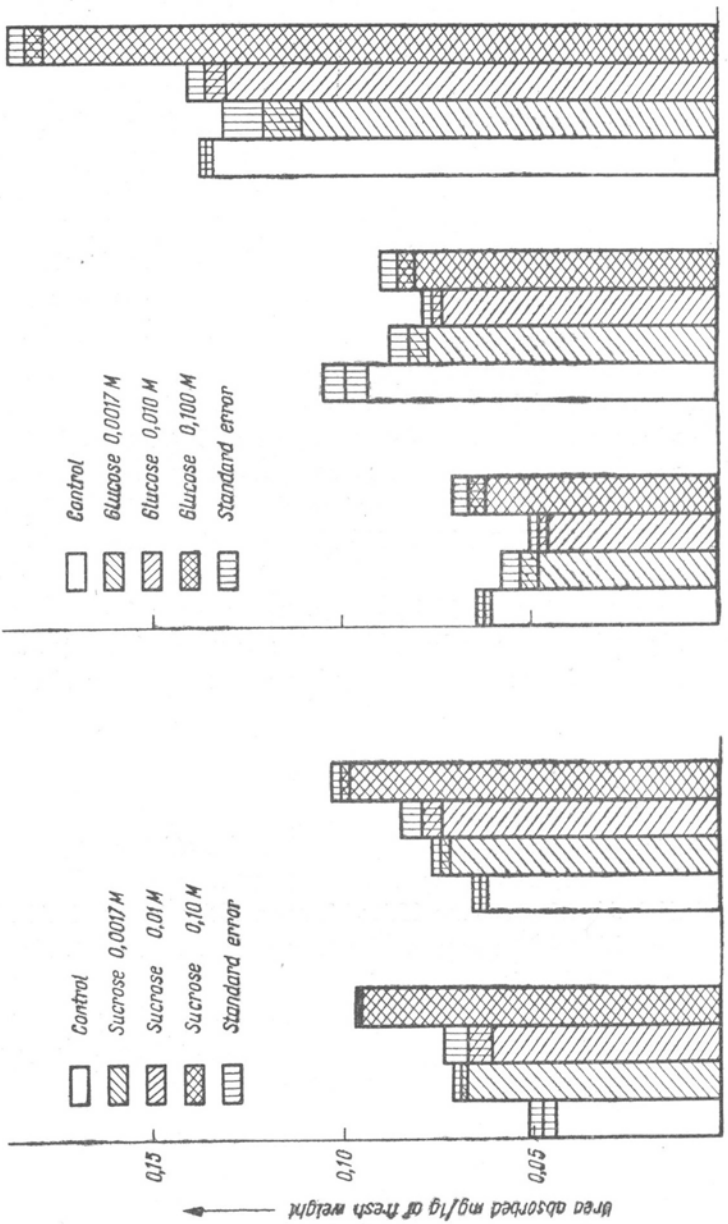

Fig. 4. Amounts of urea taken up in the presence of sugars:
a — sucrose (two experiments); *b* — glucose (three experiments)

ornithine, arginine, glutamic and aspartic acid. In the case of the latter two compounds solutions of a maximum concentration of 0.01 M (for aspartic acid) and 0.05 M (for glutamic acid) were prepared because of their low water solubility. The urea

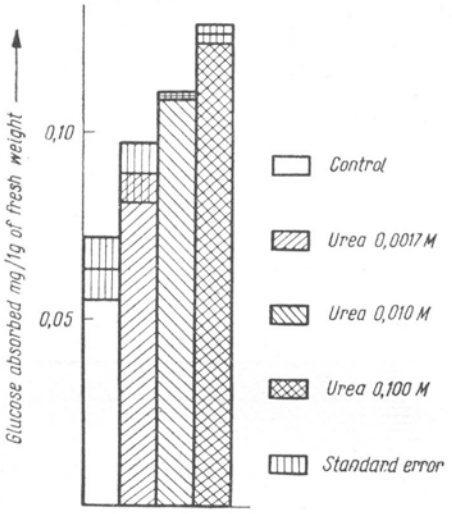

Fig. 5. Amounts of glucose taken up in the presence of urea.

uptake in the presence of sucrose is shown in fig. 4a (results of two parallel experiments), in the presence of glucose — in fig. 4b (3 parallel experiments), and in the presence of amino acids — in fig. 6.

When increasing quantities of sucrose were added to the solution, the urea uptake increased, too. These results are controversial to those of Cook (Cook 1952), who observed a decrease of urea uptake by leaves in the presence of sucrose, but this discrepancy may have been caused by different experimental material (leaves in his case, roots — in ours).

In the case of glucose addition the results were not so clear. It seems that lower concentrations of glucose (0.0017 and 0.010 M) caused a slight decrease or had no effect on urea uptake, and the highest one (10^{-1} M) had a stimulating effect.

In order to explain better the relationship between the urea uptake and glucose concentration a reverse experiment was designed: to 0.0017 M glucose -^{14}C increasing amounts of urea were added, and glucose uptake was determined after 4 hrs of absorption. The results of two parallel experiments are shown in fig. 5; the glucose uptake increased with the rise of urea concentration.

It seems obvious that the rise of sucrose concentration caused the increase of urea uptake by roots, as it supplied the material for binding NH_3 produced during urea hydrolysis. A similar effect might be expected also in the case of glucose addition, but it did not occur, and we are unable to interpret this dissimilar effect of both sugars.

There was not any distinct effect of amino acids on urea uptake, except for glutamic acid which inhibited urea uptake (fig. 6).

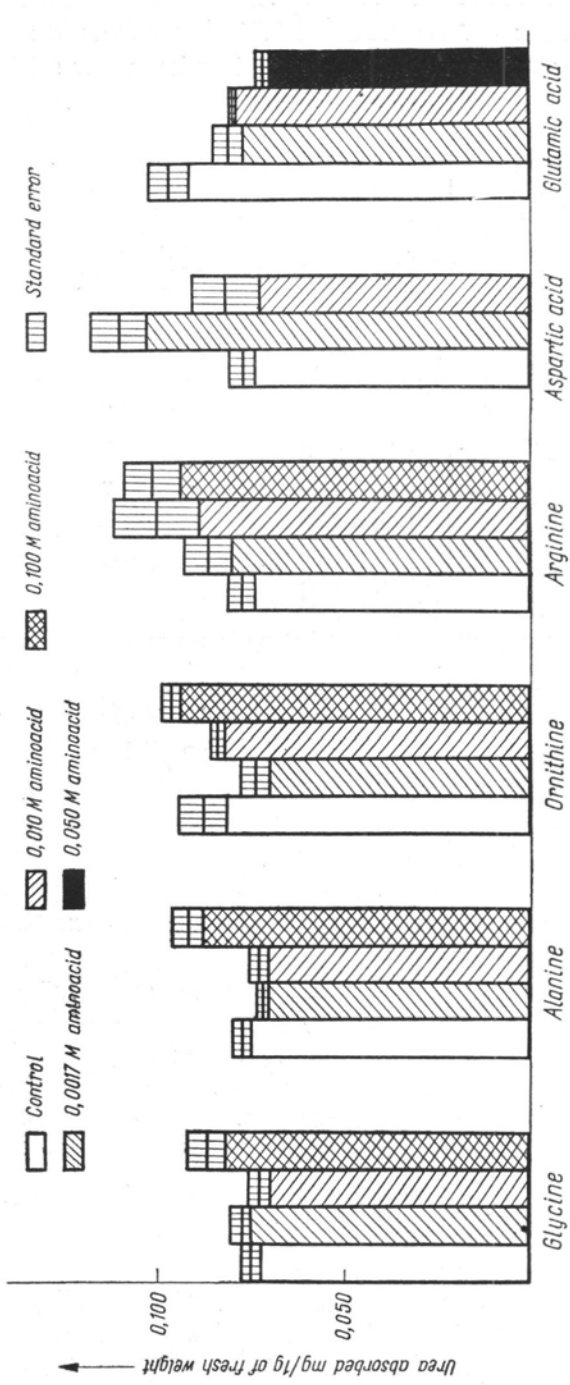

Fig. 6. Amounts of urea taken up in the presence of amino acids.

DISCUSSION

In the former paper (Olszańska 1968) it was concluded that although the mechanism of urea uptake is based on a passive process, nevertheless it is connected with metabolism by way of enzymatic urea decomposition. In the process of urea absorption three phases were distinguished, and presently the conclusions are examined in the light of results obtained in other experiments.

The temperature coefficient is ofen used as a rough criterion for estimating the character of an uptake. If $Q_{10} \approx 1$, it means that the kinetics of the diffusion process is a factor deciding of the course of absorption, and the uptake is of passive nature. A Q_{10} value of 2—4 bears evidence to the existence of a metabolic component in the uptake (Collander 1959; Rosenberg 1954; Sutcliffe 1962). In our experiments the Q_{10} values calculated on the basis of the rate of urea uptake were constant and equal to 2.55 for all linear parts of the curves, that is of the range characteristic for the biochemical reaction. These results confirmed the conclusion that in the initial phase the process of urea uptake is limited by the kinetics of diffusion, and then by the kinetics of enzymatic urea decomposition. It is known that the factor of temperature has a strong influence on the kinetics of an enzymatic reaction. Consequently, the increased amounts of urea taken up at higher temperature may be attributed to the increased rate of removal of the urea from the diffusional equilibrium between the tissue and the solution. As a result of that greater amounts of urea could pass from the solution into the tissue.

The effect of pH in the range 5—9 was not revealed in our experiments, what is consistent with our view on urea uptake. The pH value should not affect diffusion of urea as urea is very little dissociated ($K = 1.5 \times 10^{-14}$). The pH could affect the general metabolism of the tissue altering also the kinetics of urea decomposition, but pH changes of an external solution affect rather little the pH of a cell (Albaum 1937).

In the case of addition of ions to the urea solution no competitive action was observed, nor was it expected to appear. All changes of urea uptake observed in the presence of the ions may be attributed to an indirect influence of the ions — to their effect on plasma permeability or on the kinetics of urea decomposition.

The same holds true for the uptake of urea in the presence of organic substances. The stimulative effect of sucrose could be a result of addition of the material for ammonium fixation, but the different effect of glucose has not been explained. The changes of urea uptake in the presence of amino acids are probably the result of changes in NH_3 metabolism after urea decomposition but this needs also experimental evidence. For example: inhibition of urea uptake in the presence of glutamic acid may be explained by the inhibition of the reaction of amination of α-keto-glutaric acid (Kretowicz 1955, 1957, 1959; Ratner 1961):

$$\alpha\text{-keto-glutaric acid} + NH_3 \rightarrow \text{glutamic acid}$$

The increase of the glutamic acid amounts observed in the presence of urea (Baker 1962; Malavolta 1957; Webster 1955) indirectly confirms such a possibility.

Ornithine, arginine, aspartic acid might affect the urea metabolism through their relation to the ornithine cycle. Although we have found no data as to the existence of that cycle in maize roots, its presence is not excluded.

From the results reported here it is concluded that the relations observed in the experiments can be interpreted without implying the participation of an active component in the urea uptake.

If we assume an essential role of the reaction of enzymatic decomposition of urea for its uptake, it is obvious that any factor affecting urea decomposition will also affect its absorption. It will not be, however, an active uptake in the classical sense of the term. This kind of "pseudo-active" uptake may also occur in the case of other organic compounds, especially in the case of the metabolically active ones. Therefore, special care should be taken to avoid the confusion of an active uptake with the uptake enhanced by the metabolism of the compound taken up.

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SUMMARY

The effect of temperature, pH, and the presence of some ions and organic substances on urea uptake was investigated.

The contribution of an active component to the uptake has not been confirmed. All relations of urea uptake with the external factors may be explained by their influence on the reaction of enzymatic urea decomposition.

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Badania nad mechanizmem pobierania mocznika przez korzenie

II. Wpływ niektórych czynników zewnętrznych na pobieranie mocznika

Streszczenie

Przebadano wpływ temperatury, pH oraz obecności jonów i substancji organicznych w roztworze na pobieranie mocznika. Obecność aktywnej składowej w pobieraniu mocznika nie została stwierdzona. Wszystkie przebadane zależności pobierania mocznika od czynników zewnętrznych dadzą się wytłumaczyć ich wpływem na reakcję enzymatycznego rozkładu mocznika.