Studies on the mechanism of urea uptake by plant roots*

I. Kinetics of uptake, effect of concentration and inhibitors

B. OLSZAŃSKA

It is to be expected that the uptake of urea as a whole unsplit molecule will be regulated by a mechanism different than that of ion uptake. At present there is no more doubt that urea may be taken up by plants in the form of a whole molecule, without its previous decomposition to NH_3 and CO_2 (Allison 1954; Arnou 1953; Baker 1962; Cain 1956; Dilley 1961; Ellner 1955; Freiberg 1957; Hattori 1957; Hirose 1961; Kaniuga 1958; Mitsui 1962; Mothes 1961; Mokronosow 1964; Mosołow 1963; Ostromecka 1961; Pate 1964; Pawłow 1960; Walker 1952; Yamaguchi 1930). So far, investigations on the mechanism of urea uptake have been rather fragmentary, their results were sometimes controversial and incomplete. The aim of this work was to investigate the kinetics of urea uptake and also the effect of some factors on the course of this process, in order to obtain some evidence concerning the mechanism of the uptake.

METHODS

Seeds of maize (*Zea mays*, variety Sterling) were germinated at 25°C on glass plates covered with wet filter paper. On the 4—5th day after sowing, the primary roots were cut off, carefully dried with filter paper and cut into segments of about 1.5 cm length. The root samples were incubated with urea solution labelled with ^{14}C (1 $\mu\text{Ci/ml}$) at the ratio of 1 g roots: 5 ml solution, at 20°C.

After an incubation period the roots were separated from the solution and the activity of the solutions was measured with a GM window-counter.

The amounts of urea taken up were calculated on the basis of the

* The work is a part of the author's Ph. D. thesis, and has been performed at the Department of Applied Atomic Physics and Radiochemistry Timiriaziev Agricultural Academy, Moscow.

difference in solution activity before and after incubation with roots, according to the formula (R a c z y ń s k i 1962):

$$S = \frac{v \cdot c}{g} \left(1 - \frac{A}{A_0} \right);$$

- S — quantity of urea absorbed (g),
 v — solution volume (ml),
 c — concentration of urea solution (mg/ml),
 g — root mass (g),
 A — radioactivity of the solution after incubation,
 A_0 — radioactivity of the solution before incubation.

The experiments were performed in 3 replications and 10 measurements of radioactivity for each sample were done. The statistical error of the radioactive decay did not exceed 2%, and the general error of the

experiments — 10% $\left(d = \sqrt{\frac{\sum x^2}{n(n-1)}} \right)$. In view of the method used,

the first part of the kinetic curves (1 min — 1 hr) is burdened with the biggest error, since during that time the differences in the measured radioactivities of the solutions are the smallest.

From some batches of plant material samples were taken for determination of dry weight, in order to recalculate the urea concentration to the water content of plant tissue.

The methodical error, caused by the passing of carbon from tissue into the solution, could not be avoided in our experiments. After urea decomposition the radioactive ^{14}C could pass into the solution increasing its radioactivity, so that the results obtained would be lower than the actual urea uptake. Nevertheless, this should not change the general relationships observed in the experiments.

RESULTS

Preliminary investigations

a) Although the maize seeds were germinated in an atmosphere saturated with water vapour, it was necessary to verify if small changes of water content in the root tissue could affect the urea uptake. One group of roots was dipped into water for 5 min and then, after drying with filter paper, put into urea solution (A). The other part of roots was not treated with water (B). The time of incubation was 1 hr. The urea uptake in both cases was the same:

A) urea uptake — $0,0520 \pm 0,0053$ mg/1 g of fresh weight

B) urea uptake — $0,0498 \pm 0,0045$ mg/1 g of fresh weight

b) There was a possibility that cutting of the roots into segments

might influence the measured values of urea uptake. In order to verify this supposition the following experiment was carried out:

Treatment	Time of absorption	Urea uptake mg/1g of fresh wt.
Whole roots	4 hrs 45 min	0,0700
(cut off from seeds)	9 hrs	0,1529
roots cut into	4 hrs 45 min	0,0678
segments	9 hrs	0,1471

In the first treatment vaseline was applied on the place where the roots were cut off from the seeds.

The results of this experiment show that the urea uptake in the case of whole roots and roots cut into segments was the same — the difference being nonsignificant.

c) It was tested whether shaking of the test tubes during the experiment and the access of air would affect the urea uptake. The results obtained during 4 hrs incubation were:

- 1) test tubes closed, not shaken — 0,0948 mg of urea taken up/1 g fr. wt.
- 2) test tubes closed, shaken — 0,0945 mg of urea taken up/1 g fr. wt.
- 3) test tubes opened, not shaken — 0,0950 mg of urea taken up/1 g fr. wt.

In all three treatment the urea uptake was the same, so all the following experiments were carried out in closed not shaken test tubes.

d) One of the sources of error in the method used for urea uptake determination could be the decomposition of urea in the ambient solution and the escape of radioactive CO_2 . Although urease as a protein compound should not be given off from the plant tissue into the solution (Garnick 1938), nevertheless, it had to be checked, whether in our experimental conditions there were some losses of radioactivity out of the solution apart from the urea uptake.

The root samples (1 g) were put into test tubes containing 5 ml of water and left for 21 hrs at 20°C . Then the roots were removed and 0,05 ml of ^{14}C -urea solution was added to each tube. The radioactivity of the solution in the test tubes was measured: 1) directly after adding ^{14}C -urea, 2) after 4 hrs of incubation at 21°C , 3) after the following 24 hrs of incubation at 2°C . The results are given in table 1.

Table 1

Activity of ^{14}C -urea solutions after various incubation periods (counts/100 sec)

Test tube no.	Initial activity	Activity after 4 hrs incubation at 21°C	Activity after 24 hrs incubation at 2°C
1	2489	2578	2583
2	2556	2666	2652
3	2684	2787	2758

The results show that there was no loss of radioactivity observed during the experiment. The slight (4%) rise of radioactivity after the first 4 hrs of incubation could be connected with water evaporation.

c) Identification of urea in root tissue was done by the chromatographic method. The roots (5 g) were incubated during 1 and 4 hrs in 25 ml of ^{14}C -urea solution (0,0017 and 0,10 M). The root samples were separated from the solution, washed with distilled water several times and treated with 96% boiling ethanol. Then, the roots were ground and once more washed with 25 ml of boiling ethanol for 30 min. Both extracts were mixed together and evaporated. The residue was diluted with 2 ml of distilled water and chromatographed with isobutanol: acetic acid: water (4:1:5 v/v/v) as solvent. With chromatograms obtained autoradiograms were prepared (30 days of exposure with X-ray film) and the chromatograms were then developed with p-diaminobenzaldehyde which gives a yellow colour reaction with urea (Hais, 1962). By comparing the chromatograms with the autoradiograms it was found that most of the ^{14}C radioactivity was connected with urea.

Kinetics of urea uptake

According to the method described, the maize roots were incubated with ^{14}C -urea solution (0,0017 and 0,05 M) for 1 min to 24 hrs. Similar experiments were carried out with dead roots, killed by 5-min boiling in water. The results are given in figs. 1 and 2.

Fig. 1. Kinetics of urea uptake from 0,0017 M solution, by living and dead roots.

The kinetic curves (average of 4 experiments, in 3 replications each) for living and dead roots were different. In the case of dead roots, absorption was finished within 10 min.

For living roots, after the initial period of quick urea absorption, a slower uptake was observed. The course of the kinetic curves varied in dependence on the concentration of the solution. At the higher urea

Fig. 2. Kinetics of urea uptake from 0,05 M solution by living and dead roots

concentration (0,05 M), after initial quick absorption lasting about 10 min, slow uptake followed which led to an equalizing of "urea concentration" * in the tissue and the ambient solution after about 8 hrs, and then absorption practically ceased (fig. 2).

At 0,0017 M urea concentration also the initial quick absorption was followed by slow absorption. The "urea concentrations" in the tissue and in the solution were equalized after about 3 hrs, but the process of absorption still continued, leading to an increase of "urea concentration" in the tissue above its concentration in the ambient solution. After 24 hrs absorption the "urea concentration" in the tissue was nearly 7 times of that in the ambient solution (fig. 1).

Realation of urea uptake to its concentration

Urea uptake was examined in 0,0017; 0,0050; 0,010; 0,050; 0,075 and 0,10 M solutions for living and dead roots.

The time of absorption for living roots was 1, 2 and 4 hrs. Since urea absorption by dead roots ended within 10 min, the time of absorption for dead roots was limited to 1 hr. The experimental results are presented in fig. 3.

In fig. 3 the urea concentration (recalculated to the water content of the tissue) is plotted against the equilibrium concentration of urea in

* The term "urea concentration" when used for the urea content in the tissue means rather the concentration of C-14, expressed as urea concentration (recalculated to the water content of the tissue).

Fig. 3. Relation of urea concentration in the tissue to the equilibrium urea concentration in the ambient solution.

the ambient solution. It was found that the amounts of urea taken up by living and dead roots are proportional to its concentration in the ambient solution.

In the case of dead roots, the urea concentration in the tissue and in the ambient solution became equal within 1 hr of absorption. The "urea concentration" in the living roots was lower than that in the ambient solution for 1, 2 and 4-hr absorption, with the exception of the lowest (0,0017 M) concentration, in which case as established before (fig. 1), the concentrations equalized within 4 hrs. It means that permeation of urea into dead roots occurs much easier than into living ones.

Urea uptake in presence of inhibitors

The kinetic curves of urea uptake were examined for the 0,0017 M solution in the presense of 10^{-4}N KCN, 10^{-4}N NaN_3 , 10^{-3}M DNP and 10^{-3}N HgCl_2 . The first three compounds affect the active component of uptake through the inhibition of respiration and phosphorylation reactions. Consequently, if the metabolic process participates in urea absorption, inhibition of that absorption might be expected. HgCl_2 (besides its influence on the general metabolism) is a strong inhibitor of urease (Mitsui, 1960). Three parallel series of experiments were carried out (3 replications each). In the first two series, the control curve (urea

Fig. 4 a, b, Kinetics of urea uptake in the presence of inhibitors.

uptake without inhibitors) and the experimental ones were traced separately, at different times. The results obtained in both series were similar and the average results are given in fig. 4 a. However, in order to check the correctness of the data obtained in such way, a third series of experiments was carried out, in which the urea uptake was determined simultaneously for all treatments (fig. 4 b).

The course of the curves was similar in both cases (fig. 4 a and 4 b) with the exception of DNP treatment. KCN and NaN_3 had no effect on urea uptake. DNP, during the first 8 hrs of absorption, also did not affect the course of uptake, and only after 8 hrs was there a slight stimulation (in fig. 4 a) or a slight inhibition (in fig. 4 b) of the uptake. This varying effect of DNP is difficult to understand.

In the case of HgCl_2 there was distinct inhibition of urea uptake. After about 4 hrs urea absorption ceased and the urea content in the tissue did not change any more (^{14}C disappeared no more from the ambient solution).

DISCUSSION

From the point of view of active uptake it is quite understandable that in dead roots only the passive processes — diffusion and adsorption of urea — may occur. These processes run rather quickly — within 10 min equilibrium between tissue and ambient solution was established.

In living roots, after a short period of quick absorption similar as in dead roots, slow uptake followed. This process led, after a time, to the equalization of "urea concentration" between the tissue and the ambient solution, and then an apparent increase of urea concentration above its concentration in the ambient solution was observed. Nevertheless, it should be taken into account that the method of determination of urea uptake allowed to measure the urea amount in the tissue only indirectly (by measurement of activity changes in the ambient solution), and urea was not determined in the tissues by any direct method.

Since in maize roots urease is present — although with low activity (Yamaguchi 1930) — the possibility of urea decomposition in the tissue should be considered, and consequently, the amounts of ^{14}C found should not be regarded the same as the amounts of free urea in the tissues.

It is shown in fig. 1 that after 3—4 hrs the "urea concentration" in the tissue becomes higher than its concentration in the ambient solution. If one assumes that ^{14}C amounts found are equal to the amount of free urea in the tissue, such a fact would bear evidence to the existence of an active process. Yet, if one considers the enzymatic decomposition of urea in the tissue, the curve in fig. 1 does not represent the urea accumulation, but the accumulation of the products of its metabolism, labelled with ^{14}C .

Similar kinetic curves of urea uptake were obtained by Prell (1955) and Müller (after Mothes 1961). Prell calculated the urea uptake on the basis of the total N increase in the tissue of *Taraxacum*, and he found an increase of urea concentration in the tissue above its concentration in the ambient solution. Similar results were obtained by Müller by the same method (determination of total N in the tissue), but the determination of free urea in the tissue showed that its concentration never exceeded that in the ambient solution. It may be

supposed that in Prell's experiment, although he did not detect urease activity, urea decomposition took place, and the increase of total N in the tissue was not equal to the quantity of free urea. A similar situation occurred probably in our experiments.

In the course of urea absorption one might differentiate as if three phases.

The first — adsorption of urea and its diffusion into "free space". This process runs quickly — it is ended within 10 min and occurs analogically in dead and living roots, except that in living tissue the urea concentration remains lower than in the ambient solution.

The second phase consists in slow diffusion of urea through the plasmatic membranes into the cells. As the end of this phase one may assume, conventionally, the time when the urea concentrations in tissue and ambient solution become equal. This time depends on the concentration of urea in the ambient solution.

In the third phase the process of urea decomposition acquires considerable importance. After entering the cells, urea undergoes enzymatic decomposition, and in this way its diffusional equilibrium is disturbed. In its place new amounts of urea enter and in this way the process leads to a continuous decrease of ^{14}C -urea in the solution, but this should not be interpreted as an accumulation of free urea against the concentration gradient. The strict delimitation of these phases in the course of urea uptake is obviously not possible as they overlap one another. The difference between them consists in the character of the process limiting the velocity of urea permeation during the given phase. In the first phase, such a factor limiting urea permeation would be the velocity of adsorption and diffusion of urea into "free space", as this phase is similar in dead and living roots, and is not influenced by temperature or inhibitors. Then, the urea permeation would be limited by the kinetics of its diffusion through live plasmatic membranes. This phase is lacking in dead roots, in living tissue it is not affected by inhibitors, and urea uptake during this phase shows a proportional relation to concentration. Finally, when the diffusional equilibrium between the tissue and the ambient solution is established, the kinetics of enzymatic decomposition of urea in the tissue becomes the factor limiting its absorption from the solution.

The facts observed in our experiments speak in favour of a passive mechanism of urea uptake. The urea uptake shows a linear relationship to its concentration in the ambient solution (within the range of 0,0017—0,10 M). Similar results were also obtained by other authors (Hirose 1961; Keleg 1959; Kuykendall 1954). The known metabolic inhibitors such as KCN, NaN_3 , DNP had no significant effect on the quantity of urea absorbed, but in the case of HgCl_2 — an urease inhibitor — there was a distinct inhibition of the uptake in the

period corresponding to the "third phase". It confirms the conclusion that enzymatic decomposition of urea is then decisive for its uptake.

Although we regard the process of molecular diffusion as a basis for the urea uptake, it will not be the process of free diffusion in the aqueous phase. The equalizing of the urea concentrations between the living tissue and the solution occurs much slower than in the case of the dead one, it may therefore be concluded that living plasma offers a considerable resistance to free urea diffusion into the cells. May be, this is caused by the small dimensions of pores in semipermeable membranes, or by the limited urea solubility in the lipoprotein layers of a membrane (Dawson a. Danielli 1952). Although the part of active uptake in the classical sense of the term (Rosenberg 1954; Sutcliffe 1962) has not been established in our experiments, undoubtedly the process of urea uptake is connected with metabolism by way of the reaction of enzymatic decomposition of urea by urease. This very reaction is supposed to be the main cause of the shifting of the diffusional equilibrium between tissue and solution and the direct "motoric force" of the uptake mechanism.

SUMMARY

The investigations concerned the kinetics of urea uptake by roots and the effect of its concentration and of inhibitors (KCN, NaN_3 , DNP, HgCl_2) on this process. The experiments were made with young (4—5-days-old) sections of maize roots, with ^{14}C -labelled urea.

The urea uptake was estimated as the difference in solution activity before and after incubation with roots in the ratio 1 g of roots : 5 ml of solution. The urea uptake was proportional to its concentration in the solution. Of the inhibitors tested only HgCl_2 had an inhibiting effect on urea uptake. Three phases were distinguished in the process of urea absorption.

Although an active component has not been revealed in the process of urea uptake, nevertheless this process is believed to be connected with metabolism via the reaction of enzymatic decomposition of urea by urease.

Agricultural Isotopic Laboratory
Polish Academy of Sciences
Warsaw, Rakowiecka 8

(February 18, 1967)

REFERENCES

- Allison R. K., et al., 1954, *Plant Phys.*, 29:164—168.
Arnou P., Olsen J. J., Williams J. H., 1953, *Am. Jour. Bot.* 40:100—104.
Baker J. E., Thompson J. F., 1962, *Plant Phys.* 37:618—624.
Cain J. C., 1956, *Proc. Am. Soc. Hort. Sci.*, 67:279—286.
Dawson H., Danielli J. F., 1952, *The Permeability of Natural Membrane*, Univ. Press.
Dilley D. R., Walker D. R., 1961, *Plant Phys.* 36:757—761.

- Ellner P. D., Steers E., 1955, Arch. Biochem. Biophys. 59:534—535.
- Freiberg S. R., Payne P., 1957, Proc. Am. Soc. Hort. Sci. 69:226—234.
- Garnick S., 1938, Plant Phys. 13:29—54.
- Hais I. M., Macek K., 1962, Chromatografia na bumagie, Izd. Inostr. Lit., Moskwa.
- Hattori A., 1957, Jour. Bioch. (Tokyo) 44:253—273.
- Hirose S., Goto Y., 1961, Soil Sci. a. Plant Nutr., 7:85.
- Kaniuga Z., 1958, Acta Soc. Bot. Pol. 27:313—322.
- Keleg F. M., Baghdadi H. A., Minessy F. A., 1959, Ph. D. Thesis, Univ. of Alexandria.
- Kuykendall J. R., Wallace A., 1954, Proc. Am. Soc. Hort. Sci. 64:112—127.
- Mitsui S., Namioka H., Mukai N., 1960, Soil a. Plant Food 6:25—29.
- Mitsui S., Kurichara K., 1962, Soil Sc. a. Plant Nutr. 8:219—225.
- Mokronosow A. T., Ilinych Z. G., 1964, Dokł. AN SSSR, 154:1454—1457.
- Mosołow I. W., Pylnewa P. I., 1963, Wiest. Siel-Choz. Nauki 9:42—46.
- Mothes K., 1961, Can. Jour. Bot. 39:1785—1807.
- Ostromęcka M., 1961, Roczn. Nauk Roln. 82-A-2:473—488.
- Pate J. S., Wallace W., 1964, Ann. Bot. 28:83—99.
- Pawłow A. N., 1960, Dokł. AN SSSR 134:475—477.
- Prell H., 1955, Planta 46:361—380.
- Raczyński W. W., Rustamow S., 1962, Radiochimia 4:253—260.
- Rosenberg T., 1954, Symp. Soc. Exptl. Biol., 8:27—41.
- Sutcliffe J. F., 1962, Mineral Salt Absorption in Plants, Pergamon Press, Oxford.
- Walker J. B., 1952, Proc. Nat. Ac. Sci. US 38:561—569.
- Yamaguchi S., 1930, Jour. Fac. Sci. Hokkaido Univ. ser. 5 Bot. 1:37—55.

Badania nad mechanizmem pobierania mocznika przez korzenie

I. Kinetyka pobierania, wpływ stężenia i inhibitorów

Streszczenie

Przeprowadzone badania dotyczą kinetyki pobierania mocznika przez korzenie oraz wpływu tego stężenia i inhibitorów (KCN, NaN_3 , HgCl_2 , DNP) na przebieg tego procesu. Doświadczenia wykonywano na młodych 4—5 dniowych odcinkach korzeni kukurydzy przy użyciu mocznika znakowanego ^{14}C .

Pobieranie mocznika oznaczano z różnicy w aktywności roztworu przed i po inkubacji z korzeniami, przy stosunku korzeni do roztworu 1 g: 5 ml.

Pobieranie mocznika było proporcjonalne do jego stężenia w roztworze. Z przebadanych inhibitorów tylko HgCl_2 hamował pobieranie mocznika. W procesie pobierania wyróżnione zostały trzy fazy.

Chociaż przy pobieraniu mocznika nie stwierdzono udziału aktywnej składowej, to jednak przypuszcza się, że proces ten związany jest z metabolizmem za pośrednictwem reakcji enzymatycznego rozkładu mocznika przez ureazę.

Panu Prof. W. W. Raczyńskiemu składam serdeczne podziękowanie za pomoc i cenne wskazówki oraz za umożliwienie mi wykonania tej pracy w swoim laboratorium.