Studies on the Relation between Urease in Soy-Bean Seedlings and the Nutrient Value of Urea as a Nitrogen Source

By

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(With 6 Text-figures)

The occurrence of urea in the plant body was reported for the first time in fungi by BAMBERGER and LANDSIEDL (1903). WEYLAND (1912), FOSSE (1912; 1913 a, b; 1928), IWANOFF (1923 a, b, c, d; 1924 a,b; 1925) confirmed the existence of urea in some fungi and also in many higher plants, and its physiological meaning for the plant growth was discussed by some of them. These observations have called new attention to the rôle of urea in the field of nitrogenous metabolism of higher plants. Recently KLEIN and TAUBÖCK (1927; 1930; 1931 a, b; 1932 a, b) and TAUBÖCK (1927) proved that urea exists in Angiosperms, and they made researches upon the significance and the process of the appearance of urea in the green plant, studying the relation between urea and ureïdes. TAKEUCHI (1907), for the first time, demonstrated that urease exists not only in lower organisms, but also in higher plants. From the frequent occurrence of urease in the plant body and its catalytic action in the hydrolytic process, it is affirmed that urease plays a great rôle in the utilization of urea and in its distribution in the plant body. According to KIESEL (1927) urea can not exist unchanged in the plant body, if urease occurs, and the conditions under which urease exists, are closely related with the decomposition of urea. PIRSCHLE (1929) confirmed with many higher plants, especially in the root, that an intimate relation exists between urease and urea in the culture solution. TAUBÖCK (1927) ascertained the same relation also between the action of urease, and the amount and distribution of urea in a plant body. From these investigations it may be seen that urease has a close physiological relation with the nitrogen metabolism in plant

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body, so far as urea is produced in plant body or resorbed as itself. Also TRUFFANT and BEZSSONOFF (1924), KOSTYTSCHEW (1926), ASO and YOSHIDA (1928), TANAKA (1931), YAMAGUCHI (1931, 1935), PIRSCHLE (1931) and KULTZSCHER (1932) studied on the nutritive value of urea in the nitrogen metabolism of the higher plants. In his previous papers, the writer reported the results of the studies on the resorption of urea by the root system of the higher plants and on its nutrient value as a nitrogen source, using water culture—ordinary and sterile—sand and soil cultures. In the present work further studies were conducted, and it was found how important in the determination of the nutrient value of urea are its concentration in the culture solution, the method and duration of the culture and the action of urease. Soy-bean was selected as the most suitable material for the present purpose, since many uniform seedlings can be easily obtained and they contain a large amount of urease.

I. The Change of the Action of Urease in Soy-Bean Seedling in the Cource of Growth

TAKEUCHI (1909) found urease both in the resting seed and in the seedling of soy-bean, and extracted urease with water very easily. WESTER (1921) tested the occurrence of urease in every part of soy-bean seedling, and he recognized that urea exists in larger quantity in the cotyledon than in other parts. Later, KLEIN and TAUBÖCK (1927) and TAUBÖCK (1927) proved that urea or ureïdes exist in soy-bean seedling, using xanthydrol as reagent. According to them the occurrence of urea or ureïdes in every part of a soy-bean seedling has intimate relation with the distribution and the occurrence of urease. In the following described experiments the writer attempted to investigate the relation between the change of the action of urease and the growth of seedling of soy-bean, in order to see the significances of urease in respect to the nutritive value of urea as a nitrogenous source.

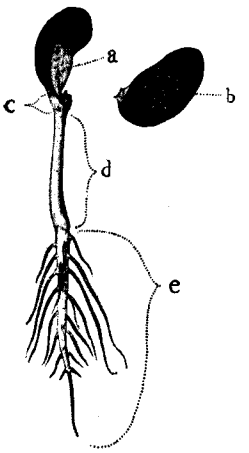


Fig. 1.

a first foliage leaf.
b cotyledon.
c attached part.
d hypocotyle.
e root.

The seeds of soy-bean were germinated in sawdust watered with tap-water, and when the primary root reached three or four cm length, the seedlings were transferred to tap-water and cultured for some days. Then the seedlings were taken out of tap-water, and after sufficient washing in distilled water, each plant body was divided into the parts of the first foliage leaf, cotyledon, hypocotyle, epicotyle, part of the cotyledon attached to the hypocotyle, and root (Fig. 1.). Each of these parts to a definite fresh weight was separately ground in a porcelain morter, and added with 30 c.c. of 30% alcohol. The thus prepared gruel was left to stand for thirty minutes, and then filtered. Filtrates were prepared from each part, and they were put into an ERLENMEYER's flask of ca 50 c.c. capacity with other substances.

Alcohol extract of sample	5 c.c.
Urea (M/100)	25 c.c.
Phosphate buffer mixture	5 c.c.
Phenolred (0.04%)	5 drops
Thymol	a small piece

The pH-value[1] of this mixed solution was 7.3 or 7.4 and remained unchanged during the experiment. After keeping this mixture for 2 hours in the thermostat of 30°C., the total free ammonia was estimated by the micro-KJELDAHL method. As control a mixed solution was used, which was prepared using distilled water instead of a 0.01 mol urea solution.

From Table I it will be seen that the occurrence of urease in soy-bean seedlings varies greatly according to the part of the plant body; it is contained to a large amount especially in the cotyledons. However, the action of urease in several parts of a seedling decreased during growth. The catalytic action of urease was not found in root, hypocotyle, epicotyle, and the first foliage leaf of the seedling cultured for about ten days. On the other hand the decrease of the urease action occurred more slowly in the cotyledon than in other parts, but this action was recognized no more in the 26 days culture. Tables II and III show, that the amount of urease in the cotyledon decreased with the growth of the seedling and in the course of the culture, also that urease was not found in the cotyledons, which became fairly hard and brown in colour. In the cotyledons, of which

1) This pH-value was reported as the optimum for the urease action (LÖVGREN, 1921).

Table I.

(Fresh weight of sample = 1 gm.)

Part of seedling		Duration of culture (days)					
		4	6	10	11	16	26
Cotyledon	Urea decomposed (mg.) (±0.03)	15.00	14.85	14.55	7.98	3.89	0.09
	Percentage of urea decomposed	100.00	99.00	97.00	53.20	25.81	1.80
Attached part of cotyledon	Urea decomposed	14.52	10.20	—	0.15	0.48	0
	Percentage of urea decomposed	96.80	6.80	—	1.00	3.20	0
First foliage leaf	Urea decomposed	—	0.81	0	0.03	0	0
	Percentage of urea decomposed	—	5.40	0	0.20	0	0
Hypococyle	Urea decomposed	—	—	0	0	0	0
	Percentage of urea decomposed	—	—	0	0	0	0
Epicotyle	Urea decomposed	—	10.08	0	0	0	0
	Percentage of urea decomposed	—	67.20	0	0	0	0
Root	Urea decomposed	13.74	1.58	0	0.06	0	0
	Percentage of urea decomposed	91.60	10.60	0	0.40	0	0

the colour changed from fair green to yellow brown, the action of urease decreased extremely. The duration of the cultivation may, however, be regarded as the more important index for the amount or activity of urease. The culture of long duration resulted in change in the hardness of the cotyledon. KIESEL and TROITZKI (1922) stated

Table II.

Duration of culture (days)	Colour of cotyledon	Number of cotyledon	Urea decomposed (±0.03 mg.)
4	Green	5	13.80
8	Yellow	5	0.09
20	Brown	5	0 04

Table III.

(Cultivated for 21 days)

Colour of cotyledon	Weight of cotyledon (gm.)	Urea decomposed (mg.) (±0.03)	Percentage of urea decomposed
Light gray green	1.0	0.33	2.20
Light green yellow	1.0	0.09	0.60
Yellow	1.0	0	0
Yellow brown	1.0	−0.03	0

that dryness of material checks the catalytic action of urease. From Table III it will be seen that the amount of urease was very little in the case of the long culture, though the colour of the cotyledons was fresh green.

As accumulation of ammonia in plant body has injurious effect on the plant growth or acts additively to the similar injury of urea, it is not unreasonable to consider that the decomposition of urea causes such an unfavourable condition. In fact the injurious effect often observed in the young stage of development of soy-bean, when the large amount of urease was found in its body. Old seedlings were not so strikingly influenced by the accumulation of urea or ammonia as young seedlings, though the former were cultured in a high concentration of urea. This is due to the fact that the action of urease is weaker and the amount of carbohydrates is larger in the old seedlings than in the young.

II. The Decomposition of the Resorbed Urea and the Change of Hydrogen Ion Concentration in the Seedlings of Soy-Bean

As the catalytic action of urease is very quick, its occurrence in the plant body may affect the change or transportation of urea. In a writer's previous paper (1931), the occurrence of a very small amount of urease was demonstrated in the part of embryo and scutellum of the seedlings of *Zea Mays*, but in the other parts it was not detected. Applying the xanthydrol method after FOSSE (1913),[1] it was

1) KLEIN and TAUBÖCK (1927) and MEVIUS (1931) pointed out that this reaction is not limited to urea, but also can be applied to ureïdes. From the writer's experiment it may be reasonable to say that the crystals which appear in the tested material after the addition of the reagent indicate the reaction of urea or at least ureïdes which can be derived from the resorbed urea.

found that the resorbed urea was transported up to the leaves without change. One part of the resorbed urea was detected unchanged in the drops of the guttation and the amount of urea exuded was variable in accordance with the concentration of urea in the culture solution. On the contrary, in the case of soy-bean, urease was found not only in the cotyledon, but in every other part, in such amount that the resorbed urea was very rapidly decomposed in the plant body. In order to test the decomposition of urea the following experiment was carried out: After sufficient washing with tap-water the young seedlings were transferred into tap-water, and afterwards the urea in the cell sap of seedlings was tested every day with xanthydrol. Urea in the cell sap of the seedlings cultured in a low concentration soon disappeared, while it was detected as itself in the seedlings cultured in a high concentration (0.05–0.01 mol) for 2–3 days.

It is not yet certain, whether the injurious effect of the accumulation of ammonia is due to the decrease of the intracellular hydrogen ion concentration or to other more important factors. It is not, however, unnecessary to test the change of the actual acidity which is probably caused by the decomposition of urea. Young seedlings which had been previously cultured for 10 days in tap-water, were transferred into solutions containing 0.1, 0.05, 0.01, 0.005 and 0.001 molar urea respectively; the hydrogen ion concentration of the expressed sap of each seedlings was determined in the following manner, using the indicators of CLARK and LUBS: A very small drop of indicator was put on non-alkaline objective glass. After drying of the indicator solution, a drop of the expressed sap of the shoot was added to it, and the pH-value was determined. From Table IV

Table IV.

Concentration of urea (mol)	pH of expressed sap of shoot	
	after 1 day	after 2 days
0.1	7.6	8.6
0.05	7.0	7.8
0.01	6.4	6.8
0.005	5.6	5.8
0.0001	5.5	5.7
0	5.5	5.5

it may be seen that the hydrogen ion concentration of the cell sap decreased in accordance with the decomposition of the resorbed urea, which depends upon the amount of urea in the culture solution and the duration of the culture.[1]

III. The Influence of Photosynthesis on the Nutrient Value of Urea and the Growth of Soy-Bean

The accumulation of a large amount of ammonia in the plant body poisons it. As already stated by TANAKA (1931), KULTZCHER (1932) and YAMAGUCHI (1935), such an unfavourable effect is diminished by the addition of glucose to the culture solution or by keeping carbohydrate in the plant body. The young seedling of soy-bean contains a large amount of urease in its body, as stated in the foregoing section. If young seedlings are grown in the urea culture solution, it may be expected that they would sometimes be injured by the accumulation of free ammonia produced by the decomposition of urea, because they are deficient in carbohydrate. This may be also the case, when they are cultured under condition unfavourable for the photosynthesis, where carbohydrate can be produced very poorly. On the contrary, if the decomposition of the resorbed urea has no chance to occur and the amount of carbohydrate in the plant body is large enough, the ammonia poisoning seems scarcely to happen. The following experiment was carried out in order to ascertain these relations.

Young seedlings of soy-bean cultured previously in tap-water for some days, were transferred into a 0.008 molar urea solution. One series of the cultures was kept in the dark and the other in the light[2] for 10 days. All cultures were carried out in the green house. The appearance of shoot and the leaf colour in the dark were poorer than those in the light, and more abnormal in the urea solution than in tap-water (Fig. 2). This result was just as expected, and the poor growth or sometimes the injury in the dark can be due partly to the want of the potosynthesis product, that is to say to the accumulation of ammonia in excess in the plant body. Further experiments were carried out for the same purpose, using various concentrations of urea.

1) A similar relation was mentioned above.
2) The shoot was illuminated, but the root was kept in the dark. The same culture condition may be applied to other similar experiments.

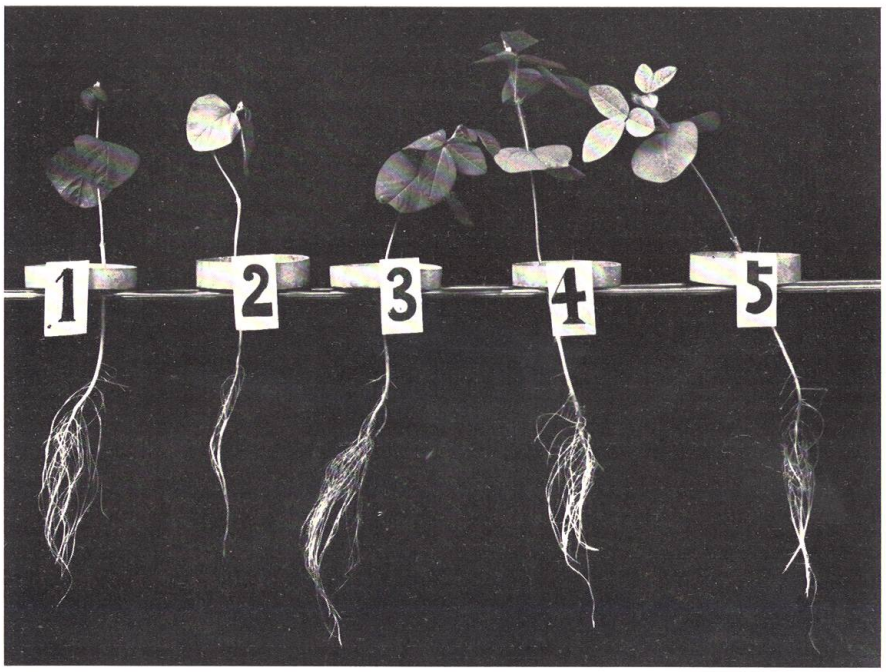


Fig. 2.

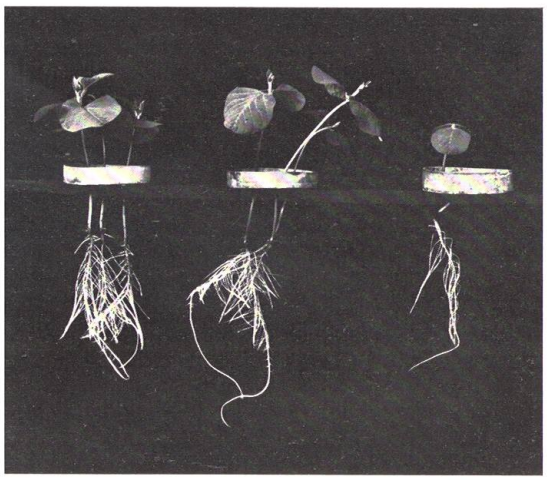
1. dark, control (no urea)
2. dark, urea (0.008 mol)
3. light, control (no urea)
4. light, urea (0.008 mol)
5. light, urea (0.008 mol)

Young seedlings, were transferred into separate culture vessels, each containing 400 c.c. of 0.01, 0.008, or 0.005 molar urea respectively, and kept in the dark and in the light for 9 days. Cotyledons were kept or removed. The results are shown in Table V. The growth of seedlings and the leaf colour in the light was better and more uniform than in the dark, in every concentration of urea. In the dark the growth and the injury occurred variously and the leaf colour developed differently, according to the concentration of urea (Figs. 3, 4, 5 and 6). That the preserved cotyledons benefit the development of seedlings is chiefly due to the supply of the non-nitrogenous substances which are utilized as nutrient matters on the one hand and on the other hand are useful for the antidotal action to the ammonia or urea poisoning.

In the foregoing experiments it was shown that carbohydrates, either produced by photosynthesis or delivered from the cotyledons,

Table V.

Concentration of urea (mol)	Cotyledon	In the dark	In the light
0.010	With	Leaf very small and yellow	Leaf small, green
	Without	Shoot and root very poor, root injured	Leaf small, green
0.008	With	Leaf small, slightly yellow, root poor	Leaf green
	Without	Shoot and root poor, root injured	Leaf green, root not injured
0.005	With	Leaf and root small	Leaf and root normal
	Without	Leaf and root very small	Leaf and root normal
Not given	With	Shoot and root very good	Growth very good
	Without	Shoot and root good	Growth normal

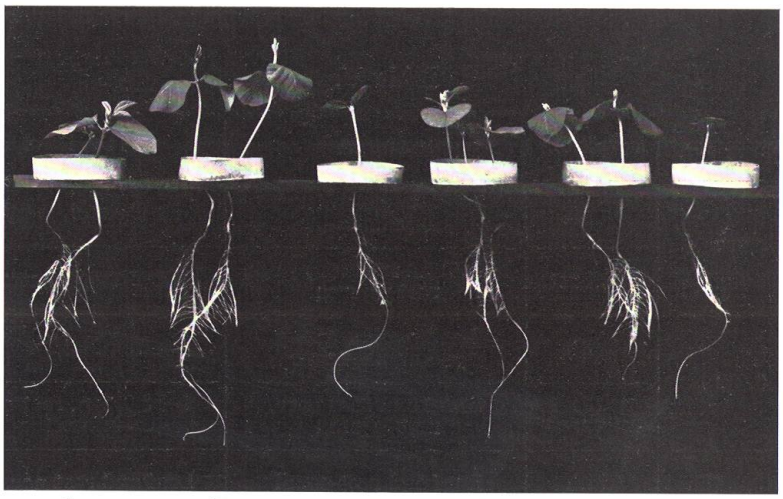


1 2 3

Fig. 3. (without cotyledon)

0.005 molar urea

1. light 2. dim light 3. dark

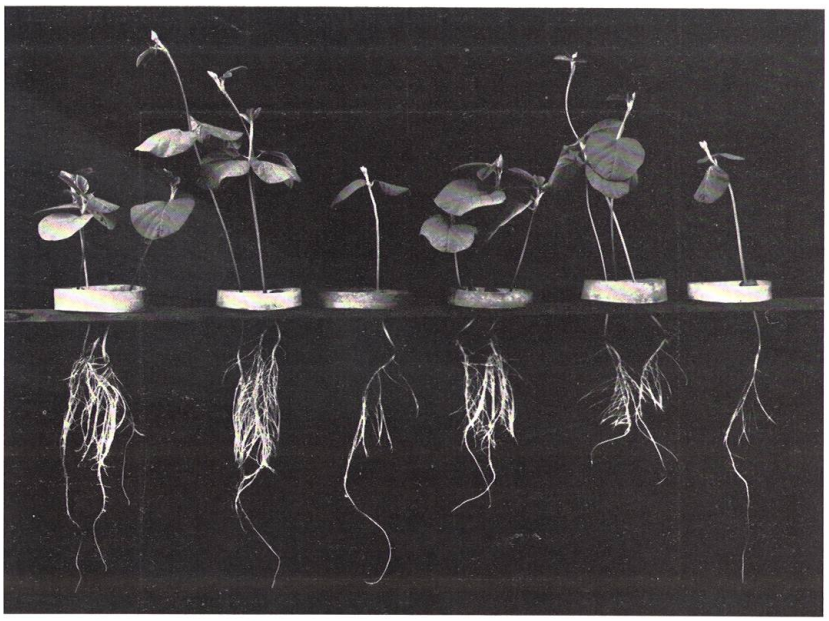


1 2 3 4 5 6

Fig. 4. (without cotyledon)

0.008 molar urea
1. light
2. dim light
3. dark

0.01 molar urea
4. light
5. dim light
6. dark

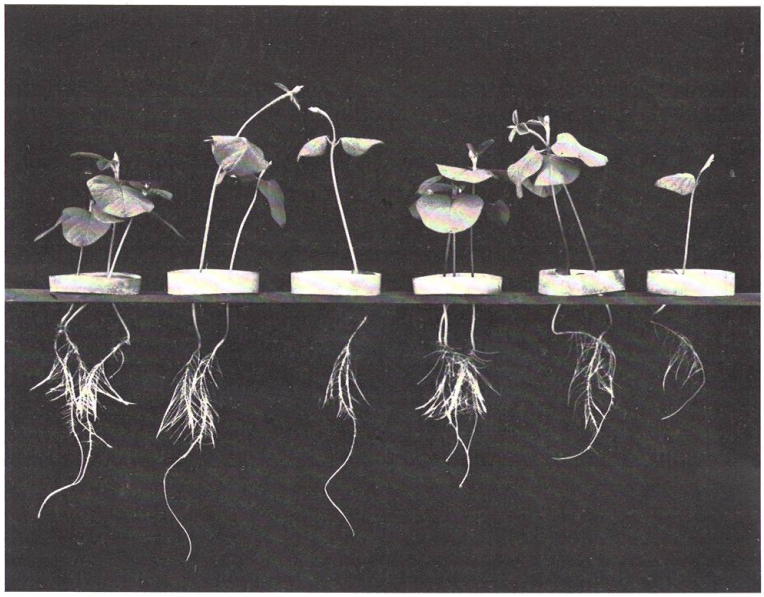


1 2 3 4 5 6

Fig. 5. (with cotyledon)

control
1. light
2. dim light
3. dark

0.005 molar urea
4. light
5. dim light
6. dark



1 2 3 4 5 6

Fig. 6. (with cotyledon)

0.008 molar urea	0.01 molar urea
1. light	4. light
2. dim light	5. dim light
3. dark	6. dark

act as the partner of urea in protein synthesis. If the plant is cultured in the light and the photosynthesis occurs so strongly that urea is utilized as soon as it is resorbed by plant root, ammonia, which is produced from urea, can not accumulate in the plant body. It has been also proved that the hydrogen ion concentration in the plant body decreases in accordance with the accumulation of free ammonia from the resorbed urea. Therefore, it may be considered as possible that photosynthesis so acts indirectly as to prevent this decrease in the plant body. In order to ascertain this relation the change of actual and total acidity of the expressed sap was measured, which was obtained from the soy-bean seedlings cultured in urea solutions of various concentrations and under various light intensities. Each three grams of fresh material of shoot and root were ground in a porcelain mortor, and 25 c.c. of redistilled water was added; the thus prepared gruel was left to stand for about 5 minutes, and then filtered using a BUCHNER's funnel. Ten c.c. of each filtrate

of the root sap was titrated with sodium hydroxide (N/100), using phenolphthalein as indicator. The total acidity of the filtrate from the shoot could not be estimated on account of the interference of chlorophyll colour. The hydrogen ion concentration of the expressed sap was determined by the quinhydrone electrode.

Table VI.

Concentration of urea (mol)	Part of plant	Cotyledon	Light		Dim light		Dark	
			Total acidity $\frac{N}{100}$NaOH (cc.) (±0.02)	pH	Total acidity $\frac{N}{100}$NaOH (cc.)	pH	Total acidity $\frac{N}{100}$NaOH (cc.)	pH
Not given	Shoot	With	—	6.4	—	6.2	—	6.3
		Without	—	—	—	—	—	—
	Root	With	1.44	5.9	0.98	6.3	1.05	6.2
		Without	—	—	—	—	—	—
0.005	Shoot	With	—	6.6	—	6.3	—	6.4
		Without	—	6.4	—	6.3	—	6.0
	Root	With	1.39	6.6	0.80	7.0	1.40	6.5
		Without	0.92	6.9	0.80	6.7	0.28	7.2
0.008	Shoot	With	—	6.6	—	6.3	—	6.3
		Without	—	6.5	—	6.6	—	6.7
	Root	With	1.79	6.5	0.80	7.1	0.82	6.8
		Without	1.51	7.0	0.90	7.7	0.56	7.4
0.010	Shoot	With	—	6.6	—	6.4	—	6.3
		Without	—	6.4	—	6.3	—	6.8
	Root	With	1.27	6.9	1.31	6.8	1.26	7.0
		Without	2.00	6.9	0.96	6.9	1.50	7.0

From Table VI it may be seen that the pH-value of the sap obtained from the plant cultured in a urea solution was a little larger than that from the culture without urea, regardless of the light intensity. This difference of the pH-value was seen especially in the root. The total acidity of shoot and root in the illuminated culture was larger than in the case of those in the dim light or in the dark, and it was also larger in the seedlings with cotyledons than in those without them. As already shown in Figs. 3, 4, 5 and 6, the growth of seedlings with cotyledons was better than of those without them, and this relation was more remarkable in the light than in the dark.

Table VII.

Light		In the light										In the dark			
Shoot		With								Without		With		Without	
Concentration of urea (mol)		0.005				0.005		0.005	0.01	0.005	0.01	0.005	0.01	0.005	0.01
Time in tap-water (in hours)		1/2		2		2		48	48	48	48	48	48	48	48
Culture		a	b	a	b	a	b	a	a	a	a	a	a	a	a
pH	initial	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
	final	7.0	7.0	7.0	7.0	7.0	7.0	5.3	5.6	6.8	6.9	6.6	6.3	6.6	6.7
Dry weight of root (gm)		—	—	—	—	—	—	0.594	0.220	0.170	0.208	0.501	0.616	0.202	0.237
Free ammonia in tap-water (mg) ($\pm$0.034)		0	0	0	0	0	0	0	0.034	1.210	3.570	0.714	1.598	2.516	4.012
Number of seedlings		3	3	3	3	3	3	9	8	3	3	9	9	3	3

If the accumulation of ammonia is prevented, its excretion from the root is not to be expected. From this consideration it is not improbable that there is a certain indirect relation also between the photosynthesis and the ammonia excretion which does really occasionally happen. In order to prove this relation the following experiment was carried out.

After the culture in tap-water for 10 days, young seedlings of soy-bean were transferred to the urea culture solution, and cultured for 10 days. Then the seedlings were throughly washed with tap-water and cultured again in tap-water in separate culture vessels. After two days the amount of free ammonia, which was expected to be excreted from the root system into the tap-water, was estimated by the micro-KJELDAHL method. The experiment was conducted under the following conditions:

1. In the dark
   a. with shoot
   b. without shoot
2. In the light
   a. with shoot
   b. without shoot

Duration of the culture in the urea solution: Sep. 1–Sep. 7 (1932). Duration of the culture in tap-water: Sep. 7–Sep. 9. Temp.: 21°–29°C. in the green house. Concentration of urea in the culture solution was 0.005 and 0.01 mol. Each culture vessel contained 400 c.c. of urea culture solution. Table VII shows that free ammonia, which was produced in the plant body as the result of the decomposition of the resorbed urea or resorbed as itself, was excreted again into tap-water. The amount of the excreted ammonia was larger in the dark than in the light, and larger in plants without shoot than in those with it. Such a result clearly indicates that the rôle of photosynthesis must be taken into consideration in the case of studies on the resorption of urea and the utilization of ammonia resorbed by the root system of plant.

Conclusion

From the results of the experiments in the present investigation it must be remembered that the rôle of urease for the utilization of urea as a nitrogen nutrient can be estimated only under the consideration of the culture condition, especially of the supply of carbohydrate. In this connection photosynthesis and nutrient storage in cotyledons

may be considered as important factors. Although urease is regarded as an indispensable factor for the assimilation of urea, its occurrence in a large amount in the plant body is not always favourable for the sake of the plant growth, rather causes the injury. The injury is due to the accumulation of the free ammonia, as in the culture supplied with ammonium salts in a low hydrogen ion concentration. Question, whether the ammonia poisoning is attributed to the decrease of the intracellular actual or total acidity can not be clearly answered now.

LEA (1890), SHIBATA (1904), DOX (1909) and MIWA and YOSHII (1934) are of opinion that the action of urease is always intracellular and this ferment is unable to pass out, so long as the cell is living. MIQUEL (1890), on the other hand, claimed that a powerful urease, quite free from bacteria cell, could be obtained. The present investigation demonstrated that urease is not able to come into water out of the root of the soy-bean during culture. This is important to the growth of soy-bean, which contains much urease, in order to escape unfavourable extreme change of the pH-value of the culture medium with urea.

PIRSCHLE (1929), YAMAGUCHI (1935) and others found that the nutritive value of urea in the field culture depends to a large extent upon the kind of soils. It varies according to the difference of soil nature, species, number and activity of bacteria, especially of urobacteria. Therefore, it may be said that the nutritive value of urea for the higher plants depends upon many outer and inner factors, which are related with each other in a very complicated manner.

Summary

1. Occurrence of a large amount of urease was ascertained in every part of soy-bean seedlings, especially in the cotyledon. But the urease action decreases in the course of the plant growth. Richness of urease in the plant body is not always favourable for the nutrient effect of urea, and sometimes promotes the ammonia poisoning. In this connection the young seedlings are more easily injured than the old ones.

2. The hydrogen ion concentration of cell sap of soy-bean decrease in accordance with the amount of urea in the culture solution.

3. A deficit in carbohydrate in the plant body very rapidly caused ammonia or urea poisoning due to their extreme accumulation. Photosynthesis diminishes the injurious effect in the dark, the actual and total acidity of the expressed sap became weaker than in the light, and free ammonia was excreted from the root. Urease is an indispensable factor for the assimilation of urea and occasionally acts as an injurious factor for the plant life at the same time.

4. By the ordinary water culture it was found that urea is resorbed in unchanged form by the seedlings of soy-bean, and it disappears in the plant body within a short time, owing to the action of urease.

5. The nutrient value of urea for soy-bean seedlings depends upon the concentration of urea, the urease action in the plant body, the degree of photosynthesis and the preservation of cotyledons.

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Literature

ASO, K. and YOSHIDA, R. (1928): 合成尿素の水稻に於るけ肥効に就て. 大日本農會報, 第五六八號

BAMBERGER, M. and LANDSIEDL, A. (1903): Monatshr. f. Chem., **24**. cited from KIESEL 1927.

DOX, A. W. (1909): The intracellular enzymes of lower fungi, especially those of *Penicillium Camemberti*. Journ. Biol. Chem., **6**.

FOSSE, M. R. (1912): Recherches sur l'urée. C. r. Acad. Sci., Paris, **155**.

FOSSE, M. R. (1913a): Formation de l'urée par deux moisissures. C. r. Acad. Sci., Paris, **156**.

FOSSE, M. R. (1913b): Formation de l'urée par les végétaux supérieurs. C. r. Acad. Sci., Paris, **156**.

IWANOFF, N. N. (1923a): Ueber die Umwandlung der Harnstoffs beim Reifen der Früchtkörper von *Lycoperdon*. Biochem. Ztschr., **135**.

IWANOFF, N. N. (1923b): Ueber den Harnstoffgehalt der Pilze. Biochem. Ztschr., **136**.

IWANOFF, N. N. (1923c): Ueber die Bildung der Harnstoffs in Pilzen. Biochem. Ztschr., **136**.

IWANOFF, N. N. (1923d): Ueber die Anhänfung und Bildung des Harnstoffs in *Champignons*. Biochem. Ztschr., **143**.

IWANOFF, N. N. (1924a): Absorption des Harnstoffs durch Pilze. Biochem. Ztschr., **150**.

IWANOFF, N. N. (1924b): Ueber die Ursache des verschiedenen Harnstoffgehalts in Pilzen. Biochem. Ztschr., **154**.

IWANOFF, N. N. (1925): Ueber die Ausscheidung des Harnstoffs bei Pilzen. Biochem. Ztschr., **157.**

KIESEL, A. und TROITGKI. (1922): Beitrag zur Kenntnis der Verbreitung der Urease in Pflanzen. Ztschr. physiol. Chem., **118.**

KIESEL, A. (1927): Der Harnstoff in Haushalt der Pflanzen. u. s. w., Ergeb. d. Biol., **2.** Berlin.

KLEIN, G. und TAUBÖCK, K. (1927): Physiologie des Harnstoffes in der höheren Pflanze. II. Oest. Bot. Ztschr., **76.**

KLEIN, G. und TAUBÖCK, K. (1930): Harnstoff und Ureide bei den höheren Pflanzen. 1. Das Vorkommen von Harnstoff im Pflanzenreich und sein Wandel im Laufe der Vegetationsperiode. Jahrb. wiss. Bot., **73.**

KLEIN, G. und TAUBÖCK, K. (1931a): Harnstoff und Ureide bei den höheren Pflanzen. II. Untersuchungen über das Auftreten und Verschwinden von Harnstoff bei der Pflanze. Jahrb. wiss. Bot., **74.**

KLIEN, G. and TAUBÖCK, K. (1931b): Harnstoff und Ureide bei den höheren Pflanzen. III. Biochem. Ztschr., **241.**

KLEIN, G. and TAUBÖCK, K. (1932a): Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. Biochem. Ztschr., **251.**

KLEIN, G. und TAUBÖCK, K. (1932b): Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. II. Biochem. Ztschr., **255.**

KOSTYTSCHEW, S. (1926): Lehrbuch der Pflanzenphysiologie. Bd. 1. Chemische Physiologie. Berlin.

KULTZSCHER, M. (1932): Die biologische NH_3-Entgiftung in höheren Pflanzen in ihrer abhängigkeit von der Wasserstoffionenkonzentration des Zellsaftes. Planta, **17.**

LEA, S. (1890): Journ. of Physiol. II. cited from TAKEUCHI (1909).

LÖVGREN, S. (1923): Studien über die Urease. II. Biochem. Ztschr., **137.**

MEVIUS, W. (1931): Besprechungen. Zeit. f. Bot., **25.** 541–542.

MIQUEL, P. (1890): Compt. rend. III. cited from TAKEUCHI (1907).

MIWA, T. and YOSHII, S. (1934): Ueber die Bildung der Urease bei *Aspergillus niger.* Sci. Rep. Tokyo Bunrika Daigaku, Sec. B. **1.**

PIRSCHLE, K. (1929): Zur Assimilation des Harnstoffs durch die höhere Pflanze. Biochem. Ztschr., **212.**

PIRSCHLE, K. (1931): Nitrate und Ammonsalze als tickstoffquellen für höhere Pflanzen bei konstanter Wasserstoffionenkonzentration. III. Planta, **14.**

SHIBATA, K. (1904): Ueber das Vorkommen von Amide spaltenden Enzymen bei Pilzen. Hofm. Beitr., **5.**

TAKEUCHI, T. (1907): On the occurrence of urease in higher plants. Journ. Coll. Agric. Imp. Univ. Tokyo, **1.**

TANAKA, I. (1931): Studien über Ernährung der höheren Pflanzen mit den organischen Verbindungen. Japan. Journ. Bot., **5.**

TAUBÖCK, K. (1927): Nachweis und Physiologie des Harnstoffes in der höheren Pflanze. Oest. Bot. Ztschr. **76.**

TRUFFANT, G. et BEZSSONOFF, N. (1924): Sur la form de l'azote le plus favorable anx plantes susperences. C. r. Acad. Sci., Paris, **178.**

WEYLAND, H. (1912): Zur Ernährungsphysiologie mykotrophen Pflanzen. Jahrb. wiss. Bot., **151.**

WESTER, D. H. (1921): II. Vorkommen von Urease in anderen Pflanzenteilen als in Samen. Biochem. Ztschr., **122**.

YAMAGUCHI, S. (1929): On the resorption of urea by plant body and its nutrient value as a nitrogen source. Journ. Sapporo Soc. Agric. Forest., **94** (Japanese).

YAMAGUCHI, S. (1930): Studies on the resorption of urea by root of *Zea Mays* seedlings in sterile culture. Journ. Fac. Sci. Hokkaido Imp. Univ., Series V. **1**.

YAMAGUCHI, S. (1935): Comparative studies on the water, sand, and soil culture of rice plants, with special reference to the N-source and hydrogen ion concentration. Journ. Fac. Sci. Hokkaido Imp. Univ., Series V. **4**.