PHYSIOLOGICAL RÔLE OF ASPARAGINE AND RELATED SUBSTANCES IN NITROGEN METABOLISM OF PLANTS[1]

A. E. MURNEEK

Historical and theoretical retrospect

Asparagine, the amide of amino succinic acid, $COOH \cdot CHNH_2 \cdot CH_2 \cdot CONH_2$, seems to have been found in asparagus over one hundred years ago (**50**) and so named soon thereafter. MOTHES (**14**) credits HARTIG (1858) as having extracted this substance from seedlings and isolated it for the first time in crystalline form. He thought that asparagine was a translocation product of nitrogen in young plant tissues. BOUSSINGAULT (**2**), however, assigned to it a different function by expressing the belief that there is an analogy between asparagine and urea in nitrogen metabolism, both being amides and end products of protein degradation. But urea is excreted from the animal organism, while asparagine is not eliminated from plants. In presence of light, it is reutilized for the synthesis of proteins. These hypotheses of HARTIG and BOUSSINGAULT are still both supported and contended.

After extensive microchemical studies on the presence and absence of asparagine in all parts of a large number of plants, PFEFFER (1872) attempted to unite the two opposing views by concluding that: (1) Protein degradation in plants, because of formation of asparagine, differs from that in animals and from results obtained *in vitro*. (2) Asparagine is the primary, not the end product of protein breakdown and should be considered also a storage form of nitrogen. (3) Judging from its presence and solubility, it is the main translocation product of nitrogenous substances. It moves to growing regions, where it combines with carbohydrates (glucose) to form proteins. (Of interest in this respect is the fact that CHIBNALL (**4**) still seems to agree with PFEFFER that asparagine is the chief form in which nitrogen is translocated in plants).

SCHULZE (**36, 37, 38, 39, 42, 43, 44, 45**) for 30 years studied many phases of nitrogen metabolism in plants, with particular reference to asparagine, by physiological and chemical means. While he was unsuccessful in demonstrating an *in vivo* and *in vitro* parallelism of protein hydrolysis and asparagine accumulation, he did show definitely that under normal conditions asparagine is produced from proteins and not from inorganic nitrogen sources. But through further studies, largely with seedlings of legumes,

[1] Presented before the American Society of Plant Physiologists at the Symposium on Nitrogen Metabolism of Plants, Boston, December 30, 1933.

Contribution from the Department of Horticulture, Missouri Agricultural Experiment Station, Journal Series no. 401.

SCHULZE and later his coworkers (primarily PRIANISCHNIKOV and associates) supplied evidence in support of the hypothesis that asparagine may be formed readily from ammonia and organic acids.

SCHULZE found that during early stages of germination and growth of seedlings, cotyledons contain only primary products of protein hydrolysis, the amino acids (leucine, tyrosine, etc.). During later development of the plant, amino acids disappear from the cotyledons and asparagine accumulates in large amounts in the shoots. Therefore asparagine does not seem to be produced directly from proteins,[2] but results from breaking down of the amino acids.

Through his own extensive investigations and those of many coworkers, PRIANISCHNIKOV (**20–31**) seems to have established the analogy between asparagine and urea in nitrogen metabolism. Moreover, he has demonstrated that asparagine may be synthesized directly in seedlings from substances within the plant and an external supply of ammonia or carbohydrates or both.

As a result of the preceding and many other studies, the following theoretical considerations seem to have become popular (**27, 6, 14,**):

> When, in comparison with carbohydrates, there is an excess of ammonia, or proteins are not required by the plant because of shortage of carbohydrates, then through hydrolysis of proteins by means of proteolytic enzymes amino acids are formed. Some of these (especially mono-amino acids) are oxidized and amino (NH_2) groups are released. Asparagine, which contains two NH_2 groups, is formed from two molecules of amino acids. One of these is oxidized to aspartic acid, the other much further with splitting off of ammonia. A union of aspartic acid with ammonia forms ammonium aspartate, from which, through dehydration, asparagine is produced in the same manner as in the animal organism urea is formed from carbamate of ammonia. When a plant is supplied with abundance of ammonia, asparagine may be formed from one molecule of an amino acid or even from an organic acid (malic, succinic, etc.). If there is need for nitrogen, asparagine will be broken down, NH_3 released, and the rest of the molecule may be oxidized to CO_2. Asparagine, therefore, has a dual function: removal of the injurious NH_3, and storage of N.
>
> In some plants, or under some conditions in most plants, not only asparagine but *glutamine,* the amide of amino-glutaric acid (**41**), *argi-*

[2] There is the possibility, of course, that asparagine may exist as such in the protein molecule, as is suggested by SCHULZE, OSBORN, and BUTKEWITSCH. In acid hydrolysis of proteins the amide group is saponified, giving aspartic or glutamic acids; but when proteins are acted upon by proteolytic enzymes, the amides appear unaltered. In many instances, however, the quantity obtained from seedlings is entirely too large to be accounted for on this basis.

nine, guanidine-amino-valerianic acid (**39, 40**), *allantoin*[3] or allantoic acid (**1**), *urea,* $CO(NH_2)_2$(10), and possibly other substances may serve as receptors of NH_3. Organic acids (malic, succinic, oxalic) likewise may take care of ammonia by forming ammonium salts, which seem to be a characteristic feature of very acid plants.

When plants have an ample supply of carbohydrates (sugars) the process is reversed. Ammonia is released from any of the receptors and is used in the synthesis of amino acids, proteins, and other complex nitrogenous products.

Experimental evidence

No attempt, of course, is made here to present all the important evidence bearing on the subject under discussion. Only some of the typical results of investigations on certain parts of the major phases of the problem will be recounted. Those desiring to secure more complete information will naturally turn to the extensive literature in this field.

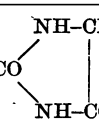
1. Nitrogen metabolism of seedlings

Changes in nitrogenous substances in the germinating seeds and seedlings have been thoroughly investigated by Schulze, Prianischnikov, and others. This has involved a study of the breakdown of proteins in the cotyledons and regeneration of nitrogen compounds in the developing stem and leaves. A conspicuous feature, often emphasized in these studies, is the abundance of asparagine in the seedlings of legumes, especially when grown in darkness. It is evident from Schulze's and other work (**4**) that with increased development of the plant there is a decrease in protein concentration and a concomitant increase in asparagine and mono-amino acids (table I). But since the amino acid fraction decreases when the seedlings

TABLE I

Nitrogen metabolism in legume seedlings (Schulze)

Age of seedlings in days	Protein	Asparagine	Other N (mostly mono-amino acids)
	%	%	%
6	5.49	1.16	1.72
12	1.71	4.02	2.39
24	1.78	5.09	1.40

[3] Allantoin,  , a diuride of glyoxalic acid.

are of considerable age, while at the same time there is a proportional increase in asparagine and no further decrease in proteins, it appears that asparagine may have come from the amino acid fraction. These plants, of course, had no external supply of nitrogen.

Similar results have been obtained not only with several legumes but also with other seedlings by many workers. Asparagine accumulates likewise in onion bulbs, asparagus roots, and similar plant structures when they are sprouted in darkness (**54, 18, 32**).

The converse of this process takes place when, for instance, seedlings are grown in light and proteins are synthesized in presence of sufficient amount of carbohydrates, or in case of development of bulbs, whose sugar content is usually high (**54, 56**). In general, the higher the amount of soluble carbohydrates in the seeds, bulbs, or roots, the less protein will be broken down owing to respiration, when growth is resumed, and there will be relatively little fluctuation in asparagine concentration.

Most oil-containing seeds produce upon germination glutamine (**41**) instead of asparagine, but the general nitrogen transformations seem to be the same. In some plant seedlings other substances than acid amides appear to play a similar rôle, as will be noted further on.

2. Nitrogen metabolism in leaves

Several investigators have demonstrated that asparagine and its homologue, glutamine, perform the same function in nitrogen metabolism of leaves as in developing seedlings. In young rapidly growing leaves, both amides may be used in synthesis of proteins (**44**). In fully developed leaves proteins are formed during the day and may be broken down and removed in soluble forms of N to other parts of the plant. Chibnall (**4, 5**), for example, has demonstrated that asparagine disappears from the leaves of the bean plant at night and that it comes largely from hydrolysis of proteins. In fruit-bearing plants nitrogen may be translocated in this form to the fruit or seed pods whence eventually it will go into the seeds (**44, 45, 53**).

Alterations in nitrogen content of starved or senescent leaves supply additional evidence of the rôle of acid amides in plant metabolism. The results secured by Miyachi are presented here (table II). They show that when leaves are starved there is a rapid breakdown of proteins and a striking increase in asparagine and other amides. Similarly, Vickery (**52**) has found that during the curing of tobacco leaves, with increasing loss of amino-N there is a more or less corresponding gain in amide-N (table III). He thinks that such change may be due to the oxidation of amino acids, the formation of ammonia, and subsequent synthesis of amides. As a result of these changes, ammonia was maintained at a relatively low level.

TABLE II

AMIDE PRODUCTION IN STARVED LEAVES OF PEONY (MIYACHI)

NITROGEN	FRESH LEAVES	LEAVES STARVED 15 DAYS
	%	%
Total	1.364	1.462
Protein	1.312	0.801
Asparagine	0.037	0.206
Other amides	0.015	0.455
	PERCENTAGE OF TOTAL N	
Protein	96.19	54.79
Asparagine	2.71	14.09
Other amides	1.10	31.12

The formation of amides, therefore, should be considered as a "defensive mechanism" for the detoxication of NH_3, which comes into operation when there is a rapid destruction of proteins and accumulation of NH_3, or when there is an excessive reduction of NO_3 to NH_3.

Probably the most extensive and complete studies of nitrogen changes in leaves have been conducted by MOTHES (**14, 15**). After investigating a series of plants that had been subjected to various environments and experimental treatments, he draws the following conclusion: When leaves are exposed to light or are fed with glucose in darkness, no amides are formed and even the original amount disappears by being used in synthesis of proteins. But when the carbohydrate content of the leaves is reduced to a low point, as a result of long exposure to darkness, then amides are produced promptly and eventually ammonia appears. Oxygen seems to be a

TABLE III

CHANGES IN AMINO ACIDS AND AMIDES IN CURING TOBACCO LEAVES (VICKERY)

TIME	AMINO–N LOSS	AMIDE–N GAIN
hr.	*gm.*	*gm.*
41	0.020	− 0.005
64	0.015	− 0.017
87	0.370	0.019
111	0.007	0.018
159	0.110	0.278
183	0.395	0.318
207	0.290	0.449
231	0.515	0.546
279	0.820	0.835
303	0.290	0.446

limiting factor in amide formation. Without its presence, neither amides nor ammonia will be produced even in leaves of a low carbohydrate content, but there will be an increase of amino, basic, and other ("rest-N") nitrogen fractions.

Oxidation of the products of hydrolysis of proteins takes place in leaves when there is a shortage of carbohydrates, the final end product being ammonia. In presence of some carbohydrates, ammonia is "neutralized" by being used for the formation of asparagine, in which form NH_3 is "stored." Whether produced within the plant or supplied from an external source, asparagine will remain as such unless carbohydrates become available, when it enters promptly into synthesis of proteins. So, too, when ammonium salts are fed to leaves high in carbohydrates, proteins will be formed quickly; when carbohydrates are short, asparagine will be produced; and when carbohydrates are absent, ammonia will accumulate in the cells until poisoning of the leaves occurs. In this respect, ammonia supplied from without behaves physiologically the same as NH_3 released within the plant. When leaves with ample carbohydrate supply are exposed to narcotics, no amides and often no ammonia will be produced.

These conclusions are in striking agreement with the results of other investigators, as the succeeding discussion will show. MOTHES seems to have verified with mature plants most of the evidence obtained with seedlings.

3. SOME FACTORS AFFECTING ASPARAGINE FORMATION

While the procedure and mechanism of accumulation and disappearance of asparagine and related substances in plant metabolism are not yet understood in all their details, we know that several factors seem to affect them. Some of these will be discussed briefly.

a. IMPORTANCE OF OXIDATION.—Proteins may be decomposed in plants with or without the presence of oxygen, the usual hydrolysis products being the amino acids (**19**). In the presence of oxygen, however, asparagine (or glutamine) often appears as the main soluble N substance. With further oxidation it is broken down to NH_3. That oxidation is really essential for the production of amides and ammonia has been demonstrated, among others, by SUSUKI (**49**) with barley (table IV) and soy beans, and by BUTKEWITSCH (**3**) with lupine (table V). SUSUKI found not only an increase in asparagine when seedlings were developed in darkness in presence of oxygen, but also a marked decrease of amino acids. This has been corroborated by WASSILIEFF (**53**). Without a supply of oxygen, amides are not formed and amino acids accumulate. With further oxidation and absence of carbohydrates, ammonia is produced in large quantities owing to the breakdown (oxidation) of asparagine (table V). SURE and TOTTING-

TABLE IV

EFFECT OF O_2 ON ASPARAGINE FORMATION IN BARLEY SEEDLINGS (SUSUKI)

	DRY MATTER	PER 100 SEEDLINGS
	%	*gm.*
At beginning of experiment	6.59	0.1427
After 45 hours in darkness with O_2	8.85	0.1818
After 45 hours in darkness without O_2	7.16	0.1336

TABLE V

EFFECT OF O_2 ON FORMATION OF AMMONIA AND AMIDE-N IN GERMINATING SEEDS OF LUPINE (BUTKEWITSCH)

NITROGEN	PER 100 SEEDLINGS	
	WITHOUT O_2	WITH O_2
	mg.	*mg.*
Ammonia	19.38	170.60
Amide	171.36	139.48
Ammonia and amide	190.74	310.08

HAM (**47**) have shown also that in the shoots of pea seedlings there is a simultaneous decrease of α-amino acids and ammonia, which indicates that "α-amino acids serve for amide production in the nitrogen metabolism of the etiolated pea plant."

b. EFFECTS OF ANAESTHETICS.—Since the time when CLAUDE BERNARD (1878) demonstrated that anaesthetics inhibit anabolism but permit catabolism, various experiments have been performed to show their effects on N metabolism in plants. BUTKEWITSCH (**3**), for example, exposed seedlings to fumes of toluol with the result that no asparagine was formed but instead large amounts of NH_3 accumulated, up to 14 per cent. of total N. This suggests that asparagine arises not as a result of tearing down but through a building up or synthetic process.

c. EFFECTS OF PRESENCE OF CARBOHYDRATES.—It has already been noted that with extreme carbohydrate deficiency the carbon structure of the asparagine molecule is broken down (oxidized) and NH_3 accumulates. Synthesis of asparagine may be effected by means of an external supply of glucose, as table VI shows. An artificial supply of sugar will not only diminish the accumulation of NH_3 but will result in a marked increase of amides (asparagine) (**3, 46**), which eventually will lead to the formation of proteins (**49, 46**). Several investigators have found that asparagine disappears when seedlings, grown in darkness, are supplied with sugar

TABLE VI

EFFECT OF GLUCOSE ON AMMONIA AND AMIDE-N CONCENTRATION IN SEEDLINGS OF LUPINE (BUTKEWITSCH)

NITROGEN	WITHOUT GLUCOSE	WITH GLUCOSE
	%	%
Ammonia	18.57	9.37
Amide	15.17	23.10

(**48, 46**). Contrariwise, an abundance of sugar prevents asparagine formation from proteins, but under the right conditions it does not interfere with and may even stimulate the synthesis of this amide.

One may conclude with PRIANISCHNIKOV (**26**), therefore, that with carbohydrate shortage, proteins will be hydrolyzed and there will be formed amino acids of the general formula $R(NH_2)COOH$, which, through oxidation and secondary synthesis, produce acid amides of the general formula $R(NH_2)CONH_2$ (asparagine and glutamine), and, in case of carbohydrate starvation, give, through further oxidation, NH_3. But when the carbohydrate supply is abundant, whatever its source, then the reverse process takes place. From NH_3 to acid amides ($R(NH_2)CONH_2$), which, with additional supply of carbohydrates, give rise to amino acids ($R(NH_2)$-$COOH$), and these in turn synthesize proteins.

The frequently observed results of formation of asparagine in light and not in darkness are due not to the direct effect of light but to the presence of carbohydrates in light, as was demonstrated by PRIANISCHNIKOV (**28**), who gives the schematic summary shown in table VII.

d. SYNTHESIS OF ASPARAGINE FROM AMMONIUM SALTS.—One of the crucial experiments in the determination whether asparagine originates directly from breakdown of proteins or comes from the synthesis of NH_3 with other oxidation products of proteins, is through feeding plants in various states of carbohydrate deficiency with ammonium salts.

TABLE VII

CARBOHYDRATE AND LIGHT RELATIONSHIPS IN THE FORMATION OF ASPARAGINE AND AMMONIA (PRIANISCHNIKOV)

EXPERIMENTAL CONDITIONS		RESULTS	
CARBOHYDRATES	LIGHT	ASPARAGINE SYNTHESIS	AMMONIA INJURY
+	−	+	−
−	−	−	+
+	+	+	−
−	+	−	+

Ever since SUSUKI (**48**), HANSTEEN (**7**), and ZALESKI (**55**) demonstrated that proteins may be formed from various nitrogenous substances supplied to the plants, much work has been done with the object of determining to what extent and under what circumstances asparagine is synthesized from N constituents of nutrient solutions. Thus PRIANISCHNIKOV (**24, 25, 26, 29**) and others (**48, 46**) have shown that by supplying salts of nitrogen to certain seedlings their asparagine content is markedly increased. For this purpose ammonia is a better source of N than the nitrates, when the physiological acidity of ammonium salts is neutralized by a base ($CaCO_3$, etc.) (**48, 25**). The legumes in particular seem to require Ca to neutralize the acid reaction of NH_4Cl and $(NH_4)_2SO_4$, which appear to inhibit the formation of asparagine (table VIII). Barley and corn seedlings, high

TABLE VIII

RESULTS OF FEEDING VETCH SEEDLINGS WITH AMMONIUM SALTS WITH AND WITHOUT CALCIUM (PRIANISCHNIKOV)

AMOUNT N IN 100 SEEDLINGS	DISTILLED H_2O	NH_4Cl	$NH_4Cl + CaCO_3$
	mg.	*mg.*	*mg.*
Protein N	85	109	90
Asparagine N	75.9	73.9	118.2
Ammonia N	0.9	0.9	1.0

in carbohydrates, will absorb NH_3 from these salts equally well either with or without the presence of calcium. But when carbohydrates are exhausted (darkness) in corn or barley seedlings, they will behave like legumes. And legumes can be put, by various methods, into a physiological state similar to that of cereal seedlings (table IX.)

TABLE IX

RELATION OF NITROGEN TO CARBOHYDRATES IN VARIOUS TYPES OF SEEDLINGS (PRIANISCHNIKOV)

GRAMINEAE	STARCH CONTAINING LEGUMES	LUPINE
1: 6	1: 2.0 – 2.5	1: 0.6

The importance of Ca ions in this respect is not clearly understood, for this ion increases even the utilization of N from nitrates (**25**). Very likely calcium has something to do with the carbohydrate metabolism. It of course neutralizes to some extent the acidity of the nutrient medium, since cations (NH_4) are absorbed faster than anions. PRIANISCHNIKOV thinks that Ca ions increase respiration and hydrolysis of proteins.

Lupine seedlings with very small carbohydrate reserves are unable to assimilate N from either NH_4Cl or $(NH_4)_2SO_4$, even in presence of Ca salts. But when exposed to light or fed glucose, they will utilize N under the above conditions. Similarly barley seedlings, after prolonged growth in darkness and loss of all starch reserves, are incapable of assimilating nitrogen. When in this state they behave like lupine seedlings. Carbohydrates and the essential internal environmental factors, therefore, are necessary for the synthesis of asparagine from inorganic nitrogen.

e. SYNTHESIS OF ASPARAGINE FROM NH_3 AND ORGANIC ACIDS.—Because of the similarity in chemical structure between some of the organic acids and acid amides, it has long been suspected that they may be interrelated. Moreover, evidence has accumulated in support of the view that organic acids are produced when proteins are broken down (**12, 34, 35**), although very likely they may be formed also from sugars (**14**). Malic and succinic acids seem to be the two that, in presence of ammonia, participate readily in the synthesis of asparagine. By feeding corn seedlings ammonium sulphate and ammonium salts of organic acids, SMIRNOV (**46**) was able to demonstrate that both malic and succinic acids enter into the synthesis of asparagine (table X), and that, in presence of glucose, eventually proteins accumulate (table XI).

TABLE X

RESULTS OF FEEDING CORN SEEDLINGS AMMONIA WITH AND WITHOUT ORGANIC ACIDS (SMIRNOV)

FORM OF AMMONIA	PERCENTAGE OF TOTAL N			
	PROTEIN N	AMMONIA N	ASPARAGINE N	AMINO ACID N
	%	%	%	%
$(NH_4)_2$ SO_4	66.62	4.19	13.34	15.85
Ammonium malate	58.42	3.87	19.30	18.41
Ammonium succinate	63.57	3.33	16.16	16.94

TABLE XI

PROTEIN SYNTHESIS IN CORN FROM ASPARAGINE IN PRESENCE OF GLUCOSE (SMIRNOV)

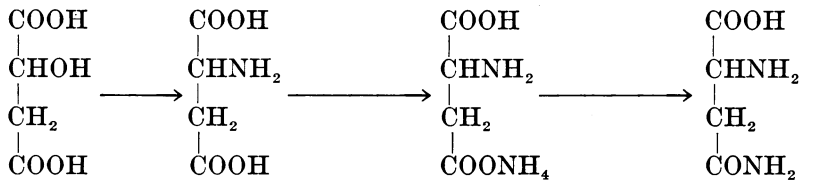
SOURCE OF AMMONIA	PERCENTAGE OF TOTAL N		
	PROTEIN N	AMMONIA N	ASPARAGINE N
	%	%	%
$(NH_4)_2SO_4$	66.79	4.4	15.10
Ammonium succinate	67.75	4.2	19.52
Ammonium aspartate	70.25	4.5	17.6

Many investigators (PRIANISCHNIKOV etc.) conceive the path of formation of asparagine from malic acid through aspartic acid and ammonium aspartate (table XII).

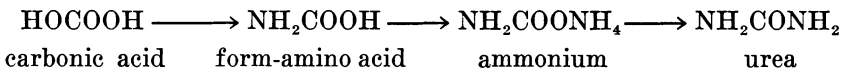
TABLE XII

ANALOGY BETWEEN ASPARAGINE AND UREA

Probable formation of asparagine in plants:



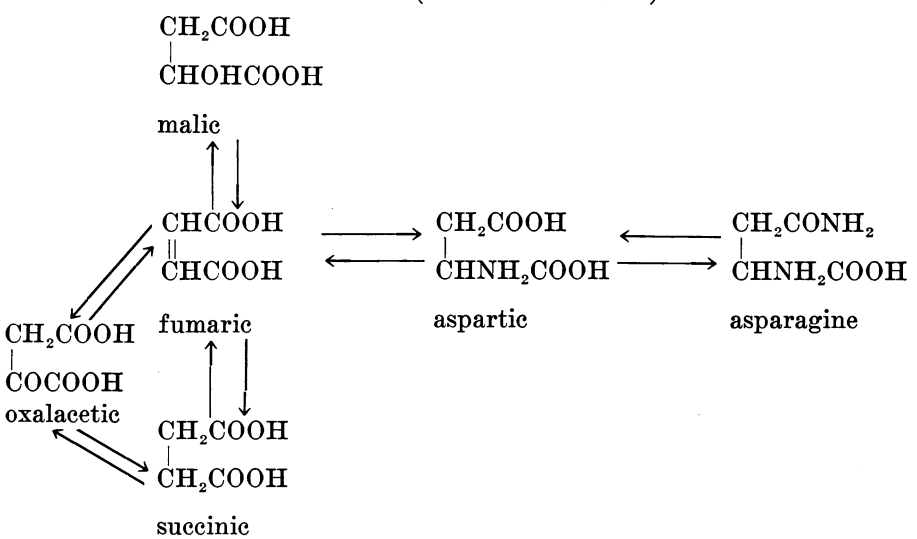
In animals:



Based on ROBINSON'S (**33**) ideas, VICKERY and PUCHER (**52**) present an additional plan, which shows the connection between asparagine and α-keto and hydroxy acids (table XIII). They state that "this purely hypothetical scheme involves, however, only reactions of known biological significance."

TABLE XIII

ROBINSON'S SCHEME SHOWING CONNECTION BETWEEN ASPARAGINE AND α-KETO AND HYDROXY ACIDS (VICKERY AND PUCHER)



Another more direct proof that ammonium salts of organic acids, although participating in the synthesis of proteins, are not so easily assimilated as asparagine, comes from experiments of feeding plants with these substances. NAKAMURA (**16, 17**), for instance, found that both phanerogams and fungi utilize more readily asparagine than ammonium succinate from a weak nutrient solution. By feeding asparagine to leaves of high carbohydrate content, PRIANISCHNIKOV and SMIRNOV were able to demonstrate protein synthesis. When carbohydrates were absent, asparagine remained intact in the leaves. This has been corroborated by NAKAMURA with barley seedlings. Hence there seems to be considerable proof in support of the above path of utilization of asparagine in protein formation.

f. ORGANIC ACIDS AS DIRECT RECEPTORS OF AMMONIA.—Recently RUHLAND and WETZEL (**34, 35**) have shown that in some highly acid plants (*Oxalis, Begonia, Rheum*) oxalic acid takes care of ammonia by forming ammonium oxalate and possibly ammonium salts of other acids. Amides are not present in any appreciable quantities in these plants, excepting in the older leaves, which are less acid. In *Rheum* both amides and organic ammonium salts may serve as receptors of NH_3 in various organs of the same plant, depending on their relative acidity. They conclude, therefore, that amides are formed in less acid plants (or tissues) and organic salts of ammonia in more acid ones.

There is much information on record showing a connection between organic acids and protein transformation. Malic, tartaric, citric, and other acids may come either from amino acids or from intermediary products of protein disintegration. But a strongly oxidized acid, like oxalic, can be derived from sugars as well as proteins.

Continuing RUHLAND and WETZEL's investigations, KULTZSCHER (**13**) found that very acid plants ($pH<5.00$) are able to store large quantities of excess nitrogen in the form of ammonium salts of organic acids. Their function is not merely a case of neutralization of NH_3 but also of storage of N. In deamination of amino acids not only NH_3 is released but also acids are formed, which then more or less automatically take care of each other by forming ammonium salts.

According to KULTZSCHER, it would seem that an equilibrium exists between amides and ammonia. In plants relatively high in actual (pH) and potential acidity, the equilibrium is shifted to the NH_3 side and ammonium salts are formed through the union of NH_3 with organic acids. The fact is emphasized by KULTZSCHER that highly acid plants are characterized by an active deamination process.

Ammonium salts of oxalic and other organic acids will take care of proportionally larger quantities of NH_3 than asparagine does, but urea of

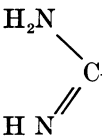
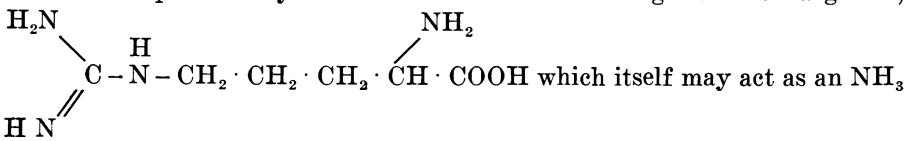
still more, and guanidine,  , most of all the compounds so far found in plants. The ratios of carbon to nitrogen in various substances that may act as receptors of nitrogen are presented in table XIV.

TABLE XIV

RATIO OF CARBON TO NITROGEN IN VARIOUS ORGANIC SUBSTANCES WHICH MAY ACT AS NITROGEN RECEPTORS IN PLANTS

	C : N
Glutamine	2.5 : 1
Asparagine	2 : 1
Ammonium malate or tartrate or succinate or citrate	2 : 1
Arginine	1.5 : 1
Ammonium oxalate	1 : 1
Allantoin	1 : 1
Urea	1 : 2
Guanidine	1 : 3

g. FORMATION OF UREA AND OTHER MEANS OF REMOVING NH_3.—Many fungi are capable of forming and even absorbing from without large quantities of urea, $CO(NH_2)_2$, but as a rule do not excrete it. According to IWANOV (**8**) some fungi (*Lycoperdon, Bovista*) may accumulate half of the total N as urea (up to 11 per cent. of dry weight). Fungi will absorb urea and thiourea from weak solutions of these substances and store it up to 15 per cent. of their dry weight (**9**).

Urea in plants may come also from the breaking down of arginine,  which itself may act as an NH_3 receptor. Ammonia is split off easily from arginine by the enzyme arginase (**11**). Arginine is supposed to have the same function in conifers as asparagine in other plants.

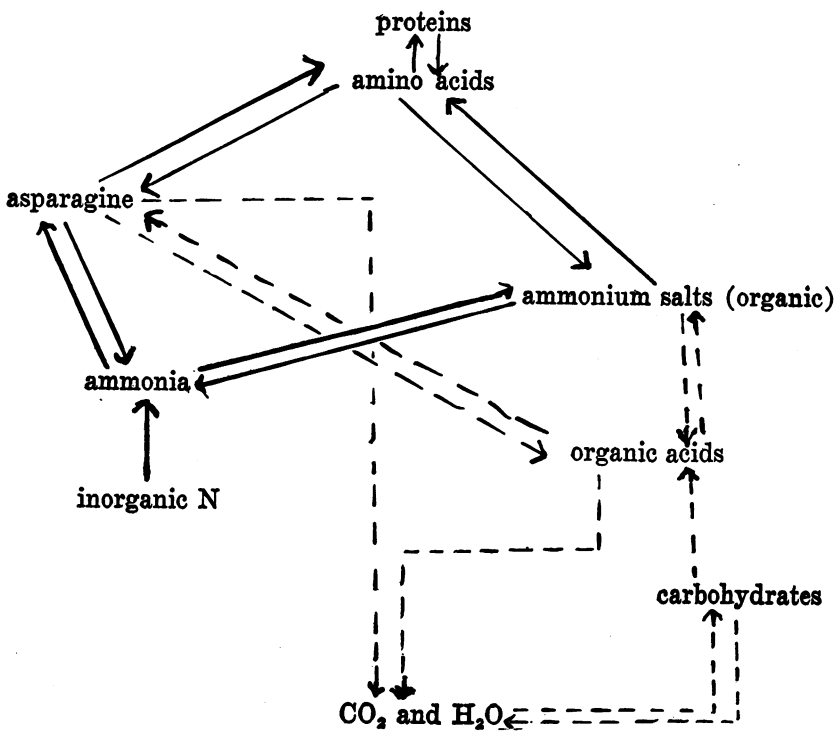
Whatever its source, urea seems to accumulate in some plants in absence of carbohydrates (**10**), and since it is used up in certain stages of the development of the organism, it should be considered as a storage form of N, and therefore analogous to asparagine and glutamine in its physiological function. Moreover, urea may be changed to asparagine. According to SUSUKI (**48**) and PRIANISCHNIKOV (**25**), it is frequently a better nutrient form of N than ammonium salts for the formation of asparagine.

From the preceding discussion it is apparent that there are at the disposal of plants various means of removing and neutralizing NH_3. The mechanism set into operation in each instance may possibly depend upon several factors, of which the most important seems to be the carbohydrate content of the plant as a whole or of specific organs, the hydrogen ion concentration and the source (meaning the rest of the molecule) from which NH_3 comes. Doubtless many environmental factors also play a rôle in this respect. Still one must be mindful of the fact that under extreme conditions of carbohydrate depletion, nitrogen may be excreted from the plant through the roots in the form of ammonia (**30, 31**).

A generalized scheme of nitrogen metabolism in plants, modified after ENGEL (**6**), is presented in table XV.

TABLE XV

SCHEME OF N METABOLISM IN HIGHER PLANTS (MODIFIED AFTER ENGEL)



4. ANALOGY BETWEEN ASPARAGINE IN PLANTS AND UREA IN PLANTS AND ANIMALS

Both amides, asparagine and urea, do not seem to be the direct products of protein hydrolysis but arise from secondary synthesis with NH_3 as the

key ion, which most often comes from oxidation of amino acids. Both amides may be synthesized in plants when NH_3 is introduced into the organism, with this difference between the two, that for synthesis of asparagine a part of the unoxidized carbohydrate molecule is necessary while for formation of urea NH_3 and CO_2 are sufficient (table XII). The physiological function of both processes appears to be the neutralization and storage of NH_3, which seems to be toxic to living organisms.

The analogy between plants and animals in this respect is that neither of them can synthesize the respective amides from ammonium salts of strong acids, but more neutral salts of ammonia will lead to such synthesis.

There is a great difference, however, in respect to the further metabolic rôle of the two amides. Urea is excreted from animals. They do not need to be so economical with N, for its intake through feeds is more or less assured and there is no caloric value in CO_2, the carbon part of the molecule. Asparagine, on the other hand, remains in the cells of plants as a reserve substance of N, which, with renewed supply of carbohydrates, can be used again for the production of amino acids and other components of proteins.

COLLEGE OF AGRICULTURE
UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI

LITERATURE CITED

1. BONNET, R. L'évolution de l'azote au cours de la germination. Bull. Soc. Chem. Biol. **11**: 1025–1061. 1929.
2. BOUSSINGAULT, M. De la végétation dans l'obscurité. Compt. Rend. Acad. Sci. Paris **58**: 917–922. 1864.
3. BUTKEWITSCH, W. Das Ammoniak als Umwandlungsprodukt stickstoffhaltiger Stoffe in höheren Pflanzen. Biochem. Zeitschr. **16**: 411–452. 1909.
4. CHIBNALL, A. C. Investigations in the nitrogenous metabolism of the higher plants. II. The distribution of nitrogen in the leaves of the runner bean. Biochem. Jour. **16**: 344–362. 1922.
5. ————. VI. The rôle of asparagine in the metabolism of the mature plant. Biochem. Jour. **18**: 395–404. 1924.
6. ENGEL, H. Beiträge zur Kenntnis des Sticksoffumsatzes grüner Pflanzen. Planta **7**: 133–164. 1929.
7. HANSTEEN, B. Ueber Eiweisssynthese in grünen Phanerogamen. Jahrb. wiss. Bot. **33**: 417–486. 1899.
8. IWANOV, N. N. Über den Harnstoffgehalt der Pilze. Biochem. Zeitschr. **136**: 1–19. 1923.
9. ————. Absorption des Harnstoffs durch Pilze. Biochem. Zeitschr. **150**: 115–122. 1924.

10. ————————. Der Harnstoff der Pilze und dessen Bedeutung. Zeitschr. physiol. Chem. **170**: 274–288. 1927.
11. KLEIN, G., and TAUBÖCK, K. Argininstoffwechsel und Harnstoffgenese bei höheren Pflanzen. Biochem. Zeitschr. **255**: 278–286. 1932.
12. KOSTYTCHEV, S., and FREY, L. Über Alcoholgärung. XI. Über die bei der Hefegärung in Gegenwart von Calciumcarbonat entstehenden Säuren. Zeitschr. physiol. Chem. **146**: 276–285. 1925.
13. KULTZSCHER, M. Die biologische NH_3-Entgiftung in höheren Pflanzen in ihrer Abhängigkeit von der Wasserstoffionen-Konzentration des Zellsaftes. Planta **17**: 699–757. 1932.
14. MOTHES, K. Ein Beitrag zur Kenntnis des N-Stoffwechsels höherer Pflanzen. Planta **1**: 472–552. 1926.
15. ————————. Zur Kenntnis des N-Stoffwechsels höherer Pflanzen. 3. Beitrag. Planta **12**: 686–731. 1931.
16. NAKAMURA, T. On the relative value of asparagine as a nutrient of phanerogams. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 465–467. 1897.
17. ————————. On the relative value of asparagine as a nutrient for fungi. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 468–470. 1897.
18. NIGHTINGALE, G. T., and SCHERMERHORN, L. G. Nitrate assimilation by asparagus in the absence of light. New Jersey Agr. Exp. Sta. Bull. 476. 1928.
19. PALLADIN, W. Ueber Zersetzungsproducte der Eiweisstoffe in den Pflanzen bei Abwesenheit von freiem Sauerstoff. Ber. deutsch. bot. Ges. **6**: 296–304. 1888.
20. PRIANISCHNIKOV, D. Zur Kenntnis der Keimungsvorgänge bei *Vicia sativa*. Landw. Vers.-Sta. **45**: 247–288. 1895.
21. ————————. Eiweisszerfall und Eiweissrückbildung in den Pflanzen. Ber. deutsch. bot. Ges. **17**: 151–155. 1899.
22. ————————. Die Rückbildung der Eiweisstoffe aus deren Zerfallsprodukten. Landw. Vers.-Sta. **52**: 347–381. 1899.
23. ————————. Zur Frage der Asparaginbildung. Ber. deutsch. bot. Ges. **22**: 35–43. 1904.
24. ————————. La synthèse des corps amidés aux dépens de l'ammoniaque absorbée par les racines. Rev. gén. Bot. **25**: 5–13. 1913.
25. ————————. Das Ammoniak als Anfangs- und Endprodukt des Stickstoffumsatzes in den Pflanzen. Landw. Vers.-Sta. **99**: 267–286. 1922.
26. ————————. Über den Aufbau and Abbau des Asparagins in den Pflanzen. Ber. deutsch. bot. Ges. **40**: 242–248. 1922.

27. ————. Asparagin and Harnstoff. Biochem. Zeitschr. **150**: 407–423. 1924.
28. ————. Sur le rôle de l'asparagine dans les transformations des matières azotées chez les plantes. Rev. gén. Bot. **36**: 108–122; 159–181. 1924.
29. ————, and SCHULOV, J. Über die synthetische Asparaginbildung in den Pflanzen. Ber. deutsch. bot. Ges. **28**: 253–264. 1910.
30. ————, and IWANOV, V. Absorption and excretion of ammonia by roots. Compt. Rend. Acad. Sci. U.S.S.R. **1929**: 327–331. (In Russian).
31. ————, and ————. Formation of ammonia during the reduction of nitrates in higher plants. Compt. Rend. Acad. Sci. U.S.S.R. **1931**: 205–209. (In Russian).
32. RAHN, H. Untersuchungen über den N-Stoffwechsel pflanzlicher vegetativer Speicherorgane. Planta **18**: 1–51. 1932.
33. ROBINSON, MURIEL E. The protein metabolism of the green plant. New Phytol. **28**: 117–149. 1929.
34. RUHLAND, W., and WETZEL, K. Zur Physiologie der organischen Säuren in grünen Pflanzen. I. *Begonia semperflorens*. Planta **1**: 558–564. 1926.
35. ————, and ————. Zur Physiologie der organischen Säuren in grünen Pflanzen. III. *Rheum hybridum* Hort. Planta **3**: 765–769. 1927.
36. SCHULZE, E. Ueber Zersetzung und Neubildung von Eiweissstoffen in Lupinenkeimlingen. Landw. Jahrb. **7**: 411–444. 1878.
37. ————. Ueber den Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. **9**: 689–748. 1880.
38. ————. Ueber die Bildungsweise des Asparagins und über die Beziehungen der stickstoff freien Stoffe zum Eiweissumsatz in Pflanzenorganismus. Landw. Jahrb. **17**: 683–711. 1888.
39. ————. Über das Vorkommen von Arginin in den Wurzeln und Knollen einiger Pflanzen. Landw. Vers.-Sta. **46**: 451–458. 1896.
40. ————. Ueber die beim Umsatz der Proteïnstoffe in den Keimpflanzen einiger Coniferen-Arten enstehenden Stickstoffverbindungen. Zeitschr. physiol. Chem. **22**: 435–448. 1896.
41. ————. Uber die Verbreitung des Glutamins in den Pflanzen. Landw. Vers.-Sta. **48**: 33–55. 1897.
42. ————. Ueber den Umsatz der Eiweissstoffe in der lebenden Pflanze. Zeitschr. physiol. Chem. **24**: 18–114. 1898.

43. ————————. Über den Abbau und den Aufbau organischer Stickstoffverbindungen in den Pflanzen. Landw. Jahrb. **35**: 621–666. 1906.
44. ————————. Studien über die Proteinbildung in reifenden Pflanzen. Zeitschr. physiol. Chem. **71**: 31–48. 1911.
45. ————————, and WINTERSTEIN, E. Studien über die Proteinbildung in reifenden Pflanzensamen. Zeitschr. physiol. Chem. **65**: 431–476. 1910.
46. SMIRNOV, A. I. Über die Synthese der Säureamide in den Pflanzen bei Ernährung mit Ammoniaksalzen. Biochem. Zeitschr. **137**: 1–34. 1923.
47. SURE, B., and TOTTINGHAM, W. E. The relation of amide nitrogen to the nitrogen metabolism of the pea plant. Jour. Biol. Chem. **26**: 535–548. 1916.
48. SUSUKI, U. On the formation of asparagine in plants under different conditions. Japanese Imp. Univ. Coll. Agr. Bull. **2**: 409–457. 1897.
49. ————————. On the formation of asparagine in the metabolism of shoots. Japanese Imp. Univ. Coll. Agr. Bull. **4**: 351–356. 1902.
50. VICKERY, H. B. Some nitrogenous constituents of the juice of the alfalfa plant. VI. Asparagine and amino acids in alfalfa. Jour. Biol. Chem. **65**: 657–664. 1925.
51. ————————, *et al.* Chemical investigations of the tobacco plant. Carnegie Inst. Publ. no. 445. 1933.
52. ————————, and PUCHER, G. W. The chemical changes that occur during the curing of Connecticut shade-grown tobacco. Connecticut Agr. Exp. Sta. Bull. 324. 1931.
53. WASSILIEFF, N. Eiweissbildung in reifenden Samen. Ber. deutsch. bot. Ges. **26a**: 454–467. 1908.
54. ZALESKI, W. Zur Keimung der Zwiebel von *Allium cepa* und Eiweissbildung. Ber. deutsch. bot. Ges. **16**: 146–151. 1898.
55. ————————. Zur Aetherwirkung auf dien Stoffumwandlung in den Pflanzen. Ber. deutsch. bot. Ges. **18**: 292–296. 1900.
56. ————————, and SHATKIN, W. Untersuchungen über den Eiweissaufbau in den Pflanzen. I. Über den Eiweissaufbau in den Zwiebeln von *Allium cepa*. Biochem. Zeitschr. **55**: 72–78. 1913.