THE DIRECT ASSIMILATION OF INORGANIC AND ORGANIC FORMS OF NITROGEN BY HIGHER PLANTS.

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In the earliest experiments on the assimilation of combined nitrogen the substances employed were, in addition to nitrates and ammonium salts, such organic compounds as occur in animal excrementitious matters and refuse, or are produced in their decomposition—urea, hippuric acid, trimethylamine, creatine, etc. Later on, when it became evident that any nitrogenous compound applied to the soil will, sooner or later, under ordinary conditions, be converted into nitrates, and so become available for plants, whether originally so or not, the question still retained its interest although from a different point of view. If it can be shown that from certain types of nitrogen compounds plants can directly obtain all the nitrogen they require, and that of such types some are more favourable than others, the results cannot fail to throw some light on the synthetical processes in plants. It must however be borne in mind that the substances supplied may undergo changes within the roots, or at the surface of the roots, and not reach the assimilating organs in their original forms.

In this connexion we would call attention to an important paper recently published by T. Takeuchi[1] who showed the presence of urease in several seeds. In experiments with urea and several other compounds it was found that the powdered seeds readily convert urea into ammonia and that biuret is slightly attacked, whilst the following compounds gave negative results: guanidine, arginine, benzamide, allantoin, leucine, alanine, tyrosine, creatine, histidine, guanine, glycine, uric and hippuric acids. The enzyme was found to be present in *Glycine hispida*, *Phaseolus vulgaris*, oats, paddy rice and buckwheat, but not in barley, upland rice, rye, maize, rape and radish.

[1] On the Occurrence of Urease in Higher Plants (*J. Coll. Agric. Tokio*, 1909, **1.** 1-14).

Further experiments in this direction seem very desirable as it is conceivable that the ability of a plant to utilise an organic nitrogen compound may depend on the presence of an enzyme capable of converting it into ammonia or perhaps some other less complex form.

With regard to the utilisation of nitrates by plants the various theories which have from time to time been suggested all assume the initial process to be a reduction. Gautier, in 1872, suggested that the nitric acid is first reduced by formaldehyde, and that hydrocyanic acid is the final product and the first form in which nitrogen is actually assimilated by combining with some carbohydrate. Treub carried the suggestion a step further and supposes that the hydrocyanic acid derived from nitrates reacts with formaldehyde and ammonia with production of amino-nitriles which yield amino-acids by hydrolysis

$$HCN + H.CHO + NH_3 = NH_2.CH_2.CN + H_2O.$$

According to Bach, nitric acid is first reduced by formaldehyde to hydroxylamine, which with a further amount of formaldehyde gives formaldoxime. The latter, by intramolecular migration, is converted into formamide.

$$\begin{array}{l} H \\ | \\ CHO + NH_2.OH \end{array} = \begin{array}{l} H \\ | \\ C:N.OH + H_2O \\ | \\ H \end{array}$$

and

$$\begin{array}{l} H \\ | \\ C:N.OH \\ | \\ H \end{array} = \begin{array}{l} H \\ | \\ CO.NH_2 \end{array}$$

Baudisch[1] has recently shown that dilute solutions of potassium nitrate are converted into nitrite by exposure to diffused daylight, and that nitrites are reduced by methyl alcohol, in diffused light, to formhydroxamate

$$KNO_2 + CH_3.OH = OH.CH:N.OK + H_2O.$$

Formaldehyde and potassium hyponitrite are also formed. Baudisch also found that both nitrates and nitrites are readily reduced by aldehydes in presence of light with production first of hydroxamic acids, and then ammonia and amines. The reduction is also brought about

[1] *Ber. deut. chem. Ges.* 1911, **44.** 1009.

under the influence of light by laevulose (with evolution of carbon monoxide) and other carbohydrates, and by phenols and naphthols.

Without going further into this question[1] it may be remarked that the above theories collectively involve the assumption that the following compounds are available to plants as sources of nitrogen: hydrocyanic acid, amino-nitriles, hydroxylamine, formaldoxime and formamide.

The more difficult question regarding the initial process in the fixation of elementary nitrogen in the root-nodules of leguminous and other plants and by independent organisms has received very little attention up to the present time; and the only evidence we have of nitrogen being brought into combination by purely chemical means at the ordinary temperature is that furnished by Loew's experiment[2] with platinum black and alkali in which it was shown that ammonium nitrite is produced.

According to Winogradsky the most probable explanation is that ammonia is formed by the action of nascent hydrogen. Whilst Gautier and Drouin suggest that the nitrogen is oxidised either to nitric or nitrous acid, Heinze[3] thinks it probable that nitrogen is at once brought into combination with a hydrocarbon and suggests that a salt of carbamic acid may be first formed, or that carbamic acid may be produced from cyanamide.

With regard to nitrogenous constituents of root-nodules[4] it may be mentioned that Stoklasa detected asparagine and that Sana[5] found *l*-asparagine and glycine in bean-nodules.

Before describing the experiments on the assimilation of the nitrogen of the various substances employed it will be desirable to show in some detail what has already been done, attention being confined chiefly to the more recent results obtained under sterilised conditions.

Inorganic Compounds[6].

Experiments by V. Meyer and E. Schulze (27) and more recently by Lutz (49) showed that hydroxylamine is not assimilated by maize and barley. Suzuki (55) found that barley failed when supplied with

[1] Compare H. Franzen, *Sitzungsber. Heidelberger Akad. Wiss.* 1910.

[2] *Ber. deut. chem. Ges.* 1890, **23**. 1447.

[3] *Landw. Jahrb.* 1906, **35**. 907.

[4] This *Journ.* 1909, **3**. 179.

[5] *Abstr. J. Chem. Soc.* 1910, ii. 993.

[6] For results obtained with ammonium salts see this *Journ.* 1909, **3**. 179; and E. Pantanelli and G. Severini: "Alcune esperienze sulla nutrizione azotata delle piante verdi con diversi sali d' ammonio" (*Staz. sper. agrar. Ital.* 1910, **43**. 449—544).

nitrogen in the form of sodium hydroxylamine disulphonate. Loew's (32) experiments with hydrazine sulphate and azoimide also gave negative results. Maeno (42) showed that amidosulphonic acid is injurious to barley and other plants.

In experiments with water plants W. Knop (5) obtained indications that nitrous oxide is assimilated. Still better results were obtained by employing a mixture of nitrous oxide and ethylene.

Birner and Lucanus (13), in 1866, showed that 0·25 per cent. potassium nitrite solutions are inimical to the life of oat plants, whilst Molisch (31) found that even 0·01 per cent. solutions may be injurious. When, however, bean plants were supplied with a 0·002 per cent. solution of potassium nitrite, it was found that the whole of the nitrite was taken up, and assimilated in from 5 to 20 hours, both the solutions and the plants failing to show any nitrite reaction. M. Schultz (57) showed that whilst solutions containing 0·5 per cent. of potassium nitrite are poisonous to plants more dilute solutions are equal to nitrates as a source of nitrogen; and that when light is deficient nitrites are more readily assimilated than nitrates. The injurious action of the stronger solutions of nitrites was found to vary with different plants, *Leguminosae* being less able to withstand their action than *Gramineae.*

Perciabosco and Rosso (72) have shown that maize will grow in sterilised 0·1 per cent. solutions of potassium nitrite, and that the produce contained a very distinctly higher percentage of nitrogen than plants grown with nitrate.

Mazé (74) in water culture experiments with maize supplied with potassium nitrite found that growth was considerably retarded at first. The final growth was however quite normal and the amount of dry matter produced equal to that obtained with nitrate.

Organic Compounds.

A large number of experiments were made by Lutz (48) in 1898 and since on the assimilation of various organic compounds containing nitrogen. The method employed was to grow various plants in sterilised sand, over dishes containing mercuric chloride covered by large bell jars. It was found that the sand remained uninfected throughout the experiments. As regards amines Lutz obtained the following results.

Methylamine was assimilated by *Cucurbita max.* and propylamine by *Cucumis prophet.*; maize and *Ipomaea purpurea* showed very slight gains of nitrogen with propylamine. Amylamine showed a gain of

6·6 mg. nitrogen with *Cucurbita* and dimethylamine a gain of 3·8 mg. with the same plant. Slight gains were obtained with butylamine and trimethylamine, whilst tetramethyl- and tetraethylammonium chlorides gave negative results: more recent experiments by Molliard (71) in which radishes were grown in ignited pumice with methyl-, ethyl-, propyl-, dimethyl- and trimethylamine chlorides gave negative results.

As regards cyanides the only recent experiments seem to be those of Suzuki (54) who showed that plants are destroyed in solutions containing as little as 0·001 per cent. of potassium ferrocyanide, and of Rana Bahadur (59) who found that sodium nitroprusside is not assimilated. Acetamide, which seems to be the only acid amide which has been employed, is assimilated according to Bente (23). Choline, in experiments by H. T. Brown (63) with excised barley embryos, was found to be assimilated; and Schreiner *et al.* showed that small amounts of choline are beneficial to wheat seedlings. Schreiner (66) found that neurine acts as a stimulant in solutions containing only 6 parts per million and that solutions containing 25 parts per million are very injurious.

Piperidine. Lutz found that *Cucumis melo* failed to assimilate nitrogen in this form.

Glycine was employed in the early experiments of Hampe, Knop and Wolf, and Wagner (21). Schreiner found that in solutions containing 0·1 per cent. or less it is beneficial to wheat seedlings. Hansteen (47) showed that *Lemna*, in absence of light, produces proteins from glycine and glucose, but not with sucrose.

Lutz obtained negative results with *Cnicus benedictus* and *Cucumis melo* supplied with glycollamide as source of nitrogen.

Betaine also gave negative results in experiments by Lutz with maize and *Ipomaea purpurea*. H. T. Brown, on the other hand, found that excised barley embryos gained 50 per cent. of nitrogen (as in the experiment with choline) with betaine; and Schreiner showed that solutions containing 5 per million were beneficial to wheat.

Alanine. Hansteen (47) obtained negative results in experiments with *Lemna*, in which the plants, kept in darkness, were supplied with alanine and glucose (or sucrose). Schreiner, Reed and Skinner found that 0·05 per cent. of alanine is toxic to wheat seedlings, whilst smaller amounts proved to be beneficial. In a series of experiments made in 1896 Prianischnikoff and Lebedeff (44) found that leucine is not assimilated by barley, and similar indications were obtained by Lutz, in 1899, with *Ipomaea purpurea*. Subsequent experiments by Lutz (61) with *Cucumis vulgaris* showed, however, gains of 0·636 and 0·723 mg. of nitrogen corresponding with about 38 per cent., and 6·5 and 3·9 mg. of dry matter. The seeds germinated normally and the growth was vigorous. Hansteen found that *Lemna* grown in absence of light failed to produce protein from leucine and glucose (or sucrose); and, more recently, Brown showed that whilst excised barley embryos gained a slight amount of nitrogen when supplied with leucine as source of nitrogen, there was no gain, but rather a loss of dry matter. Schreiner, on the other hand, found that solutions containing 0·05 per cent. of leucine, or less, were uniformly beneficial to wheat.

The first experiment with urea made under sterilised conditions seems to be that of Prianischnikoff and Lebedeff (44) who obtained negative results. In 1897, Suzuki, who made a number of experiments with yellow lupins, potatoes, *Halesia hispida*, wheat and barley, found that, with the exception of barley, all the plants produced more asparagine from urea than from ammonium salts; and that, unlike nitrates, urea gives rise to the production of asparagine in etiolated shoots. Thomson (50) also showed that the nitrogen of urea is directly assimilated by oats and that the plants contained 50 per cent. more protein nitrogen, although only slightly more total nitrogen, than plants supplied with nitrates. Sawa (53) found that solutions containing more than 0·05 per cent. of urea are injurious. Hansteen's experiments with *Lemna* showed that protein is readily produced from urea in presence of glucose or sucrose.

Kawakita (58) showed that 0·464 gram of biuret per litre killed young barley plants in a few days.

Thiosinamine. The only experiment with this substance seems to be one by Knop and Wolf who found that it is toxic.

Kawakita (58) found that solutions containing 0·5 gram of guanidine in 250 c.c. killed young barley plants in three days and that solutions four times as dilute destroyed the plants in two weeks. Schreiner showed that even 1 per million of guanidine carbonate is highly injurious. Creatine was employed in 1869 by Wagner (21) who found that it is readily assimilated, and recent experiments by Skinner (75) show that both creatine and creatinine are assimilated by wheat. Hansteen showed that *Lemna,* in absence of light, is unable to produce protein from creatine and glucose or sucrose.

Dicyanodiamide is, according to Loew (67), assimilated by *Elodea* from 0·2 per cent. solutions. Perotti (68) obtained similar indications with cereals in water culture containing 0·5 gram of dicyanodiamide per litre, and in sand cultures; Aso (70) who grew buckwheat and oats in solutions containing 0·01 per cent. of dicyanodiamide also found that it is assimilated.

Dicyanogen. Loew (67) showed that barley plants are killed by solutions containing 0·01 per cent. of dicyanogen.

Aspartic acid. Prianischnikoff and Lebedeff (44) obtained negative results, whilst Brown (63) showed that it is assimilated by excised barley embryos. According to Schreiner, Reed and Skinner, wheat plants are killed by 0·05 per cent. of aspartic acid whilst 0·01 per cent. solutions are not toxic.

Baessler (26) showed in 1886 that asparagine is readily assimilated by maize provided that the roots do not remain too long in the solutions. Otherwise they are liable to injury due, it is supposed, to the action of decomposition products. Prianischnikoff and Lebedeff failed to obtain assimilation with oats in sterilised solutions. On the other hand, Nakamura (43), using 1 per cent. solutions of asparagine, found that barley produced considerably more growth than with ammonium succinate. The more recent experiments of Brown showed that excised barley embryos produced as much dry matter with asparagine as with nitrate whilst the percentage of nitrogen in the dry matter was much higher. According to Hansteen (47), *Lemna* grown in darkness is able to produce protein from asparagine in presence of glucose but not with sucrose. In experiments with wheat seedlings Schreiner found that 0·1 per cent. solutions of asparagine were beneficial.

Glutamic acid. The only experiment with glutamic acid seems to be one by H. T. Brown who included it in the series already referred to. The results with barley showed that the effect of glutamic acid was practically the same as with aspartic acid, asparagine, and nitrate, except that the percentage of nitrogen in the dry matter was rather lower than with nitrate.

Glutamine. Hansteen found that glutamine, in presence of glucose, is converted into proteins by beans growing in absence of light, but not when sucrose is employed. The result accords with that obtained with asparagine, whilst with glycine the presence of sucrose, and not glucose, seems to be essential.

Allantoin was found by Brown to give almost identical results with choline as source of nitrogen for excised barley embryos.

Alloxan. Schreiner grew wheat seedlings in solutions of alloxan and found that whilst solutions containing 1—25 mg. per litre slightly stimulated growth, concentrations of 100 mg. per litre were toxic.

Uric acid was employed by S. W. Johnson (15) and by Hampe.

The more recent experiments by Thomson showed that it is assimilated by oats, but that its nutritive value is far less than that of urea.

Guanine. Saturated solutions of guanine only contain 40 mg. per litre, and Schreiner found that solutions of that strength slightly promote the growth of wheat.

Xanthine is also very sparingly soluble. Solutions containing 1—25 mg. per litre were found by Schreiner to be beneficial to wheat seedlings.

Caffein. Lutz showed that maize, *Helianthus annuus* and *Cucurbita maxim.* do not assimilate caffein hydrochloride and that maize, in solutions of the free base, failed altogether. Sawa, employing 0·1 and 0·25 per cent. solutions of caffein, also obtained negative results with celery.

Several experiments were made by Lutz with aniline, diphenylamine and benzylamine, but only negative results were obtained; and picric acid in an early experiment by Knop and Wolf also failed to give any result.

Hippuric acid. Numerous experiments, commencing with those of S. W. Johnson (15) and Hampe (18), have been made with hippuric acid. Of the more recent results those of Prianischnikoff and Lebedeff (44) failed to show assimilation, whilst Thomson obtained some growth with oats, but far less than with urea and nitrate.

Nitrobenzoic acid was employed by Knop and Wolf (11) in 1865 who obtained negative results.

Aminobenzoic acid. Knop and Wolf failed to obtain assimilation.

Phenylalanine. H. T. Brown (63) found that excised barley embryos supplied with phenylalanine as source of nitrogen only gained 10 per cent. dry matter.

Tyrosine. Lutz (61) grew *Cucumis melo* in solutions containing tyrosine and failed to obtain any increase of nitrogen. In later experiments, with *Cucumis vulgaris* he obtained normal germination and vigorous growth; in one experiment there was a gain of 0·211 mg. of nitrogen, or 11·9 per cent., and in a second experiment a gain of 19·5 per cent. H. T. Brown found that excised barley embryos failed to assimilate nitrogen in the form of tyrosine, whilst Schreiner (66) showed that solutions containing 0·01 per cent., are very injurious to wheat seedlings and that even 0·001 per cent. solutions are toxic.

The remaining experiments with organic compounds have all given negative results. Lutz attempted to grow maize and *Cucurbita max.* with naphthylamine hydrochloride and with pyridine, whilst Sawa found that antipyrine is toxic to celery in 0·1 per cent. solutions. Scatole, pyridine, picoline, piperidine and quinoline were all found by Schreiner to be very toxic. The only experiments with alkaloids under sterilised conditions are those of Lutz who failed to obtain growth with atropine, cocaine, quinine and morphine.

Humus.

In 1842 de Saussure (3) published some results of experiments on the assimilation of potassium humate by beans and *Polygonum persicaria*. The plants grew satisfactorily producing healthy roots, and reduced the amount of humus by from 6 to 43 mg. in a week or two; the solutions became considerably lighter in colour. From these results and the fact that the roots remained colourless, de Saussure drew the conclusion that humus is not only absorbed but assimilated. And he attributed the failure of similar experiments by Harting to the roots not having enough space to develop.

Wiegmann and Polstorff (4) soon afterwards (1845) grew *Mentha undulata* and *Polygonum persicaria* for a month in a humus solution. The plants grew well and one solution lost 16 mg. of humus. Some of the same solution exposed to air during the same period, without vegetation, lost 12 mg.

In Grandeau's (22) experiments with wheat and barley supplied with a neutral solution of ammonium humate, the solution became colourless owing to the precipitation and not to the assimilation of the humus. Grandeau supposes that the humus is dialysed by the roots, the organic matter remaining outside and the mineral matter being absorbed. As regards nitrogen it is probable that the ammonia of the ammonium humate was sufficient for the needs of the plants; and the ammonia being removed the free humic acid would of course be precipitated.

Bréal (37), in 1894, made experiments with *Poa annua*. A tuft of the grass was deprived of its roots and placed in water until new roots were formed. The tuft was then divided into two parts, one of which was supplied with a solution of potassium humate, whilst the roots of the other part were cut off and placed in a second portion of the same solution. In two or three days the humate solution was completely decolourised by the grass, whilst the solution containing the roots which had been cut off remained unchanged. In another experiment, similar to the ones made by Sachs to demonstrate the action of roots on marble, etc., it was found that the roots of *Poa* acted in like manner on a paper filter covered with insoluble humic acid.

Wiley showed that oats grown in peat soil contained about 25 per cent. more nitrogen than when grown in ordinary arable soil, and that the excess of nitrogen was mainly in the form of amides and not proteins. The results indicate that a portion of the nitrogenous matter of peat is directly assimilated and that the nitrogen thus taken up is in the form of amides.

SUMMARY OF RESULTS.

The results of all the experiments above described may be shortly summarised as follows.

Nitrites have been shown by Molisch, M. Schultz, and by Perciabosco and Rosso to be assimilated by various plants from sufficiently dilute solutions. Perciabosco and Rosso found that plants supplied with nitrogen in this form contain higher percentages of nitrogen than with nitrates as source of nitrogen. This is also the case, as was already shown, when plants take up all, or most, of their nitrogen as ammonium salts[1].

TABLE I. *Assimilation of the nitrogen of organic compounds.*

			Dry matter		Nitrogen		
			Actual (gram.)	Gain (gram.)	In dry matter per cent.	Total (mgm.)	Gain (mgm.)
Methylamine ...	Lutz (48)	Cucurbita max.	0·223	0·086	7·26	16·2	5·9
Propylamine ...	,,	Cucumis prophet.	0·169	0·016	7·42	12·5	1·6
Amylamine ...	,,	Cucurbita max.	0·195	0·058	8·65	16·9	6·6
Dimethylamine ...	,,	,,	0·210	0·073	6·70	14·1	3·8
Diethylamine ...	,,	,,	0·202	0·065	7·57	15·3	5·0
Trimethylamine...	,,	Maize	0·162	0·028	3·40	5·5	1·0
Choline	Brown (63)	Barley	0·152	0·017	2·88	4·4	1·4
Betaine	,,	,,	0·135	—	3·34	4·5	1·5
Urea	Thomson (50)	Oats	2·227	—	4·63	103·0	—
,, ...	,,	Barley	1·810	—	5·21	94·0	—
,, ...	Frank	Lupins	10·071	—	1·11	111·4	102·3
Aspartic acid ...	Brown	Barley	0·153	0·012	3·61	5·53	2·5
Asparagine ...	Prianischnikoff (44)	,,	0·710	—	—	—	—
,, ...	Brown	,,	0·155	0·020	5·22	8·1	5·1
Glutamic acid ...	,,	,,	0·156	0·021	3·75	5·9	2·9
Allantoin ...	,,	,,	0·143	0·008	3·13	4·5	1·5
Uric acid... ...	Thomson (50)	Oats	1·177	—	3·67	4·1	—
,, ...	,,	Barley	1·691	—	3·37	5·7	—
Hippuric acid ...	,,	Oats	0·472	—	2·90	—	—
,, ,, ...	,,	Barley	0·317	—	2·86	—	—

[1] This *Journ.* 1909, **3**, 191.

Experiments with other inorganic forms of nitrogen—amidosulphonic acid, hydroxylamine, hydrazine and azoimide—have all given negative results up to the present time.

As regards organic compounds the majority have given negative if not uncertain results. The results in which at least 1 milligram of nitrogen was assimilated are set out in Table I, p. 291.

EXPERIMENTAL.

The methods employed in the following experiments were the same as described in the previous paper on the assimilation of ammonium salts[1]. The seeds were sterilised by means of a mercuric chloride solution under reduced pressure, and the plants (peas) were grown in Woulffe's bottles containing the following mineral substances in about 1 litre of water.

$CaSO_4$	... ...	0·5 gram.
$MgSO_4, 7H_2O$	...	0·5 „
KCl	... ...	0·25 „
NaCl	... ...	0·25 „
KH_2PO_4	... ...	0·5 „
Fe_2Cl_6	... ...	Trace

The nitrogenous compound was added in such quantity as to supply about 80 mg. of nitrogen.

Series I. *Peas in Water Cultures with various forms of Nitrogen.* The following compounds were employed:

1. Ammonium sulphate.
2. Ethyl nitrate.
3. Tetranitromethane.
4. Acetamide.
5. Propionitrile.
6. Alanine.
7. Urea.
8. Guanidine hydrochloride.
9. Sodium aspartate.
10. Barbituric acid.
11. Hippuric acid.
12. Peptone.

The seedlings were placed in the solutions in October 1908. A satisfactory beginning in either one or both bottles was obtained with ammonium sulphate, acetamide, alanine, urea, guanidine, sodium aspartate, hippuric acid and peptone, whilst ethyl nitrate proved

[1] This *Journal*, 1909, **3**. 179.

unsatisfactory, and the plants supplied with tetranitromethane and with propionitrile failed altogether.

Owing, however, to the late time of the year the plants made comparatively little growth and by January all of them died off. In April 1909 the whole experiment was repeated with fresh solutions, when necessary, the only difference being that calcium carbonate was added to the solutions containing tetranitromethane.

Of the different substances employed urea gave the best results. With ammonium sulphate the amount of growth was a good deal less than in the previous series in which calcium carbonate was added. Acetamide gave more growth than ammonium sulphate, whilst with guanidine, peptone and sodium aspartate the growth was somewhat less, and with alanine distinctly less. With barbituric acid, propionitrile, ethyl nitrate and tetranitromethane the plants failed although they seemed to have taken up, if not assimilated, small amounts of nitrogen in most cases.

At the conclusion of the experiments all the solutions were found to be free from nitrates, and the whole of the nitrogen remaining in the ammonium sulphate bottles was found to be unchanged. The guanidine bottles gave indications of infection and this experiment was repeated in the next series.

Samples of the culture solutions were carried over to Omelianski's medium for the detection of nitrifying organisms and to nutrient- and urea-gelatin to test for the presence of organisms related to putrefactive and urea bacteria. All the solutions were shown to be free from nitrifying bacteria, while the occurrence of other organisms is indicated in Table II.

On the whole there were no great differences in the amounts of growth in the plants which remained alive except in the case of one of the urea plants which was distinctly better than any of the others, not only as regards leaf and stem, the roots being in every respect more normal (see photos, Plate V). The roots in the ammonium sulphate solution had about the same length as those of the urea plant but were less bushy.

Those of the sodium aspartate plant were very short, but very thick; whilst the acetamide plant had roots of great length, about 18 inches. The peptone plant was altogether small and produced very little root. As peptone has been shown to occur in various seedlings[1],

[1] Mack, W. R., "Über das Vorkommen von Pepton in Pflanzensamen," *Inaug.-Diss.*, Leipzig, 1903.

it might be expected to be available as a source of nitrogen and the results in Table II indicate that a small amount has in fact been utilised by the pea plant. It seems possible, however, that whilst peptone and certain other nitrogen compounds may be taken up and to some extent utilised by plants, they may be unable to furnish the whole of the nitrogen required, or, at any rate, to supply the plant with sufficient rapidity.

TABLE II. *Peas growing in Water Cultures,* 1909.

		Nitrogen in Solutions		Dry produce	Nitrogen		
		at commencement	at conclusion		in dry subst.	total in dry subst.	gain or loss[1]
		mgm.	mgm.	gm.	p.c.	mgm.	mgm.
Ammonium sulphate	a	80	68	0·368	5·250	19·3	8·9
	b	80	65·5	0·451	5·075	22·9	12·5
Ethyl nitrate	a	80	—	—	—	14·6	4·2
	b	80	—	—	—	12·3	1·9
Tetranitromethane + $CaCO_3$	a	80	—	—	—	14·8	4·4
	b	80	—	—	—	13·5	3·1
Acetamide	a	78	61	0·696	3·701	25·8	15·4
	b	78	—	—	—	12·3	1·9
Propionitrile	a	80	—	—	—	14·5	4·1
	b	80	—	—	—	11·3	0·9
a-Aminopropionic acid	a	79	74	0·269	5·777	15·5	5·1
	b	79	—	—	—	7·8	-2·6
Urea	a	80	58	0·533	5·358	28·6	18·2
	b[2]	80	43	0·758	5·818	44·1[2]	33·7
Guanidine hydrochloride	a	75·5	63	0·301	5·930	17·9	7·5
	b[2]	75·5	64	0·425	4·447	18·9[2]	8·5
Sodium aspartate	a[2]	81	71	0·367	5·188	19·0	8·6
	b[2]	81	73·5	0·264	6·841	18·1[2]	7·7
Barbituric acid	a	80	—	—	—	16·3	5·9
	b	80	—	—	—	9·7	-0·7
Hippuric Acid	a	80	—	—	—	8·9	-1·5
	b	80	78	0·299	3·629	10·9	0·5
Peptone	a	98	—	—	—	8·7	-1·7
	b	98	94	0·329	4·553	15·0	4·6

Series II. *Peas in Water Cultures with Organic Nitrogen.* These experiments were in all respects similar to the last series.

The substances employed were:

[1] Deducting 10·4 mg. which was the average amount present in the seed. The variations in the individual seeds amounted to 4 mg.

[2] Culture infected.

1. Trimethylamine hydrochloride.
2. Methyl carbamate.
3. Guanidine hydrochloride.
4. Cyanuric acid and $CaCO_3$.
5. Oxamide.
6. Humus.

The humus solution was prepared from garden soil and was neutralised as nearly as was possible without precipitating. The seedlings were placed in the solutions by July 10, 1909. The plants all made a good start with the exceptions of those supplied with methyl carbamate, one of which failed altogether. The humus plants, which finally gave the best results, were distinctly behind the other plants during the first two or three weeks.

The guanidine plants were the first to lose their colour, but by August 17 most of the plants had dried up except those in humus solution and those supplied with cyanuric acid and trimethylamine, and the latter had very little green remaining.

On taking out the plants the following root measurements were made (in cm.).

	a	*b*	av.
1.	25	27	26
2.	6	11	8·5
3.	19	22	20·5
4.	33	29	31
5.	37	24	30·5
6.	29	25	27

The bacteriological examination of the solutions showed that the conditions of sterilisation had been maintained to the end.

The results set out in Table III show that the peas failed to utilise trimethylamine and methyl carbamate. Both these compounds are of interest as possible sources of nitrogen. Trimethylamine is known to occur in a small number of plants, and as a constituent of such substances as choline, betaine, caffeine and the lecithins etc. is very widely distributed; whilst as a constituent, or decomposition product, of fish manure it must frequently come into contact with the roots of plants. As regards carbamic acid, it has been suggested by Heinze[1] that this may be one of the first compounds produced when free nitrogen is brought into combination by nitrogen-fixing organisms. It is possible, of course, that the methyl salt employed is an unfavourable form in which to supply the compound for consumption by plants.

[1] *Landw. Jahrb.* 1906, **35**. 907.

There is, however, no choice since the metallic salts are too unstable to undergo the process of sterilisation.

TABLE III. *Peas grown in Water Cultures*, 1909.

		Nitrogen in Solutions		Dry produce	Nitrogen		
		at commencement	at conclusion		in dry subst.	total in dry subst.	gain or loss[1]
		mgm.	mgm.	gm.	p.c.	mgm.	mgm.
Trimethylamine hydrochloride...	a	81·4	73·7	0·273	3·97	10·9	0·5
	b	81·4	74·0	0·190	3·42	6·5	−3·9
Methyl carbamate	a	60	65·7	0·109	4·04	4·4	−6·0
	b	60	65·2	0·205	3·28	6·7	−3·7
Guanidine hydrochloride	a	75·3	74·1	0·232	4·50	10·4	0
	b	75·3	70·0	0·321	4·08	13·1	2·7
Cyanuric acid + $CaCO_3$	a	84·9	82·4	0·318	3·68	11·7	1·3
	b	84·9	85·0	0·161	3·17	5·1	−5·3
Oxamide	a	52·4	46·4	0·312	4·49	14·0	3·6
	b	52·4	51·2	0·242	3·62	8·8	−1·6
Humus	a	—	54·9	0·577	2·83	16·3	5·9
	b	—	54·6	0·449	3·32	15·0	4·6

Guanidine hydrochloride was assimilated to a slight extent by one plant. Previous experiments had always given negative results with guanidine probably owing to the strongly alkaline base having been employed, or to too great concentration of the solutions. Oxamide was also assimilated in one case; whilst humus gave much the best results of any in this series.

Series III. *Peas in Water Cultures with different forms of Nitrogen, chiefly organic.* The culture solutions contained in addition to the usual mineral nutrients, as previously described, the following compounds as sources of nitrogen:

1 *a* and *b*. No Nitrogen.
2 *a* and *b*. Formamide.
3 *a* and *b*. Glycine.
4 *a* and *b*. Hexamethylenetetramine.
5 *a* and *b*. Hydroxylamine hydrochloride.
6 *a* and *b*. *para*-Urazine.
7 *a* and *b*. Alloxan.
8 *a* and *b*. Barbituric acid and Calcium carbonate.

There were also two bottles, 9*a* and 9*b*, containing garden soil heated at 120° for two hours.

[1] See footnote on p. 294.

Sterilised pea seedlings were placed in the first six pairs of bottles in July 1910, and in the last three pairs in the following September.

TABLE IV. *Peas grown in Water Cultures*, 1910.

			Dry matter		Nitrogen		
			Total	Gain ±0·025	In dry substance	Total	Gain or loss[1]
			gm.	gm.	p.c.	mgm.	mgm.
1	Without Nitrogen	a	0·210	−0·065	4·72	9·9	−0·5
		b	0·200	−0·075	5·38	10·35	—
2	Formamide	a	0·320	0·045	4·42	14·1	3·7
		b	0·410	0·135	4·60	18·9	8·5
3	Glycine	a	0·350	0·075	4·62	16·2	5·8
		b	0·150	−0·125	5·96	8·9	−1·5
4	Hexamethylenetetramine	a	0·170	−0·105	5·38	9·2	−1·2
		b	0·180	−0·095	7·30	13·1	2·7
5	Hydroxylamine hydrochloride	a	0·190	−0·085	4·88	9·3	−1·1
		b	0·210	−0·065	5·59	11·7	1·3
6	*para*-Urazine	a	0·252	−0·023	5·61	14·1	3·7
		b	0·166	−0·109	5·46	9·1	−1·3
7	Alloxan	a[2]	0·318	0·043	4·60	14·6	4·2
		b	0·467	0·192	3·73	17·4	7·0
8	Barbituric acid and calc. carb.	a[2]	0·278	0·003	5·69	15·8	8·4
		b	0·698	0·423	3·92	27·4	17·0
9	Garden soil	a[2]	0·367	0·092	4·48	16·5	6·1
		b[2]	0·475	0·200	4·68	22·2	11·8

The plants made a good start on the whole, but with the exception of those supplied with alloxan and barbituric acid and the ones growing in garden soil, there was not very much final growth. The results (see Table IV) show however that formamide and glycine were undoubtedly assimilated, although less readily than alloxan and barbituric acid, whilst the results obtained with hydroxylamine, hexamethylenetetramine and *para*-urazine are doubtful, as although one plant in each case contained more nitrogen than the original seed (which might be due to absorption without assimilation), there was a decided loss of dry matter.

The garden soil experiment was inconclusive as it was found that the soil in both bottles had become infected and contained considerable amounts of ammonia. It is perhaps worthy of note that, in the case of plants supplied with barbituric acid and alloxan, a slightly greater assimilation occurred where the sterile conditions were maintained throughout the experiment, than where infection occurred.

[1] See footnote on p. 294. [2] Infected.

Summary and Conclusions.

The various compounds may be divided into the following groups according to their availability, or otherwise, as direct sources of nitrogen for peas:

I. Readily assimilated:
- Ammonium salts.
- Formamide.
- Acetamide.
- Urea.
- Barbituric acid $CO\left\langle\begin{matrix}NH.CO\\NH.CO\end{matrix}\right\rangle CH_2$.
- Alloxan $CO\left\langle\begin{matrix}NH.CO\\NH.CO\end{matrix}\right\rangle CO$.
- Humus.

II. Assimilated:
- Glycine.
- α-Aminopropionic acid.
- Guanidine hydrochloride.
- Cyanuric acid $CO\left\langle\begin{matrix}NH.CO\\NH.CO\end{matrix}\right\rangle NH$.
- Oxamide.
- Peptone.

III. Doubtful or not assimilated:
- Hippuric acid.
- Trimethylamine.
- *para*-Urazine $CO\left\langle\begin{matrix}NH.NH\\NH.NH\end{matrix}\right\rangle CO$.
- Hexamethylenetetramine.
- Ethyl nitrate.
- Propionitrile.
- Hydroxylamine hydrochloride.
- Methyl carbamate.

IV. Toxic:
- Tetranitromethane.

The results so far obtained are not sufficiently numerous to make it possible to trace any connexion between the assimilability or the reverse of the nitrogenous compounds and their constitution. Apart from humus which, as a mixture, cannot be said to have a constitution, the best results were obtained with urea and barbituric acid the former assimilating rather more nitrogen than the latter whilst barbituric acid gave the greater amount of dry produce. The similarity of the two results is probably to be accounted for by the fact that barbituric acid is readily decomposed with production of urea and malonic acid.

The next best result was obtained with acetamide which gave nearly as much dry produce as barbituric acid, although less nitrogen was assimilated. After acetamide the highest results as regards the amounts of nitrogen assimilated were those obtained with ammonium sulphate (without calcium carbonate), formamide and alloxan which assimilated 12·5, 8·5 and 7 mg. of nitrogen respectively. Glycine, alanine and peptone come next with 5·8, 5·1 and 4·8 mg. of nitrogen. Then oxamide (3·6) and guanidine (2·7 mg. nitrogen) and lastly cyanuric acid.

With humates the nitrogen assimilated amounted to only 5·9 mg.; the amount of dry produce was however comparatively high being more than was obtained with urea.

The above grouping is of course only provisional and applies only to peas. It is quite possible that other plants may be able to utilise some of the substances which with peas have given negative results.

The ability of a plant to utilise the nitrogen of any particular compound will depend not only on the power of detaching the nitrogen but on the nature of the carbon compound or compounds remaining.

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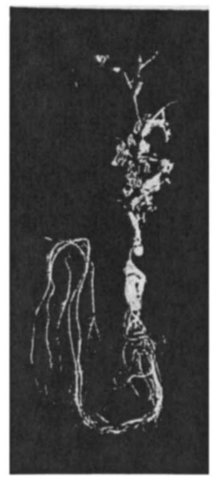
EXPLANATION OF PLATE V.

Pea plants supplied with nitrogen in the form of

1. Ammonium sulphate.
2. Urea.
3. Acetamide.
4. Guanidine hydrochloride.
5. Sodium aspartate.
6. Peptone.

1

2



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