SYNTHESIS OF PROTEINS IN PLANTS. PART I. CONVERSION OF NITRATES INTO PROTEIN IN *HELIANTHUS ANNUS*, LINN.

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Although the study of synthesis of proteins in plants has drawn the attention of a large number of workers, yet very little definite information is available about this important branch of plant physiology. This is chiefly due to want of adequate technique for controlling the intake of nitrogen by plants as also to lack of accurate methods for estimating small quantities of nitrogenous substances formed at various stages in the plant.

A number of useful observations have been recorded by individual workers but the uncertainty regarding the first product of photosynthesis has invariably led to a good deal of confusion in interpreting these observations. At various times hydrocyanic acid, ammonia or potassium nitrite has been mentioned as reacting with formaldehyde and thence the mechanism of protein-synthesis elaborated. The fact that protein-synthesis is not dependent on light but can proceed even in the dark has been a great drawback in these explanations. Several theories have also been postulated on the basis of observations made under *in vitro* conditions but most of them have only academic interest.

The theories regarding the mechanism of protein-synthesis can be broadly divided into three groups—(1) those that assume the preliminary formation of the several amino acids and their subsequent condensation to proteins, (Trier, G. Einfachen-flanzenbasen und ihre Beziehungen zum Aufbau der Eiweisstoff und Lecithin, Berlin, 1912; Baudisch u. Meyer, *Central Bakt;* Abt. II. 1912, **45**, 1771; Baudisch u. Klinger, *Ibid.*, 1916, **49**, 1167; Lob, *Zeit. f. Elektrochem.*, 1906, **12**, 282), (2) those that assume direct interaction between the sugars formed during photosynthesis and potassium nitrite or ammonia arising out of the decomposition of absorbed nitrate, resulting in the synthesis of protein in plant (Baly *et al.*, *J.C.S.*, 1922, **121**, 1078; Huppert, Konstitution und Konifiguration der Eiweisstoff, Leipzig, 1925; Loew, *Biochem. Z.*, 1912, **41**, 224; *Chem. Ztg.*, 1912, No. 7 and *Ber.*, 1913, **46**, 684), and (3) those that assume a preliminary formation of amides, especially asparagine and their subsequent transformation into proteins (Muller, *Landwirtschaft Versuchs-St.*, 1886, **33**, 311; Nakamura, *Bull.*

Agric. Coll. Tokyo, 1897, 465; Saposchnikow, *Bot. Zent.*, 1895, **63**, 246; Primanischnikoff, *Russ. J. Expt. landwirt*, 1912, **13**, 5; *Rev. gen. Bot.* 1913, **25**, 5).

The object of the present investigation is to throw some fresh light on this important problem by directly feeding the plant with various nitrogenous constituents and following the attendant biochemical changes. The present paper relates to the changes undergone by nitrates in the sunflower plant (*Helianthus annus*, Linn.).

EXPERIMENTAL.

The experiments were conducted with sand-cultured plants in glazed earthenware pots. The sand was repeatedly washed with water and, after drying, filled in at the rate of 60 lbs. per pot. It was then mixed with lime at 50 g. per pot and left to weather for about 4-5 weeks, after which potassium acid phosphate (4.8 g.) and potassium sulphate (2.7 g.) were added.

The sunflower plants (Sutton's Giant Yellow variety) were raised on manured beds until the stems attained a thickness of about 8 mm. and then transplanted into the pots. The potted plants were watered at the rate of 2 litres for every alternate day.

After about 15 days when the plants had established themselves. they were injected with distilled water and their ability to take in aqueous nutrients tested from day to day. The technique of injection developed after numerous trials and adopted for this investigation was as follows :—

A glass tube about 1 cm. in diameter and 10 cms. long was fused to another one of diameter 3 mm. The smaller tube was bent twice at right angles. To the end of the smaller tube was attached one inch of rubber tubing and to this was fixed an 'Esco' record syringe needle of stainless steel. To inject a plant, the needle was inserted slantwise into the stem as near the soil as possible so that it was well fixed in the plant. It was then removed and the bore cleaned with a brass wire and reinserted. The point at which the injection was made was protected with a little beeswax. Distilled water was placed in the side tube which acted as a reservoir and observed for a couple of days to ensure that the uptake was continuous and that there was no choking in the needle. When required, the distilled water was pipetted out and the experimental solution introduced in known quantities. The

tube was held in position by means of a zinc clip attached to an iron rod and a test tube inverted over the reservoir to minimise evaporation.

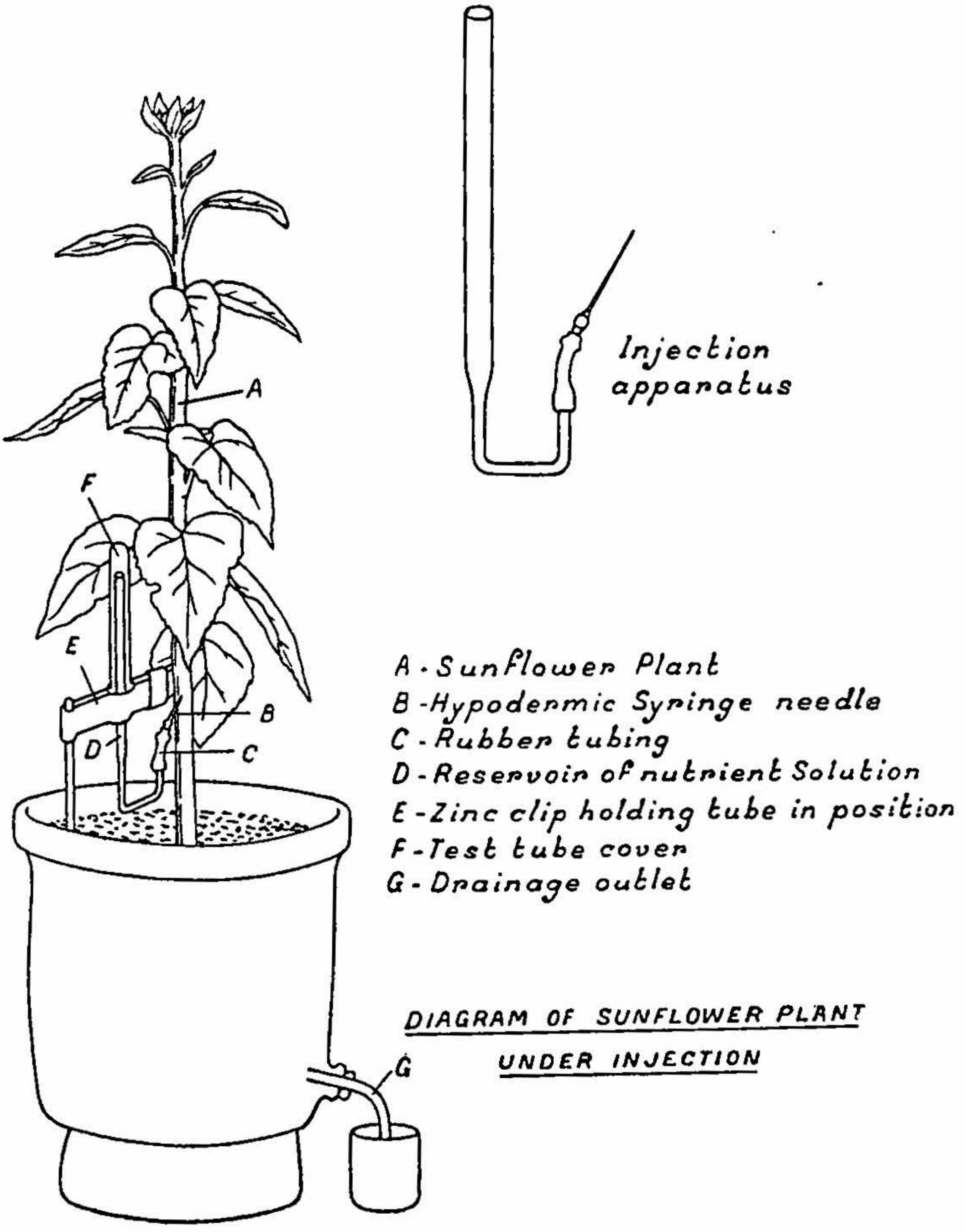

DIAGRAM OF SUNFLOWER PLANT UNDER INJECTION

It was observed that almost all the plants took in water quite readily and continued to grow healthy though the leaves soon began to turn yellow owing to lack of nitrogen.

After ensuring that the passage of water into the plant was steady and continuous, the distilled water was pipetted out and known quantities of standard nitrate solution (2.5 per cent.) placed in each of the reservoir tubes. Several plants were left with water only to serve as controls.

The above treatment with nitrate solution was continued for about a week. Specimens of whole plants were collected at intervals, biological activity stopped by heating for 4 hours at 100° and drying completed at 60°. The dried specimens were analysed for total weight, total nitrogen including nitrates, humin N, amide N, basic N, and non-basic N. The quantity of nitrate absorbed by each plant at the time of observation was also determined by the difference between the quantities introduced at the beginning and that left unabsorbed when sampling.

A preliminary trial was conducted in July 1930 and subsequently repeated in greater detail in February 1931 and 1932 respectively. It was observed that the plants recovered their normal colour within 48 hours and remained healthy throughout the experiment. The results show that the injection method of feeding plants is a highly efficient form of introducing large quantities of nutrients into plants without any detrimental effect.

The total nitrogen was found according to the Arnold-Gunning modification of the Kjeldahl method. Considerable difficulty was experienced in selecting suitable methods for estimating small quantities of the different forms of nitrogen in plant material, owing to the amount of material available for analysis being small. Thus the estimation of nitrates presented great difficulty and several standard methods were tried and found unreliable. The most consistent results were obtained by repeated extraction with hot water followed by reduction with Devarda's alloy.

In regard to the distribution of nitrogen in the plant, the method of differentiating it in terms of solubility in various solvents like water, alcohol and sodium chloride would not lead to any conclusive figures. Van Slyke's method of analysis is not applicable to a material like dried plant. In view of the above the micro method of determining Hausmann numbers developed by Thiemann (*Biochem. J.*, 1926, **20**, 1190) was employed. The material was dropped into boiling 20 per cent. hydrochloric acid and hydrolysed for 36 hours. The hydrolysate was then separated into humin, amide, basic and non-basic fractions. The nitrate content of each sample was also determined.

A large mass of useful data were collected during the three seasons in which the experiments were conducted but with a view to facilitating discussion only the more representative figures have been given in the following tables :—

TABLE I.

Effect of nitrate injection on the dry weights of plants.

No. of days under injection	Treated Plants		Control Plants	Percentage increase in dry weight over controls
	Nitrate absorbed in mg.	Dry weight in gms.	Dry weight in gms.	
1	8·5	11·78	10·8	+10·0
2	14·9	7·51	6·42	+16·9
3	6·4	6·00	7·73	−23·0
4	7·9	11·21	11·85	− 5·4
5	11·1	11·27	9·09	+23·8
6	16·9	9·66	6·60	+46·3
7	13·2	13·28	8·40	+58·0
8	4·1	11·02	9·69	+13·4
11	3·6	12·45	6·69	+86·4
12	7·3	10·11	7·05	+43·4
13	5·7	8·27	9·91	−17·0
14	6·5	10·61	7·51	+41·3
15	9·8	12·65	7·44	+70·0
17	?	10·82	7·71	+40·2
18	8·1	12·18	9·79	+24·5
19	10·4	11·63	8·44	+37·9
20	?	17·63	11·24	+57·0
21	11·5	13·02	9·59	+35·5
22	12·6	13·71	11 20	+22·3
23	9·4	9 58	9·14	+ 5·8
24	0·2	12·25	7·87	+55 5
25	9·9	13·82	10·47	+32·0
26	11·7	16·39	12·74	+28·7
27	5·9	9·59	9·65	− 0·7
28	9·7	11·74	10·27	+14·3

Average increase in dry weight = 25·6 per cent.

TABLE II.

Season—1931.

Number of days under injection	Total nitrogen in plant	Nitrate nitrogen in plant
	mgms.	mgms.
2	67·1	10·7
4	92·5	4·2
5	112·6	10·6
7	105·4	13·2
11	205·2	26·4
12	126·9	1·9
15	122·6	7·2
18	99·5	2·3
20	233·2	19·2
21	101·5	5·5

TABLE III (a).

Season—1930.

Date of commencement of injection, 25-8-1930.

Number of days under injection	Total nitrogen per cent.	Humin nitrogen per cent.	Percentage of humin N. in total N.
4	0·79	0·33	41·0
9	0·94	0·40	43·2
15	0·83	0·34	41·0

TABLE III (b).

Season—1931.

Number of days under injection	Percentages on dry weight			
	Humin N.	Amide N.	Diamino N.	Non-basic N.
2	0·11	0·10	0·15	0·40
4	0·11	0·06	0·10	0·53
5	0·12	0·06	0·10	0·67
6	0·14	?	0·13	0·61
7	0·12	0·05	0·13	0·39
8	0·11	0·01	0·13	0·50
11	0·12	0·15	0·11	1·77
12	0·12	0·08	?	0·91
13	0·13	0·05	0·13	0·45
15	0·14	0·05	0·09	0·67
17	0·13	0·04	0·14	0·42
19	0·12	?	0·11	0·57
20	0·16	0·05	0·09	0·51
22	0·11	0·09	?	0·65
23	0·13	0·08	0·16	0·45
25	0·12	0·06	?	0·47
26	0 13	0·07	0·14	0·88
27	0·14	0·08	0·12	1·77

The effect of nitrate injection on the dry weights of plants was determined as follows: pairs of plants which were very nearly alike with regard to height, girth and general development at the time of the commencement of the experiment were taken out at stated intervals and their respective dry weights compared. Since the specimens were independent plants and had absorbed varying amounts of nitrate, the quantitative effect of the treatment with time could not be evaluated. The plants were therefore treated as representatives of two sets of population, one subjected to injection and the other left untreated and the effects on corresponding specimens compared. The results (Table I) show that the treatment has been beneficial and that the majority of

plants have shown distinct increase in dry weight as the result of nitrate injection. The above observation being perhaps the first record of plant growth being favoured by direct feeding with a mineral nutrient, the experiments were repeated carefully during successive seasons and the results confirmed.

The absorption of nitrate (as shown in column 2, table I) being a mechanical process dependent on such variable factors as temperature, humidity, transpiration etc. does not show any regularity. The results show, all the same, that the plants have absorbed useful amounts of nitrogen and have grown normally without, in any way, being affected by either the injection or the high concentration of the nutrient fed into it.

From column 3 of table II, it might be seen that there is increase in the nitrate content upto the 11th day followed by during successive days. There is however no regularity about this accumulation nor does the quantity bear any proportion to the total nitrogen in the plant thereby suggesting that there is continuous transformation of nitrate into other forms of nitrogen.

The large difference with regard to the humin nitrogen obtained during the 1930 and 1931 series is quite striking. In the former, the humin [table III (a), column 3] accounted for about 40 per cent. of the total nitrogen while in the latter [table III (b), column 2] it was less than a third of that quantity. The difference between the two sets of figures is quite inexplicable particularly when considering that the hydrolyses were conducted under identical conditions. Such high figures as those obtained in the first season were not repeated at any later stage but even those of the latter ones should be regarded as excessive so that the accuracy of the distribution of nitrogen has to a large extent been affected by the precipitation of quite considerable quantities in the form of the comparatively unknown entity humin.

The variation observed with regard to the figures for amides [column 3, table III (b)] suggest the formation of those compounds as intermediary products, in the building up of proteins. This supports one of the existing theories (*loc. cit.*) regarding the mechanism of protein synthesis. Whether this is due to the formation of asparagine or any other amide, it is not possible to say at this stage of the investigation. It is hoped that later research will throw more light on this aspect of the problem.

The figures for diamino nitrogen [table III (b), column 4] representing the combined values for arginine, histidine, lysine and cystine

fractions do not lead to any conclusion. Fractionation of the constituents by micro methods is now in progress and will form the subject of a later communication.

The figures for non-basic nitrogen (column 5) show a correlation with those for amide. The rise and fall correspond inversely to similar alteration in the amide value showing a relation between those two classes of substances. Thus, from table III (b), it is clear that the transformation of nitrate in the plant is accompanied by periodic accumulation and disappearance of amides and corresponding reverse changes in the non-basic fractions.

With a view to shortening the interval between the observations the whole series of experiments were repeated in 1932 sampling the plants day and night at intervals of two hours. The first sample was taken 2 hours after the injection of nitrate and the last 50 hours later. The figures for the analysis of the various samples collected at six-hour intervals are given in table IV.

TABLE IV.

Time of sampling hrs.	Total dry weight of plant in gms.	Total N in whole sample in mg.	Nitrate N in whole sample in mg.	Humin N	Amid N	Diamino N	Non-basic N
				per cent.			
0	14·62	118·2	0·6	0·16	0·13	0·16	0·41
0	14·08	124·9	2·8	0·16	0·12	0·14	0·32
6	19·05	144·0	0·8	0·15	0·13	0·13	0·35
12	16·05	202·7	1·3	0·19	0·12	0·16	0·79
18	18·94	...	3·7	0·12	0·09	0·12	0·52
24	17·66	...	2·1	0·17	0·16	...	0·66
30	17·24	159·1	0·7	0·16	0·11	0·17	0·48
36	16·20	372·6	4·5	0·30	0·19	0·37	1·40
42	37·65	349·7	1·5	0·14	0·09	0·13	0·57

It may be seen from the above that the nitrate content in the plant remains comparatively small throughout the period of observation. The total nitrogen does however show a tendency to increase, thereby indicating that conversion of the nitrogen into other forms takes place at a very rapid rate so that nitrate content is not high at any stage during the experiment.

The amide nitrogen under observation shows the same type of rise and fall as that observed before thereby indicating that the nitrate passes through the amide stage. But there is no correlation between the amide content of the plant and the figures for non-basic nitrogen as was noted before.

Although the foregoing observations have not revealed any striking feature in the mechanism of synthesis of proteins in plants they have yet shown that the technique of direct feeding offers immense possibilities for future study. Further work is therefore in progress with a view to refining the technique and standardising the conditions for estimation of the different forms of nitrogen.

SUMMARY.

A direct method of feeding of plants with nutrients is described. This technique has been applied to the study of protein synthesis by feeding nitrogen-starved sunflower plants with potassium nitrate solution and studying the attendant changes.

There is evidence to suggest that there is continuous conversion of nitrate into other forms of nitrogen. The added nitrogen would appear to pass through the amide stage before being converted into protein.

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