CXLV. THE NITROGEN METABOLISM OF THE PEA SEEDLING.

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(Received March 10th, 1936.)

THE investigation of the lupin [McKie, 1931], an asparagine-producing plant, has been followed by that of the pea, in which no asparagine or other amide is formed in any amount, with a view to elucidating still further the part played by the various nitrogen metabolites. Similar conditions have been observed, and at the same time a parallel investigation of certain aspects of etiolated pea seedlings has been made.

EXPERIMENTAL.

Pea seeds (*Pisum sativum*), about 6–12 per pot, were grown in batches of 50 in a good potting mixture from January to April, 1935. The temperature of the greenhouse varied from 50 to 67° F. For no batch were conditions extreme or in any way adverse. The harvesting and preparation of material for analysis were carried out as for the lupin [McKie, 1931].

Drying. The temperature recommended by Link and Schulz [1924] was finally adopted. The seedlings, from spirit, were dried at 65° for 3 hours, then ground to a fine powder in a mill and the meal thinly spread on filter-paper and dried in an air incubator at 65° for 48 hours. Analyses for total N (Kjeldahl), were made on this dried material.

Extraction of material. The dried and weighed material (6–7 g.) was intimately ground for 20 min. with 50 ml. of ammonia-free water and filtered through fine (mesh 0.017 cm.) weighed and dried muslin. This process was repeated, 6 times in all, using 30 ml. of water for each subsequent extraction, each muslin being pressed as dry as possible. The residue was dried to constant weight at 105°. Analyses were carried out as follows:

Residue. Total-N was estimated by Kjeldahl and expressed as “Insoluble N”.

Filtrate. The combined washings were made up to 500 ml. (250 ml. for 0 (*a*) and 3 (*a* and *b*) days and 1000 ml. for 32 days), with ammonia-free water and analysed for total N and individual water-soluble N compounds. Analyses for total N, proteose, ammonia, amide and nitrate were performed as described for the lupin.

Protein. Owing to the presence in the pea of legumin, a protein soluble in acidulated water and incompletely coagulated by heat, a modified method of precipitation and filtration based on a study of the optimum conditions was adopted. The p_H of the extract was adjusted to 4.5, the isoelectric point of legumin, by addition of acetic acid, and the extract gently boiled for 1 hour. The coagulum was filtered hot through hardened filter-paper (Whatman No. 50) under slight pressure, repeatedly washed with hot water and dried to constant weight at 105°. Analyses for total N were then made on this material.

Proteose. Since some legumin inevitably remains after coagulation, and since this residuum is precipitated by Na_2SO_4 , the estimation of proteose by the method of Wasteneys and Borsook [1917] gives proteose *plus* this residual protein. 5 ml. of extract were used for each determination.

Amino-acid. The Van Slyke method was adopted, 3 ml. of extract being used for each determination.

Fig. 1. Distribution of various forms of nitrogen.

Table I. *Variation of nitrogenous constituents.*

Age days	Seedling		Insoluble material			Coagulable protein		
	Dry wt. g.	Total N g.	g.	N %	N % dry wt.	g.	N %	N % dry wt.
0 (a)	12.11	0.48	3.79	1.46	0.46	4.40	7.90	2.87
(b)	12.23	0.49	3.59	1.41	0.42	5.02	7.32	3.09
3 (a)	6.05	0.25	4.26	4.29	3.03	0.80	4.98	0.66
(b)	8.76	0.36	6.35	4.58	3.33	0.97	5.00	0.55
5	5.01	0.24	2.57	3.86	1.98	1.44	5.69	1.64
9	7.20	0.38	3.08	4.74	2.03	2.05	6.33	1.80
13	4.65	0.26	1.50	4.55	1.47	1.49	6.41	2.05
18	3.56	0.36	0.81	3.26	0.74	1.09	5.86	1.79
22	6.02	0.35	2.41	4.43	1.78	1.57	6.84	1.78
32	7.71	0.39	4.27	5.3	2.94	0.76	6.1	0.60
Age days	Protease-N % dry wt.	Amino-N % dry wt.	Amide-N % dry wt.	Ammonia-N % dry wt.	Nitrate-N % dry wt.	Insol. N/ Water-sol. N		
0 (a)	0.47	0.09	0.05	0.005	—	7.71		
(b)	0.34	0.10	0.04	0.010	—	8.62		
3 (a)	0.27	0.01	0.05	0.02	—	0.38		
(b)	0.03	0.09	0.04	0.023	—	0.23		
5	0.55	0.33	0.04	0.028	—	1.31		
9	0.27	0.64	0.05	0.026	—	1.37		
13	0.67	1.25	0.19	0.039	—	2.86		
18	1.02	1.54	0.15	0.066	0.27	6.52		
22	0.78	0.61	0.13	0.073	0.19	2.01		
32	0.55	0.29	0.21	0.027	—	0.67		

Over a period of 32 days' growth from seed, it is seen (Fig. 1, Table I) that insoluble N, protein-N and amino-N represent the greater part of the total N. Small quantities of amide-N and ammonia-N occur at all stages but never amount to more than 5 %, and in the earlier stages of growth are only about 1.4 % of the total N.

The most significant fact is the increase of α -amino-acids from 2 % of the total N in the seed to 25 % at the 18th day. This is paralleled by the decrease of insoluble nitrogen compounds, which, after a perplexing maximum at the 3rd day, reach a minimum on the 18th day. Protein, after a rapid fall during the first 3 days of growth, rises to a fairly steady level, representing about 40 % of the total N. The seedling growth of the pea is slower than that of the lupin, and at each stage the plant has a smaller proportion of leaves; hence active synthesis of protein is delayed. Thus the ratio of insoluble to soluble nitrogen compounds falls, and the active metabolites show a tendency to accumulate (Table I). The curve for proteose-N follows closely that for coagulable protein, as would be expected, since this fraction in the pea contains part of the actual protein (legumin). Amides never accumulate. They are present to the extent of 1 % in the seed, and keep at this level through the early stages of growth, showing a tendency to increase in the leafy seedling. Ammonia follows a parallel course but in still less amount. Nitrates have been detected at the 18th and at the 22nd day. Thus the behaviours of the labile metabolic products, other than asparagine and amino-acids, in the lupin and the pea show a close parallel.

Amino-acids represent the chief nitrogenous metabolic products in the pea during the early stages of growth, when hydrolysis of protein is the principal metabolic process. After the 18th day leaf formation accelerates and amino-acids are removed. Comparison with the lupin [McKie, 1931] shows that the rôle of amino-acids in the pea is played by asparagine in the lupin. The amide content of the pea is at all times negligible and asparagine has never been detected. On the other hand, the amino-N content of the lupin never reaches 1 % of the total-N.

Etiolated pea seedlings, grown in complete darkness to the 18th day, show that amino-acids still represent the major water-soluble nitrogen compounds

Table II. *Effect of etiolation on nitrogen distribution.*

	9 days		18 days	
	Etiolated	Green	Etiolated	Green
Ammonia-N (% total N)	0.6	0.48	0.96	1.1
Amide-N (% total N)	3.7	1.05	13.4	3.4
Amino-N (% total N)	5.6	12.01	20.06	25.3
Complex N	—	—	65.6	58.9
Water-sol. N	—	—	34.4	33.4
Complex N	—	—	65.6	58.9
Amide-N	—	—	13.4	3.4
Amino-N	—	—	1	1
Amide-N	—	—	0.67	0.13

(Table II). Thus, while the ratio of complex N to water-soluble N is approximately the same in both types, indicating that light does not appear to affect the extent of hydrolysis, the ratio of amino-N to amide-N changes from 1/0.13 in light to 1/0.67 in the dark, showing that the course of hydrolysis is considerably different. Total N (as % dry weight) is parallel in etiolated and green plants to the 9th day, but is 5 % in the former as compared with 6 % in the latter at the

18th day. If this difference is real—it is difficult to ensure that growth is exactly parallel—it might indicate that nitrogen metabolism, as well as following a different course, is lowered by virtue of decreased intake.

Evidence has been adduced [McKie, 1931], in line with other workers, that asparagine, when it is produced as a nitrogen metabolite, is probably a soluble, mobile translocation product. In the absence of asparagine, amino-acids seem to serve predominantly for translocation.

There is some evidence [Allison, 1934; Maskell and Mason, 1930; Montiverdi, 1889; Prianschnikov, 1922, 2; 1924; Schulze, 1901] that the nature of the translocation products is affected by the concentration of carbohydrate, *i.e.* asparagine formation seems to be greatest in seedlings which have low non-nitrogenous reserve, and this is now substantiated by the comparison of green and etiolated seedlings in the pea. Failing an adequate carbohydrate supply oxidation of amino-acids occurs, with separation of ammonia, which is then transformed into asparagine (*vide* the suggestive finding of Butkewitsch [1909] that, in an asparagine-forming plant, ammonia accumulates if asparagine formation is inhibited by anaesthetising). In the pea, a non-asparagine plant, carbohydrate reserve is comparatively high and amino-acids accumulate until protein synthesis becomes active. The occurrence of asparagine in the lupin is strikingly accompanied by a low carbohydrate reserve. Further, when carbohydrate formation is artificially hindered by absence of light, the amino-acid content falls and is accompanied by amide formation. Thus, in the first days of growth, when the carbohydrate values are identical, no difference is observed between the green and the etiolated seedling. Only when secondary carbohydrate supply, available to the green plant by photosynthesis, fails does oxidation of amino-acid and formation of amide become evident.

SUMMARY.

1. Soluble and insoluble protein-N in the pea together account for about 87 % of the total N of the seed, as compared with 100 % in the lupin. Small quantities of proteoses, amides and amino-acids are also present.
2. Insoluble nitrogen compounds reach a minimum on the 18th day, corresponding with a maximum content of amino-acids.
3. Amino-acids appear to represent the chief metabolic product and to have the same significance in the pea as does asparagine in the lupin.
4. The possible rôle of asparagine as a labile translocation product, formed in plants with low carbohydrate reserve by the oxidation of amino-acids, is confirmed by the comparison of green and etiolated seedlings. Amide is produced at the expense of amino-acids when carbohydrate is depleted by the absence of light.

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