

SUMMARY

1. Extracts of *Pisum sativum* at seven different stages of development were analysed for total nitrogen, non-dialysable nitrogen, total and non-dialysable amino nitrogen and total and non-dialysable amide nitrogen; total non-protein nitrogen and peptide nitrogen were determined by difference.

2. The protein distribution in the extract was determined from ultracentrifuge diagrams.

3. In the early stages of development the incoming nitrogen was distributed about equally among the different fractions. With ensuing desiccation, however, there occurred first a sharp increase in peptide nitrogen and later a sharp increase in protein nitrogen at the expense of peptide nitrogen.

4. The data offer strong evidence in favour of peptide intermediates in the synthesis of the seed protein.

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Protein Synthesis in Ripening Pea Seeds

2. DEVELOPMENT OF EMBRYOS AND SEED COATS

BY ILSE DOROTHEA RAACKE*

Biochemical Institute, University of Uppsala, Sweden

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In seeds of the Leguminosae, the constituent parts do not all develop simultaneously. For example, it is known that the endosperm develops first, only to be later absorbed by the developing embryo (including the cotyledons). This means that while protein is being built up in some parts of the seed, it is being broken down in others, so that a study of the development of the whole seed gives only the net result of protein formation. A truer picture might be obtained by studying independently the development of different parts of the seed. As far as the author is aware, such a study has not previously been carried out.

* Present address: Chemical Laboratory, University of Cambridge.

EXPERIMENTAL

The material consisted of peas of the same lot as those used in the preceding paper (Raacke, 1957). They were grouped into stages as previously described, but the groups represented here are not exactly the same as those of Part I of this investigation, although they roughly correspond. The embryos of peas belonging to stages I, II and III were too small to be separated, so that only embryos of peas of stages IV, V, VI and VII were used.

The seed coats of peas in stages IV, V and VI were removed by making a small incision in the coat, through which the embryo could be squeezed out. The ripe peas, stage VII, were allowed to soak overnight in water at 2° before removal of the coat.

The material designated as 'cotyledon IV' is a whole embryo of peas belonging to stage IV. From peas at stages V,

VI and VII, the hypocotyls and epicotyls were dissected out and only the cotyledons used. The hypocotyls of stages V and VI were pooled and designated as 'Hyp'.

The seed coats, cotyledons and hypocotyls were minced in a Waring Blender and lyophilized in the manner described in Part 1 of this investigation. The lyophilized material was then analysed for moisture, Kjeldahl and amino nitrogen, and extracted with 'standard buffer'. The extracts were analysed as previously described (Raacke, 1957).

RESULTS

A comparison between Kjeldahl and amino nitrogen of cotyledons and Kjeldahl and amino nitrogen of seed coats is given in Table 1. It is seen that both become relatively poorer in nitrogen as ripening progresses.

Table 2 gives the nitrogen distribution in extracts of the different seed parts. As in the previous paper, amino and amide nitrogen represent dialysable forms only, and amino and peptide nitrogen represent nitrogen in amino and in peptide links respectively. The figures show a sharp increase of protein and a decrease of amino, amide and peptide nitrogen in the cotyledons as maturity is approached. In the beginning (stage IV) only 29% of the total nitrogen is present as protein, 31% as amino, 31% as peptide and 9% as amide nitrogen. In the ripe cotyledons, however, 82% of the total nitrogen is present as protein, and only 5% as amino, 13% as peptide and none as amide nitrogen. Fig. 1 shows the absolute distribution of nitrogen in the extracts of cotyledons. It is seen that, as in the picture for whole peas, there is a divergence of the curves representing protein and non-protein nitrogens between stages V and VI.

In the seed coats, in contrast to the cotyledons, the relative distribution of the different nitrogenous fractions remains relatively constant throughout.

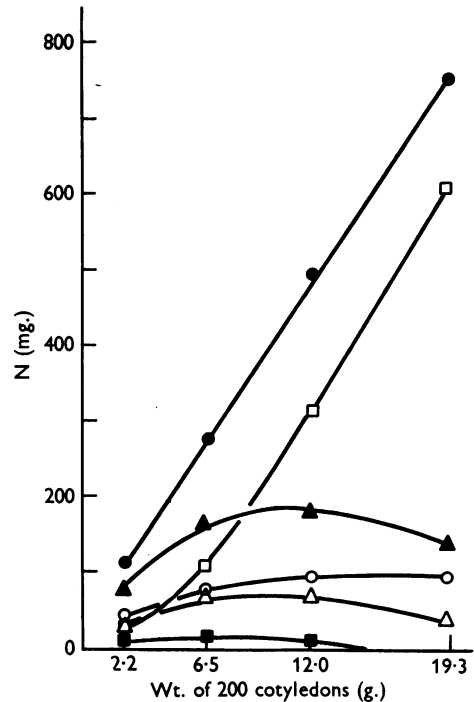


Fig. 1. Nitrogen distribution in extracts of cotyledons at different stages of ripening. ●, Total N; □, protein N; ▲, total non-protein N; ○, peptide N; △, amino N; ■, amide N.

Table 1. *Partial nitrogen analysis of cotyledons and seed coats*

Peas (stage)	Cotyledons		Seed coats		Hypocotyls	
	Total N	Amino N	Total N	Amino N	Total N	Amino N
IV	5.81	2.23	3.56	1.45	7.69	1.00
V	4.99	1.46	3.10	0.88	—	—
VI	4.87	0.83	2.26	0.64	—	—
VII	4.45	0.35	0.66	—	—	—

(N as % dry wt.)

Table 2. *Nitrogen distribution in extracts of cotyledons and of seed coats*

Material	Stage	Total N	Protein N	Non-protein N			
				Total	Amino	Amide	Peptide
				(% dry wt.)			
Cotyledons	IV	5.11	1.48	3.63	1.57	0.49	1.57
	V	4.25	1.69	2.56	1.09	0.21	1.26
	VI	4.14	2.65	1.49	0.60	0.08	0.81
	VII	3.90	3.18	0.72	0.21	0	0.51
Seed coats	IV	2.94	0.43	2.51	1.38	0.11	0.89
	V	2.27	0.39	1.88	0.86	0.09	0.93
	VI	1.70	0.24	1.46	0.62	0.09	0.75
	VII	0.18	0.07	0.11	0.03	0.02	0.06
Hypocotyls	—	—	1.33	1.51	0.56	—	—

About 15% of the nitrogen is present as protein, 40% as amino, 40% as peptide and 5% as amide nitrogen. There seems to be a depletion of the soluble nitrogen forms in the ripe seed coats, but the figures are too low to be trustworthy.

The distribution of free and bound amino nitrogen, and free and bound amide nitrogen in the cotyledons and seed coats respectively is shown in Table 3. The bound amino nitrogen in the cotyledons is found to increase from about 7% of the total amino nitrogen in the unripe seeds to 40% of the total in ripe ones. During the same period the bound amide nitrogen increases from 20 to 100% of the total amide nitrogen. In the seed coats, the amino nitrogen distribution remains approximately

constant, but there is a constant decrease in the bound amide nitrogen.

The protein distribution in the extracts of cotyledons was determined from ultracentrifuge diagrams. The results are given in Table 4 and Fig. 2 shows typical diagrams of the four stages investigated. The seed coats contain only albumin; the different stages were not investigated separately.

DISCUSSION

Separate analyses of the development of the seed coats and of the cotyledons show that the maturation process as observed with whole peas represents the net result of two superimposed trends. In the seed coats, although synthesis is undoubtedly occurring, the breakdown of protein is predominant. The figures in Table 2 show very clearly how the nitrogenous materials from the seed coats (comprising also the endosperm) are gradually absorbed by the developing embryo, thus corroborating chemically the long-known histological observations. In the cotyledons, on the other hand, the balance of metabolic activities lies overwhelmingly on the synthetic side, as is shown by the rapid increase in protein (Fig. 1). The marked rise and subsequent drop in the concentration of peptides observed previously with whole peas (Raacke, 1957), is due largely to the peptide fraction in the seed coats, the peptide level in the cotyledons remaining relatively constant. It would appear, then, that an important function of the seed coats is in the maintenance of a definite peptide pool in the cotyledons. Failure to maintain this pool at an appropriate level, as occurs in the last stage of

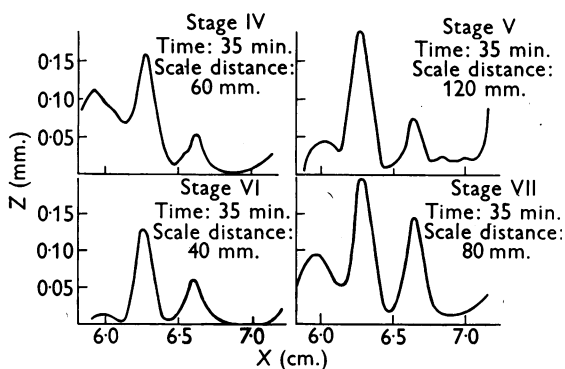


Fig. 2. Representative ultracentrifuge diagrams of extracts of cotyledons at four different stages of development. The ordinate is proportional to the gradient of refractive index; abscissa is distance from the centre of rotation.

Table 3. Free and bound amino and amide nitrogen in extracts of cotyledons and of seed coats

Material	Stage	Amino N (% dry wt.)			Amide N (% dry wt.)		
		Total	Bound	Free*	Total	Bound	Free*
Cotyledons	IV	1.68	0.11	1.57	0.61	0.12	0.49
	V	1.23	0.14	1.09	0.35	0.14	0.21
	VI	0.72	0.12	0.60	0.28	0.20	0.08
	VII	0.33	0.14	0.21	0.30	0.30	0
Seed coats	IV	1.45	0.17	1.38	0.24	0.13	0.11
	V	0.92	0.06	0.86	0.11	0.02	0.09
	VI	0.67	0.05	0.62	0.12	0.03	0.09
	VII	0.03	0	0.03	0.02	0	0.02

* Difference between amino N before and after dialysis of the extracts.

Table 4. Protein distribution in extracts of cotyledons

Stage	Legumin (% of total protein)	Vicilin	Ratio: vicilin/legumin	Albumin (% of total protein)	Ratio:
					albumin/globulin
IV	15	44.5	2.94	40.5	0.68
V	25.5	54	2.12	20.5	0.26
VI	37	59	1.59	4	0.04
VII	36	46	1.28	16	0.19

maturation when the seed coats are depleted of nitrogenous materials, causes protein synthesis to stop.

The picture of a constant-size peptide pool fits in well with the hypothesis of definite intermediate stages in the build-up of high-molecular proteins, since on one side there is the well-established necessity for an adequate amino acid pool within the cell, and on the other, a build-up and maintenance of a certain size albumin pool as a preliminary for the synthesis of the pea globulins.

The development of the different protein components can be seen more clearly in the cotyledons than in the whole peas, because in this case the seed coat with its large amount of albumin is eliminated. It is again found that vicilin appears first. From the diagrams there is also clearly discernible a decrease of the concentration of albumin and then again a final increase. This confirms an observation reported in the previous paper. This final increase is not evident from the series of analyses on whole peas since, as was mentioned above, the albumin from the seed coats obscures the picture. It should be noted that Danielsson (1952) also observed an increase of the albumin/globulin ratio in the last stage.

The figures in Table 3 are notable as they show the gradual and ultimately complete incorporation of the amide nitrogen into the protein of the cotyledons. It has often been suggested that amide occupies a pivotal position in the nitrogen metabolism of the seed (Schulze, 1900; Schulze & Castoro, 1903; Prianischnikow, 1922; Mothes, 1926; Vickery, Pucher & Clark, 1936; Vickery & Pucher, 1943; Gregory & Sen, 1937; Steward & Preston, 1941). From the present data, however, it appears that amide is also utilized directly and in much the same manner as other amino acids in the build-up of the pea proteins. This, of course, does not mean that amides do not have any special functions, since there is, indeed, much evidence

that these compounds serve as a storage product of ammonia. But it serves to emphasize that amide metabolism in the plant does not follow a single pathway, and that in normally synthesizing tissues, which are not subjected to any stress such as etiolation or unbalanced supply of nutrients which calls upon the defence mechanism of the plant, at least part of the amide nitrogen does not suffer a fate different from that of the amino nitrogen.

SUMMARY

1. Cotyledons and seed coats of *Pisum sativum* at four different stages of development have been analysed. It is shown that as ripening progresses the nitrogenous materials from the seed coats (including the endosperm) are absorbed by the cotyledons.

2. The study of the nitrogen distribution in the cotyledons corroborates the evidence obtained from studies of whole peas to the effect that protein synthesis proceeds through various stages which include peptides and 'proteoses'.

3. It was found that in cotyledons amide nitrogen is incorporated gradually but completely into the protein; the relationship of this finding to current views of amide metabolism in plants is discussed.

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Protein Synthesis in Ripening Pea Seeds

3. STUDY OF THE PODS

By ILSE DOROTHEA RAACKE*

Biochemical Institute, University of Uppsala, Sweden

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It is well known that there is a translocation of nutrients from the pods of the Leguminosae to the developing seeds. This was first observed by Emmerling (1880) for *Pisum sativum* and later confirmed by Schulze & Winterstein (1910) and Bisson & Jones (1932), as well as by Wassilieff

(1908), for *Lupinus albus*, and by Pfenninger (1909) for *Phaseolus vulgaris*. The available evidence indicates that the nitrogenous nutrients do not pass directly from the leaves to the seeds through the pods, but are at least in part at first stored in the pods in the form of protein, which is then used to supply the developing seeds.

* Present address: Chemical Laboratory, University of Cambridge.