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; Prianschnikow, 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

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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.

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Many workers have studied the transfer of the pod proteins to the seeds, but although it is certain that the proteins must be broken down before translocation, the form in which the nitrogen passes to the seeds is still rather uncertain. Because of large amounts of asparagine present in the stems and pods of several species of Leguminosae, Schulze & Winterstein (1910) concluded that nitrogen was translocated in the form of that amide, rather than in the form of amino acids. However, in addition to these two possibilities, it is also conceivable that peptides play a part in the transfer; hence, it seemed of interest to determine peptide nitrogen in the developing pods.

EXPERIMENTAL AND RESULTS

The experimental material for the present investigation consisted of pods of *Pisum sativum*, variety Fenomen, grown and sampled as described in Part I of this investigation (Raacke, 1957). The

Pods were divided into seven groups according to the physical characteristics given in Table 1. Unfortunately, it was not possible to obtain ripe, i.e. dry pods, so that all seven groups represent immature stages.

The stages do not correspond to the stages of peas described previously (Raacke, 1957), because the peas were regrouped after shelling. So, for example, pods I yielded only peas I, but pods II yielded peas I and some II, pods III gave peas I and II and some III, and so on; pods VII yielded mainly peas VI, and some V and IV.

The pods were minced and lyophilized as previously described. In the more mature pods, long fibres which could not be minced were sifted out and discarded. The lyophilized material was subjected to moisture, Kjeldahl, and Van Slyke determinations, the results of which are given in Table 2.

Extractions were carried out with standard buffer and the extracts analysed as previously described. The extraction yields varied from 74 to 84 % of the total nitrogen and 76–88 % of the amino nitrogen of the dry material, so that the results obtained from the extracts can be taken as representative. The relative nitrogen distributions can be seen in Table 3. Fig. 1 shows the changes in the absolute amounts of the various nitrogenous fractions in the pods as maturation proceeds.

Ultracentrifuging of the extracts was also carried out. Because of the low protein content of the pods, the extracts were dialysed, lyophilized and the protein redissolved in 'standard buffer'. There was no obvious difference between the different stages,

Table 1. *Physical characteristics of pods of peas at different stages of development*

Stage	Wet wt. (g.)	Dry wt. (g.)	Length of pods (cm.)	Thickness of pods (cm.)
I	23	3.6	2-3	0.1
II	48	5.1	3.5-4.5	0.3
III	106	10.2	5.0-5.5	0.3-0.4
IV	285	29.4	6.0-7.5	0.4
V	336	36.0	8.0-9.0	0.4
VI	338	36.0	7-9	0.6
VII	337	36.0	7-8	0.8-0.9

Table 2. *Partial analysis of pods of Pisum sativum during maturation*

Pods (stage)	Total N		Amino N		Total N/100 pods (mg.)	Amino N/100 pods (mg.)	Moisture (%)
	Wet (%)	Dry (%)	Wet (%)	Dry (%)			
I	0.94	7.09	0.21	1.62	255	58	86.7
II	0.57	5.34	0.20	1.85	272	94	89.4
III	0.47	4.87	0.16	1.70	497	173	90.4
IV	0.42	4.60	0.16	1.83	1352	538	89.6
V	0.40	4.57	0.16	1.87	1645	673	89.3
VI	0.41	4.56	0.15	1.69	1642	609	89.5
VII	0.35	4.11	0.12	1.36	1480	490	89.5

Table 3. *Nitrogen distribution in extracts of pods during their development*

Stage	Total N	Protein N	Non-protein N			
			Total (% dry wt.)	Amino	Amide	Peptide
I	4.88	1.65	3.23	1.20	1.05	0.98
II	4.26	1.02	3.24	1.56	0.93	0.75
III	3.71	0.79	2.92	1.34	0.91	0.67
IV	3.56	0.68	2.88	1.30	0.88	0.70
V	3.83	0.83	3.00	1.53	0.83	0.64
VI	3.45	0.53	2.92	1.28	0.83	0.77
VII	3.41	0.59	2.82	1.09	0.85	0.88

the bulk of the protein being contained in an ill-defined albumin peak with low sedimentation coefficient ($\approx 1-2$), although a small peak of high sedimentation coefficient (16-22) was consistently present. This may be present originally in the pod, but more probably it is an aggregation product, as the varying sedimentation coefficients would tend to indicate. A typical diagram is shown in Fig. 2.

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

Fig. 2. Typical ultracentrifuge diagram of a protein preparation from pods of *Pisum sativum* (stage V). Time: 20 min., scale distance: 40 mm. The ordinate is proportional to the gradient of refractive index; abscissa is distance from the centre of rotation.

DISCUSSION

As can be seen from Table 3, the decrease in total nitrogen concentration is closely paralleled by a decrease in protein concentration, whereas the concentration of amino, amide and peptide nitrogen remains remarkably constant throughout the ripening process. These data, together with those in Fig. 1, illustrate once more very clearly that protein is first synthesized in the pods and then broken down again and transferred to the developing seeds. It is worth noting, however, that it is not broken down completely to amino acids, but that a considerable portion remains in peptide linkage. As the amino nitrogen comprises the free amino group of asparagine and also the free amino groups of peptides, it can be seen that very little exists in the form of amino acids. Since, however, there is very little amide in the pod protein (about 1.5 %) the large amounts of free amide must be formed by secondary reactions from amino acids and carbohydrate residues, as with seedlings (Prrianischnikow, 1922).

The question of the role of amide in the transport of nitrogen in the plant has been extensively discussed by Mothes (1926). On the basis of detailed analyses of different anatomical parts of plants this author concluded that amides undoubtedly figure in the translocation of nitrogen. One would expect, however, that not only amide but any low-molecular product present in appreciable concentration would be translocated. The present data would seem to indicate that peptides resulting from proteolysis, as well as amides, are in this category.

Another point brought out in the course of this investigation is the influence of water content in the regulation of protein synthesis. As it has been seen from the results on whole peas, ensuing desiccation brings about important changes in the composition of the seeds, which become apparent in the form of a sharp increase in protein. By examining the data in Fig. 1, it can be seen that at the stage corresponding to ensuing desiccation of the seeds (it should be remembered that pods VI yielded mainly peas of stage V), the breakdown of nitrogenous products in the pods is faster than the synthesis, as can be seen from the sharp breaks in the curves. (The sharp increase in peptide nitrogen in the seeds together with the sharp decrease in protein nitrogen in the pods should be noted.) This is despite the fact that the moisture content of the pods remains remarkably constant throughout (see Table 2), which shows the interdependence of metabolic processes in various organs of the plants, and indicates, as well, the fact that no generalization concerning the influence of water content in the formation of protein can be made. In the seeds, reduction of moisture causes an increase in protein; in the pods protein decreases whereas moisture

remains constant; and in the leaves (Petrie & Wood, 1938) the net rate of protein synthesis increases with increased water content.

SUMMARY

1. Pods of *Pisum sativum* at seven different stages of development have been analysed for total, protein, amino, amide and peptide nitrogen.

2. Protein is first formed, then broken down and transferred to the developing seeds in the form of amide and peptides.

3. The moisture content of the pods has no influence on protein hydrolysis, which, rather, is shown to be determined by the metabolic state of the seeds.

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The Effect of Parathormone on the ^{32}P Uptake by the Bones of Rabbits and Cats

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The two major hypotheses explaining the mechanism of action of parathormone on bones are those of Albright & Ellsworth (1929) and Thompson & Collip (1932). Albright & Ellsworth (1929) suggested that the main action of parathormone is on the kidney, inhibiting the reabsorption of phosphate by the renal tubules. This results in a tendency to a decreased serum inorganic phosphate level, which induces a withdrawal of phosphate from bone to restore the $[\text{Ca}] \times [\text{PO}_4]$ product in the serum to normal. The hypothesis of Thompson & Collip (1932) postulated a direct demineralizing effect of parathormone on bone. Subsequent investigations by Heller, McLean & Bloom (1950), on histological changes in bone after parathormone treatment, and by Barnicot (1948), on the local demineralization of bone with parathyroid tissue grafted in apposition to it, have increasingly supported the view that parathormone has a direct effect on bone, producing demineralization. Engel (1952) suggested that this demineralization was due to a destruction of osteoid matrix. The effect of parathormone on the renal tubules cannot be excluded, as Harrison & Harrison (1941) and Jacobs & Verbanck (1953)

have demonstrated a decreased tubular reabsorption of phosphate in normal animals. Other workers (Dent, 1953; Stoerck & Silber, 1949) found, however, that parathormone had no phosphate diuretic effect in normal subjects but did show this effect in parathyroidectomized preparations. This led to the concept that two parathormones may exist, one of which exerts a maximum effect on bone, whereas the other, by affecting the kidneys, produces a phosphate diuresis.

The ^{32}P uptake by bone could depend on the operation of the following processes: an ion exchange between phosphate ions on the mineral crystal surface and ions in the surrounding fluid, a recrystallization of the bone crystal which would reduce the availability for re-solution of ions already deposited and also active growth and remodelling of bone which would, presumably, depend on cellular activity (Neuman & Weikel, 1954). The distribution of ^{32}P in bones of animals injected with the isotope has been investigated by two different techniques: autoradiography and radioactivity measurements. Autoradiography (Amprino & Engström, 1952) demonstrates in a qualitative way