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AMINO ACID-DEPENDENT ATP-PYROPHOSPHATE EXCHANGE IN NORMAL AND BORON DEFICIENT BEAN ROOTS

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Abstract—Amino acid-dependent ATP–pyrophosphate exchange activity in extracts from root sections of normal and boron deficient *Vicia faba* var. *minor* was measured in the presence of single, and mixtures of, amino acids. The activity decreased away from the tip of the root and was less in boron deficient than in normal tissue. The changes in deficient tissue could be observed before deficiency was manifest as reduced growth rate. The results have been interpreted in terms of the maturation, or differentiation, which continues towards the root tip despite the decreasing elongation in the deficient root.

INTRODUCTION

BORON functions as an essential trace element for the growth of plants; but the mechanism of its action is unknown. While several hypotheses have been proposed they have not been substantiated sufficiently for acceptance (see reviews by Gauch and Dugger[1] and Skok[2]). Boron, as borate, is required for continued elongation of the roots of the bean (*Vicia faba* var. *minor*),[3] and we have used these roots for biochemical studies on normal and borate deficient tissues (see also following papers[4, 5]). Since previous investigations of biochemical activities in boron deficient tissue have directed attention largely to catabolic processes, the present studies have been related more to synthetic aspects of metabolism. Amino acid-dependent adenosine triphosphate ATP–pyrophosphate exchange has been measured as being of possible relevance to the activation of amino acids for protein synthesis. The activities with single, or mixtures of many, amino acids have been compared for boron deficient and normal roots, and for different regions of both types of roots. The content of individual amino acids in extracts of sections of normal and deficient roots have been determined.

RESULTS AND DISCUSSION

The soluble fraction (105,000*g* supernatant) from homogenates of bean roots was shown to be active in catalysing an amino acid-dependent ATP–pyrophosphate exchange. Under the assay conditions used the rate of exchange was constant over at least the first 10 min of incubation. Initially 5 min of incubation was used for the assay of activity. The results in Table 1 show that, when expressed on a soluble protein basis, the activity with a mixture of

[1] H. G. GAUCH and W. M. DUGGER, JR., *Maryland Univ. Agr. Exp. Sta. Bull.* A 80 (Tech.).
[2] J. SKOK, In *Trace Elements* (Edited by C. A. LAMB, O. G. BENTLEY and J. M. BEATTIE), p. 227. Academic Press, New York (1958).
[3] T. F. NEALES, *Australian J. Biol. Sci.* **13**, 232 (1960).
[4] R. W. HINDE and L. R. FINCH, *Phytochem.* **5**, 619 (1966).
[5] S. CORY, L. R. FINCH and R. W. HINDE, *Phytochem.* **5**, 625 (1966).

seventeen added amino acids was greatest in the tip of the normal root and decreased up the root away from the tip. In the borate deficient root the activity was less than in the normal root, and again decreased up the root. The high endogenous pyrophosphate exchange activity (in the absence of added amino acids) was probably due to the high levels of free amino acids present in the tissue extract.

TABLE 1. ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM NORMAL AND BORON DEFICIENT ROOTS

	Normal tissue			Deficient tissue		
	Tip (5 mm)	Section 2 (5 mm)	Section 3 (5 mm)	Tip (5 mm)	Section 2 (5 mm)	Section 3 (5 mm)
ATP–pyrophosphate exchange (nmoles/gm protein/min)						
No added amino acids	55	40	25	54	24	24
Plus seventeen amino acids (0·1 mM)	69	48	32	52	26	22
Content of endogenous amino acid in incubation mixture (μmoles)						
	2·7	3·2	3·8	4·8	6·7	5·7

Roots were harvested after 47 hr in nutrient solution at 22°, those in boron deficient medium having ceased to elongate at 41 hr. 105,000g supernatant extracts were prepared and assayed for exchange activity and amino acid content as described under Materials and Methods.

TABLE 2. ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM NORMAL AND BORON DEFICIENT ROOTS

	Normal tissue		Deficient tissue	
	Tip (2·5 mm)	Section 2 (2·5 mm)	Tip (2·5 mm)	Section 2 (2·5 mm)
ATP–pyrophosphate exchange (nmoles/mg protein/min)				
No added amino acids	29	45	48	36
Plus seventeen amino acids (0·1 mM)	76	70	66	56
Content of endogenous amino acid in incubation mixture (μmoles)				
	1·2	2·0	1·8	3·2
Protein content of incubation mixture (mg)				
	0·62	0·53	0·62	0·39

Roots were harvested after 36 hr in nutrient solution at 22°. Those in boron deficient medium ceased to elongate at 39 hr. 105,000g supernatant extracts were prepared and assayed for exchange activity and amino acid content as described under Materials and Methods.

In the experiment presented in Table 1 the boron deficient roots had ceased elongating, approximately 6 hr before harvesting. In the subsequent experiment (Table 2) the roots were harvested at, or before, the point of cessation of growth. Again the pattern shown in Table 1 was apparent, though less marked. To confirm these results the experiment was repeated with triplicate assays on tips from normal roots and deficient roots harvested slightly before the point of cessation of growth. The results are presented as averages in Table 3. In the experiments presented in Tables 2 and 3, the endogenous exchange activities were lower than for the experiment presented in Table 1 because the supernatant extract was diluted further,

before assay, in order to decrease the concentration of endogenous amino acids in the incubation mixture.

The endogenous amino acid contents shown for the incubation mixtures in the three tables are representative of the relative amounts of free amino acids in the extracts from the different tissues. Determination of the content of individual amino acids in sections of normal and deficient roots (Table 4) has shown that asparagine is the major amino acid component in all sections. The contents of many amino acids in the tissues are affected by deficiency, both increases and decreases being observed, frequently with different effects in different sections of the root. Examination of the results in Table 4 would suggest that the increased total levels, reported in Tables 1–3, for amino acids in the soluble fractions from sections of deficient roots are due partly to increased concentrations in the deficient tissue, but mainly to the increased fresh weight of the sections.

TABLE 3. ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM TIPS OF NORMAL AND BORON DEFICIENT ROOTS

	Normal tissue (3 mm tips)	Deficient tissue (3 mm tips)
ATP–pyrophosphate exchange (nmoles/mg protein/min)		
No added amino acids	32·7 ±3·7 (3)	33·7 ±0·9 (3)
Plus seventeen amino acids (0·1 mM)	69·3 ±2·4 (3)	59·0 ±1·5 (3)
Content of endogenous amino acid in incubation mixture (nmoles)		
	1·27±0·07(3)	1·40±0·06(3)
Soluble protein extracted per 100 roots (mg)		
	3·4 ±0·06(3)	3·63±0·29(3)
Fresh weight of tissue per 100 roots (g)		
	0·277±0·033(3)	0·337±0·022(3)

Roots were harvested after 31 hr in nutrient solution at 22°, under conditions identical to those for the experiments quoted in Tables 1 and 2. 105,000*g* supernatant extracts were prepared and assayed for exchange activity and amino acid content as described under Materials and Methods. Three samples of 100 roots from each growth medium were extracted and assayed. The results are reported as the mean ± standard error of the mean for the given number of samples.

The levels of amino acids and amines can be considered to be the resultant of the rate of supply or synthesis and the rate of utilization or destruction. Presumably there are differences in these factors in the different tissues, bringing about the observed variation in levels. While the results presented do not suggest what these differences may be, they could provide a starting point for further investigation.

The finding of a decreased ATP–pyrophosphate exchange for deficient tissue, with the mixture of amino acids, poses the question as to whether the effect depends on all, or only some, of the amino acids present in the mixture. To investigate this, the exchange was assayed in the presence of individual amino acids, for extracts from three sections of both normal and deficient roots. The results of two experiments are shown in Table 5 for the 3 mm tips, in Table 6 for the 5 mm second sections and in Table 7 for the 5 mm third sections. The results in the first column of each table (for the normal roots harvested at 24 hr in the first experiment) are based on the protein content in the corresponding extracts from deficient tissue. Since

the protein content of the deficient extracts has been found to be higher than that of the normal ones, the values for the exchange so calculated are likely to err on the low side.

Some general conclusions seem possible from the results. There were no consistent changes in the patterns of activity with the various amino acids for the different tissues. As in the earlier experiments, the activity measured on a soluble protein basis was greatest in the root tip and decreased in the sections further up the root. For most amino acids a decrease

TABLE 4. AMINO ACID CONTENT OF VARIOUS SECTIONS OF NORMAL (B_+) AND BORON DEFICIENT (B_-) ROOTS

Amino acid	Tip (3 mm)		Section 2 (5 mm)		Section 3 (5 mm)	
	B_+	B_-	B_+	B_-	B_+	B_-
	(μmoles/g fresh weight)					
Alanine	2·90	1·00	2·00	0·88	1·65	1·15
Asparagine	10·6	14·3	18·0	12·0	20·0	38·8*
Aspartic acid	2·28	2·25	2·28	2·30	1·58	1·65
Cysteine	1·60	—	0·35	0·08	0·09	—
Glutamic acid	4·75	0·68	1·25	0·38	0·48	0·50
Glycine	0·25	0·85	0·20	0·24	0·17	0·30
Histidine	1·08	2·35	1·18	1·03	2·90	1·28
Isoleucine	0·20	0·35	0·28	0·25	0·25	0·25
Leucine	0·18	0·19	0·13	0·12	0·10	0·11
Lysine	0·35	0·19	0·055	0·045	trace	0·68
Methionine	—	0·028	0·022	0·028	trace	trace
Phenylalanine	0·60	0·55	0·58	0·63	0·35	0·80
Proline	—	trace	0·58	0·15	0·13	0·11
Serine	1·83	1·18	1·40	3·58	3·25	*
Threonine	1·15	0·90	0·45	1·10	0·90	1·18
Tyrosine	1·33	0·45	0·70	0·70	0·45	0·98
Valine	0·83	0·98	1·10	1·10	0·95	1·25
Phosphoserine	1·43	3·40	4·50	2·56	4·00	6·00
Glycerophosphoethanolamine	0·078	—	0·195	0·075	0 325	0 013
Phosphoethanolamine	0·11	0·10	0·33	0·17	0·21	0·11
Taurine	trace	0·04	2·10	0·80	1·93	0·03
Urea	2·45	0·55	0·35	1·33	2·08	0·25
Ornithine	0·58	0·40	—	—	—	0·70
Ethanolamine	1·15	0·68	0·24	0·24	0·04	0·04
3-Methyl histidine	—	0·55	0·38	0·30	0·13	—
β-Alanine	trace	0·168	trace	0·168	0·043	0·048
Fresh weight per 100 sections (g)	0·20	0·34	0·39	0·70	0·45	0·92

* The value tabulated for asparagine represents the sum of asparagine plus serine. Experimental details as described under Materials and Methods.

in the exchange was also observed for deficient as compared with normal tissue. The differences in the activities for the two types of tissue were generally greater for roots harvested at 48 hr than for those harvested at 24 hr.

The amino acid-dependent exchange presented is the difference between the exchange values determined in the presence and absence of added amino acid and is thus subject to the errors in both determinations. A major source of error in these values would lie in variation of the retention of (^{32}P)-pyrophosphate on the charcoal used for trapping the labelled ATP. The range of magnitude of the retention, as found from incubations without enzyme, was

TABLE 5. AMINO ACID-DEPENDENT ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM TIPS OF NORMAL (B_+) AND BORON DEFICIENT (B_-) ROOTS

Added amino acid(s) (3 mM)	Roots harvested at 24 hr				Roots harvested at 48 hr			
	Expt. 1		*Expt.* 2		*Expt.* 1		*Expt.* 2	
	B_+*	B_-	B_+	B_-	B_+	B_-	B_+	B_-
	Added amino acid-dependent ATP–pyrophosphate exchange (nmoles/mg protein/min)							
Alanine	1	8	3	0	2	1	2	3
Arginine	0	0	1	0	1	1	6	4
Asparagine	4	6	9	6	8	8	18	6
Aspartic Acid	5	5	5	2	3	4	5	5
Cysteine	14	11	16	11	16	12	15	0
Glutamic Acid	2	1	1	0	2	2	1	5
Glutamine	0	1	0	1	0	1	1	3
Glycine	1	0	0	2	4	0	1	0
Histidine	12	18	19	13	10	14	20	15
Hydroxyproline	0	0	1	1	3	1	0	1
Isoleucine	9	12	12	9	10	8	15	10
Leucine	61	50	44	36	52	47	59	39
Lysine	16	14	14	5	14	7	21	15
Methionine	7	3	6	4	6	2	9	2
Phenylalanine	18	15	14	16	16	10	17	10
Proline	16	13	13	13	13	7	15	9
Serine	7	3	7	4	1	6	5	6
Threonine	20	16	16	13	19	11	18	9
Tryptophan	6	8	7	9	10	6	12	9
Tyrosine	5	5	11	10	9	8	13	10
Valine	23	17	27	25	26	21	35	19
Nineteen amino acids	111	134	148	141	144	110	158	103
	Content of endogenous amino acid in incubation mixture (nmoles)							
	26	26	26	26	20	22	22	30
	Protein content of incubation mixture (μg)							
	—*	11·2	16·6	17·5	9·5	11·0	12·0	17·7
	Cumulative growth of roots at harvest (mm)							
	13·6 ± 1·3	13·1 ± 0·8	12·6 ± 0·8	14·1 ± 0·7	35·4 ± 2·5	22·1 ± 2·1	37·7 ± 2·3	22·1 ± 1·3

* Protein content of B_+ extract not available. The values for exchange presented in this column are based on the protein content of the concurrent B_- extract (see text).

On harvesting after the stated periods in boron deficient (B_-) or normal (B_+) nutrient solution at 26°, fifty roots were cut into sections with lengths of 3, 5 and 5 mm respectively, proceeding from the tip. 105,000g supernatant extracts were prepared in a final volume of 8–9 ml. The extract was used undiluted for the determination of protein and amino acid content. For assay of pyrophosphate exchange as described under Materials and Methods the extract was diluted 25-fold with medium A and 0·5 ml of the diluted material was used per 1 ml incubation mixture. The mixture of nineteen amino acids included all except cysteine and tyrosine of the twenty-one used individually. The cumulative growth of the roots at harvest is given as the mean ± the standard error of the mean for the increase in length of sixteen roots from the time of planting in normal and boron deficient liquid media to the time of harvest.

equivalent to 2 to 6 nmoles/mg protein/min. After correction for this retention the exchange in the absence of added amino acid (endogenous exchange) ranged from 4 to 10 nmoles/mg protein/min. It would be unwise to place emphasis on single values for the activation of an amino acid where the values fall below this range.

TABLE 6. AMINO ACID-DEPENDENT ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM SECOND SECTIONS OF NORMAL AND BORON DEFICIENT ROOTS

	Roots harvested at 24 hr				Roots harvested at 48 hr			
	Expt. 1		*Expt.* 2		*Expt.* 1		*Expt.* 2	
Added amino acid(s) (3 mM)	B_+*	B_-	B_+	B_-	B_+	B_-	B_+	B_-
	Added amino acid-dependent ATP–pyrophosphate exchange (nmoles/mg protein per min)							
Alanine	3	4	1	3	3	2	6	0
Arginine	0	2	3	2	3	2	4	0
Asparagine	5	4	8	4	5	9	15	4
Aspartic Acid	5	3	5	2	3	4	5	11
Cysteine	11	7	20	6	11	12	10	5
Glutamic Acid	3	7	1	3	1	0	3	1
Glutamine	3	1	6	0	2	2	0	0
Glycine	7	2	1	1	2	3	1	0
Histidine	13	11	15	11	10	2	15	7
Hydroxyproline	3	5	2	—	3	2	2	0
Isoleucine	11	9	12	5	7	5	17	5
Leucine	40	37	39	32	37	33	51	32
Lysine	11	8	8	1	12	7	12	6
Methionine	3	2	4	8	3	6	0	1
Phenylalanine	12	8	10	10	9	7	14	7
Proline	9	8	6	10	11	4	7	4
Serine	7	6	9	1	7	7	5	2
Threonine	13	11	9	10	11	6	12	3
Trytophan	9	9	11	5	10	15	12	7
Yyrosine	8	4	9	4	5	10	10	6
Valine	20	18	22	14	16	19	18	17
Nineteen amino acids	94	90	128	130	91	81	161	85
	Content of endogenous amino acid in incubation mixture (nmoles)							
	64	62	60	64	50	72	52	68
	Protein content of incubation mixture (μg)							
	—*	9·7	11·4	14·1	8·2	9·5	8·2	12·6

* Protein content of B_+ extract not available. The values for exchange presented in this column are based on the protein content of the concurrent B_- extract (see text). For experimental details see Table 5.

Another possibility which could limit the precision of this method for the determination of the activation of individual amino acids would be the presence of appreciable concentrations of the amino acid in the extract and therefore in the endogenous assay. The total concentrations of endogenous amino acids in the incubation mixtures lay in the range 2×10^{-5} to $8{\cdot}8 \times 10^{-5}$ mM. The results given in Table 4 for the contents of individual amino acids would indicate that only asparagine would be present at levels greater than one-tenth of this range. Thus, the exchange measured in the presence of a single added amino acid would not

be expected to have been greatly affected by the amount of the compound present endogenously.

Assessing the exchange for single amino acids in terms of the overall results an approximate classification can be made. Those amino acids—alanine, arginine, glutamic acid,

TABLE 7. AMINO ACID-DEPENDENT ATP–PYROPHOSPHATE EXCHANGE ACTIVITY FROM THIRD SECTIONS OF NORMAL AND BORON DEFICIENT ROOTS

	Roots harvested at 24 hr				Roots harvested at 48 hr			
	Expt. 1		*Expt.* 2		*Expt.* 1		*Expt.* 2	
Added amino acid(s) (3 mM)	B_+*	B_-	B_+	B_-	B_+	B_-	B_+	B_-
	Added amino acid-dependent ATP–pyrophosphate exchange (nmoles/mg protein/min)							
Alanine	5	0	0	1	1	3	—	3
Arginine	5	1	0	1	0	3	9	5
Asparagine	2	4	5	2	7	6	7	8
Aspartic acid	3	3	3	2	0	4	6	3
Cysteine	4	6	14	—	11	8	5	3
Glutamic acid	3	1	8	1	5	0	2	4
Glutamine	2	1	4	0	2	2	3	5
Glycine	0	2	3	3	2	0	1	0
Histidine	0	8	14	—	9	7	8	12
Hydroxyproline	0	2	4	—	2	0	2	3
Isoleucine	6	4	7	6	8	6	11	7
Leucine	23	29	32	22	30	37	32	32
Lysine	5	7	7	12	11	6	2	8
Methionine	0	6	3	4	1	4	3	5
Phenylalanine	4	8	14	7	8	9	12	9
Proline	6	5	8	11	9	3	3	5
Serine	3	4	10	—	4	10	0	5
Threonine	6	6	8	8	12	4	4	6
Tryptophan	3	5	10	6	5	10	8	12
Tyrosine	2	6	—	3	2	6	5	9
Valine	11	—	17	15	12	15	15	21
Nineteen amino acids	57	68	105	123	76	68	96	88
	Content of endogenous amino acid in incubation mixture (nmoles)							
	74	88	72	74	58	80	58	74
	Protein content of incubation mixture (μg)							
	—*	9·5	11·6	13·0	3·7	9·5	6·6	9·2

* Protein content of B_+ extract not available. The values for exchange presented in this column are based on the protein content of the concurrent B_- extract (see text). For experimental details see Table 5.

glutamine, glycine and hydroxyproline—which show low and variable values may be stimulating very little exchange under the conditions used. Those values which are low but consistent, as with asparagine, aspartic acid, methionine, proline, serine and tyrosine, can be considered as indicating some exchange. The final group stimulating high or very high exchange includes cysteine, isoleucine, leucine, lysine, phenylalanine, threonine and valine.

Overall the results show a decrease in the amino acid-dependent ATP–pyrophosphate exchange in boron deficient tissue as compared with the same sections of normal tissue. The

effect appears general for all amino acids and the difference between the differently treated tissues increases with the increased severity of the deficiency. In its distribution along the root the activity is greatest, on a soluble protein basis, in the tip and decreases up the root. Thus, in showing decreased activity in deficiency, a given section behaves more like a section found further from the tip in the normal root.

The distribution of enzymic activities should be considered in relation to the morphology of the tissues involved. In normal roots grown under the conditions used, the meristematic area lies within the region extending approximately 1·5 mm back from the tip, although some cell division is still occurring 3–4 mm from the tip. The main zone of cell elongation is approximately from 3–9 mm from the tip with little or no elongation occurring within 3 mm from the tip. There are definite indications of differentiation from 8 mm onwards with the first signs of vessel formation appearing 10–12 mm up the root.

For deficient roots, which have just ceased to grow and are showing thickening at the tip, that is, after approximately 48 hr in boron-free medium, there are still dividing cells in the first millimetre, although the procambial strand is already evident approximately 1–2 mm from the tip. The procambial strand is well formed in the region 3–8 mm from the tip but there are still some cells which are not fully elongated. Lateral initials are sometimes present in the 9–13 mm region and the presence of root hairs in this zone has been observed. As noted by others[3, 6] the distance of the region of differentiation from the tip seems to be dependent on the rate of growth. Once growth ceases the region is soon located in close proximity to the tip.

The changes in enzymic activities observed in deficiency can thus be accounted for by the continuance of the maturation, or differentiation, of the tissue, coupled with decreased elongation, so that tissue of a given stage of physiological maturity is found closer to the tip as deficiency progresses. The resultant change in the physiological nature of the tissue in a given section might be expected to give a parallel change in the biochemical properties observed in the section.

Thus, despite the fact that the changes in enzymic activities presented here and in the succeeding paper[4] have been observed at or before the point of cessation of growth of the deficient tissue, it seems likely that they are only secondary effects to physiological or morphological alterations in the tissue. This conclusion is supported by the finding of such changes in the activities of a variety of enzymes investigated in tissue at, or near, the point of cessation of elongation (see also 4). An implication of the results presented here and in the accompanying paper is that, in attempting to determine the direct action of boron in plant growth, no reliance can be placed on comparisons between normal tissue and tissue which is at, or past, the point of becoming macroscopically deficient. This criticism would apply to the majority of such studies presented in the literature to date.

MATERIALS AND METHODS

"Tick" or field beans (*Vicia faba* var. *minor*) were imbibed overnight in slowly running tap water. After discarding any damaged or infected seed, they were planted on to moist vermiculite or "Perlite" in a tray which was then placed in a polythene bag. In later experiments moist "Perlite" covered with blotting paper was used in place of vermiculite. The seed was germinated in the dark at either 22° or 26° for approximately 56–60 hr.

The germinated seedlings (radicle length 18–25 mm) were then cultured by the method of

[6] W. J. WHITTINGTON, *J. Exp. Botany* **10**, 93 (1959).

Neales,[3] in square polythene dishes (11 in. across, 5 in. depth, taking a volume of 9·6 l.). Up to 230 roots were grown per dish with growth measurements being made on 15 or 16 roots from each. In boron sufficient growth solutions, the micro-nutrient was supplied at a concentration of 0·5 ppm.

The bean roots were harvested and cut into the appropriate lengths with a scalpel, the root being laid out on a thin piece of glass over graph paper. On cutting, the sections were placed in chilled beakers in ice and were then ground in the cold in a glass mortar. The resulting fibrous pulp was transferred to a chilled glass homogenizer where it was dispersed in a small volume (usually 5 ml/100 sections) of Medium A (sucrose 0·35 M; $KHCO_3$ 0·035 M; KCl 0·025 M; $MgCl_2$ 0·004M)[7] by five passes with a loose fitting nylon pestle. The homogenate was then centrifuged in the cold at 15,000*g* for 15 min. The supernatant was again centrifuged at 105,000*g* for 60 min in a Model L Preparative Spinco centrifuge. The supernatant of this second centrifugation was the extract used for enzyme assay and is referred to as the "soluble" fraction.

Since tissues from normal and deficient roots, and even from different sections of the same roots, appear to differ in resistance to disruption, it is conceivable that the degree of extraction of cellular components achieved may vary considerably. Thus activities extracted per unit of tissue might not be comparable for different tissues. To overcome this difficulty total protein in the soluble fraction was used as the basis of comparison for results from different tissues. This assumes that changes in the extractability of the tissue components investigated are proportional to changes in the extractability of the soluble protein.

ATP–pyrophosphate exchange was assayed by the procedure of Hele and Finch.[8] The materials and methods for the earlier experiments (Tables 1–3) were essentially the same as those given by Finch and Birt[9] with incubation, for 5 min at 27°, of a mixture containing 3 μmole $Na_4^{32}P_2O_7$, 100 μmole tris–HCl buffer (pH 7·5), 6 μmole Na^5MgATP, 0·1 μmole of each of seventeen amino acids and 0·5 ml of enzyme extract in a total volume of 1 ml.

In experiments to assay the ATP–pyrophosphate exchange with individual amino acids (Tables 4–6), a number of modifications were tested and used to increase the sensitivity of the method. The extract was diluted 25-fold with Medium A (made 0·5 mM with respect to EDTA), before assay, to reduce the content of endogenous amino acids in the incubation mixture. The concentration of Na_2MgATP was increased to 12 mM and that of amino acids to 3 mM, while that of $Na_4^{32}P_2O_7$ was decreased to 1 mM. The incubation period was increased to 10 min, and the specific activity of the $Na_4^{32}P_2O_7$ was increased to allow small rates of exchange to be detected. To reduce the counts resulting from adsorption of pyrophosphate onto the charcoal, 2 ml of unlabelled $K_4P_2O_7$ (0·5 M, pH 7·2) was added to the charcoal in the filter before the trichloroacetic acid treated incubation mixture was decanted onto it. Using this method the retention of (^{32}P)-pyrophosphate onto the charcoal was reduced to 0·02–0·06 per cent of the added counts. This represented 0·2–0·6 nmoles of pyrophosphate corresponding to an exchange of approximately 2–6 nmoles/mg protein/min under the experimental conditions used.

^{32}P was counted in an M·6 liquid counter of 9 ml capacity (20th Century Electronics). Inorganic phosphate was estimated by the method of Allen.[10]

Protein was determined by the method of Cleland and Slater.[11]

[7] E. B. Keller and P. C. Zamecnik, *J. Biol. Chem.* **221**, 45 (1956).
[8] P. Hele and L. R. Finch, *Biochem. J.* **75**, 352 (1960).
[9] L. R. Finch and L. M. Birt, *Comp. Biochem. Physiol.* **5**, 59 (1962).
[10] R. J. L. Allen, *Biochem. J.* **34**, 858 (1940).
[11] K. W. Cleland and E. C. Slater, *Biochem. J.* **53**, 547 (1953).

Amino acid content of the soluble fraction was determined with ninhydrin[12] on the supernatant obtained after deproteinisation of the fraction with two volumes of ethanol. Results have been expressed as μmole amino acid using the colour yield of L-isoleucine as a standard.

For estimation of the content of individual amino acids, roots were harvested after 45 hr growth in a nutrient solution. After weighing, the tissue was ground and homogenized in 1% picric acid (5 ml/100 sections) and the homogenate centrifuged at 20,000*g* for 15 min. The resulting pellet was washed with a further volume of picrate, and the supernatant and washing combined and adjusted to a constant volume. The solution was acidified with conc. HCl to approximately pH 2, and the picric acid removed by shaking with ether. This ether extraction was repeated until the solution was clear. Ammonia in the extract was removed by adjusting the solution to pH 11–12 with 10 M KOH and holding it under vacuum for 3 hr. After re-adjustment to pH 7 and the addition of 0·2 ml of 0·5 M sodium sulphite, the extract was allowed to stand for a further 4 hr to remove glutathione.

The sample was then evaporated to dryness and dissolved in 5·0 ml of buffer (sodium citrate 2 H_2O, 19·6 g; conc. HCl, 16·5 ml.; thiodiglycol, 20 ml; BRIJ-35 detergent solution, 2 ml, and caprylic acid, 0·1 ml, in a total volume of 1 l.). The samples were then analysed according to the standard procedure[13] in a Beckman Amino Acid Analyser Model 120 B, using the 30°–50° run for physiological fluids.

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[12] G. E. CONNELL, G. H. DIXON and C. S. HANES, *Can. J. Biochem. Physiol.* 33, 416 (1955).
[13] D. H. SPACKMAN, *Instruction Manual for Beckman Model* 120 *B Amino Acid Analyser.* (1962).