The Requirement for Organic Nitrogen in Zea mays Embryos [1, 2]

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Previous investigations have shown that, during germination, there is a transfer of organic nitrogen from storage tissues to the developing embryo (16). Indeed in both barley (10) and pea (5) specific storage proteins are the first components to disappear. If this supply of storage nitrogen represents an obligate requirement for the developing embryo, the growth of excised embryos in culture with an adequate supply of carbohydrate should show abnormal features reflecting this deficiency.

Oat and barley embryos which grow feebly when removed from their storage parts are at least partially revived by additions of amino acids (9, 12). This suggests that the systems for the transformation of carbohydrate into amino acids are inadequate in the young embryo. The fact that Edelman et al. (8) found very little C^{14} from glucose in the proteins of cereal embryos supports this view. Excised maize embryos, which show normal increases in dry weight and shoot length when supplied with glucose (7), and which have been grown to maturity (1), present an apparent exception to the general picture. However, Nason (18) has shown that excised maize embryos have abnormally low levels of trypotophan, and when our own preliminary investigation showed that the amount of protein in such embryos was drastically reduced, an investigation was made of the importance of organic nitrogen normally supplied by the endosperm. A progress report of this work has appeared previously (20).

Methods

Maize grain (hybrid variety Wf 9 × 38–11) was sterilized briefly with chlorox, rinsed, and allowed to germinate on a thin layer of agar (1.5%) in the dark at 26°. When the root was 2 cm long (about 40 hr) the embryos (including scutella) were removed by sterile excision. Three or four normal seedlings (intact) or excised embryos were transferred to 10 ml of a mineral-salts solution (14, 18) supplemented with 2 % glucose. After a period of 50 to 70 hours in liquid culture in the dark the embryos were washed and placed in an oven at 70° for 12 hours. Nitrogen was determined by the Kjeldahl method (15) on 10 to 12 mg samples before and after extraction with 80 % ethanol.

In those cases where the concentrations of soluble amino acids were measured the embryos were extracted directly with 80 % ethanol. The ethanol extract was dried in vacuo at 35° and made up to volume in H_2O. Aliquots of this solution were used for soluble nitrogen and α-amino nitrogen determinations. The amino acids were washed free of other components in the alcohol extract by passage through a 6 × 1-cm column of Dowex-50 × 8 (H^+) cation exchange resin and eluted with 1N NH_4OH. They were further fractionated into acidic, neutral, and basic amino acids by the procedures of Hirs, Moore and Stein (13, 17). In this procedure glutamic and aspartic acids were adsorbed on a 20 × 1-cm Dowex-1 × 10 (acetate) column. Glutamine and asparagine in the water effluent from this column were hydrolyzed in 1N HCl for 4 hours at 100°. The resulting glutamic and aspartic acids were adsorbed on a second Dowex 1-× 10 (acetate) column. The water effluent from this column was passed through a 15 × 1 cm Dowex-50 × 98 (Na^+) column to adsorb the neutral and basic amino acids. The neutral amino acids were eluted with 15 ml of 0.1 M Na citrate (pH 5.0) and the basic amino acids, including γ-amino butyric acid, with 0.5 M Na citrate (pH 7.5). Glutamic and aspartic acids were eluted serially from the Dowex-1 × 10 (acetate) columns with 0.2 N acetic acid. After each step in the procedure the eluates were dried and the α-amino nitrogen of each fraction was determined with ninhydrin (24).

Results

Transfer of Nitrogen from the Endosperm to the Embryo. Results with intact maize seedlings (fig 1) showed that during germination the alcohol-insoluble nitrogen fraction increased in the embryo while it declined in the endosperm. The level of alcohol-soluble nitrogen increased temporarily in the endosperm and had not fallen below the initial level at 96 hours. There was a progressive increase in this fraction in the embryo after 20 hours. Usually about three-quarters of the loss from the endosperm was recovered as embryo nitrogen. This picture is sufficiently similar to well documented results obtained with barley (10, 11) to assume that organic nitrogen is transferred as amino acids or peptides derived from the hydrolysis of storage protein.

The Effect of Glucose on Embryo Growth and Nitrogen Content. In confirmation of earlier work (7) it was found that excised embryos grown on glucose or sucrose increased in dry weight at essentially the same rate as control (attached) embryos

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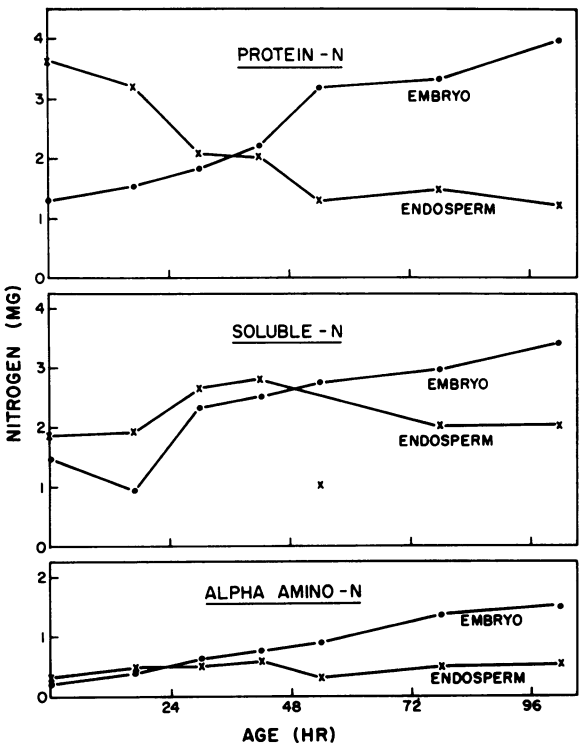

FIG. 1. The transfer of nitrogen from the endosperm to the embryo. The experimental conditions were described in table I. The age represents the time in liquid culture, and the nitrogen values are for 3 embryos or endosperms.

(table I). Maltose was less effective. With each of these substrates, however, the increases in total nitrogen were only a fraction of those shown by the controls. When the glucose content in the medium was increased to 5% there were increases in dry weight but the nitrogen content was not significantly higher. In addition an increase in sugars (soluble hexoses and those hexoses released by a 4 hour hydrolyses at 100° with 0.5 N HCl) corresponding to the increase in dry weight was observed. Light enhanced the accumulation of nitrogen in both control and excised embryos; however the nitrogen content of the excised embryo was still less than that of the control.

The results in table II show that during a second 72 hour incubation period there was a fivefold increase in organic nitrogen in the excised embryos. This almost equaled the maximal increase in the control embryos which was observed in the first 72 hour period. When conditions favor a normal accumulation of nitrogen (fig 1, table III) roughly half the organic nitrogen is found in the alcohol-insoluble residue. Therefore it is assumed that the increase in nitrogen found in the second 72 hour period represents a real increase in protein nitrogen. There was no change in the total nitrogen content of embryos excised after the first 72 hour period. These results suggest that the supply of organic nitrogen from the endosperm in some way curtails the endogenous synthesis within the embryo. After a lag period during which the levels of soluble nitrogen remain low the ability to synthesize organic nitrogen develops.

Table II

Evidence for a Lag in the Synthesis of Organic Nitrogen by Excised Embryos

The experimental conditions were described in table I. The initial value was determined at 40 hours. After the first 72 hour period fresh medium was added.

Conditions	mg/embryo		
	Control	Excised	
		at 40 hr	at 72 hr
Intial	1.15	1.15	...
72 hr	2.90	1.43	2.90
144 hr	3.60	2.80	2.85
	Increase/72 hr		
72 hr	1.75	0.28	...
144 hr	0.70	1.37	nil

Table I

The Effect on the Carbon Source and Light on the Dry Weight and Total Nitrogen of the Excised Embryos

After a germination period of 40 hours on agar, 4 embryos were incubated in 10 ml of a mineral-salts solution (see Methods) for an additional 50 hours.

	Dark		Light*	
	Dry wt (mg/embryo)	Nitrogen (mg/embryo)	Dry wt (mg/embryo)	Nitrogen (mg/embryo)
Initial (40 hr)	32.4	1.04	33.0	1.16
Intact	71.9	2.24	98.4	3.95
" + 2% glucose	79.4	2.36	...	...
Excised				
" + 2% glucose	64.0	1.15	62.7	2.28
" + 5% glucose	80.6	1.23	...	...
" + 2% sucrose	69.9	1.16	...	...
" + 2% maltose	50.1	1.15	...	...

* Seedlings exposed to 900 ft-c of white light for 16 hours each day.

Table III

The Effect of Glucose on the Soluble Amino Acid Pools

The experimental conditions were described in table I. The initial value was determined at 44 hour, the others after an additional 72 hours. In the intact embryo 2.19 mg of nitrogen were lost per endosperm during the incubation period whether glucose was present or not.

	mg/embryo			μg/embryo				
			α-amino	Acids		Amides		Neutral + Basic
	I*	S*	nitrogen	Glutamic	Asparatic	Glutamic	Aspartic	Amino acids
Initial	1.21	0.27	0.178	25.0	5.2	15.6	19.9	76
Intact	2.91	1.68	0.646	33.4	30.3	28.4	232.0	312
Intact + 2% glucose	2.43	1.31	0.534	51.0	25.7	8.9	51.0	398
Excised + 2% glucose	1.42	0.40	0.221	48.5	15.5	5.1	59.2	93

* I is the alcohol-insoluble and S the alcohol-soluble nitrogen.

Soluble Amino Acids in Control and Excised Embryos. The results in table III show that at a time when protein levels had increased in the control embryos there were also striking accumulations of soluble nitrogenous compounds. Glucose added to the control embryos had little effect either on the loss of nitrogen from the endosperm or on the increase of total nitrogen in the embryo. With it however there was a marked reduction in asparagine. By contrast, much smaller increases in nitrogen were observed in the excised embryos. The large difference in the soluble nitrogen levels between the excised and control embryos was accounted for by the neutral and basic amino acids. The amounts of glutamic and aspartic acids and their amides in excised and glucose-grown control embryos were comparable at 72 hours. Other experiments not reported here showed the same basic changes in the α-amino nitrogen components from 12 to 96 hours after the removal of the endosperm. Altered levels of alcohol-insoluble nitrogen were not observed until 24 hours after excision.

The differences in the individual neutral and basic amino acids are shown in table IV. The concentrations of the neutral amino acids in the excised embryo were less than the initial levels. The drop was most marked with proline. There were slight increases in the amounts of some of the basic amino acids, but these increases were small compared to those which occurred in the control embryos. After excision the embryo was apparently able to maintain more or less normal levels of glutamic and as-

Table IV

Soluble, Neutral, and Basic Amino Acids in Corn Embryos

The experimental conditions were described in table I. The initial value was determined at 36 hours, the others after an additional 72 hours. A Technicon Amino Acid Autoanalyzer was used to determine the individual pools, and usual procedures for the total values of each fraction.

	Initial (μg α-amino nitrogen/ embryo)	Intact (μg α-amino nitrogen/ embryo)	Excised (μg α-amino nitrogen/ embryo)
Neutrals	55.3	186	25.3
Threonine	4.0	17.1	2.5
Serine	8.0	22.9	4.7
Proline	26.0	23.8	2.4
Glycine	7.7	32.4	4.1
Alanine	9.2	31.6	7.6
Cystine*	trace	+++	trace
Valine	5.9	25.3	2.8
Methionine*	trace	+++	trace
Isoleucine	1.1	12.2	1.1
Leucine	1.6	13.6	1.2
Tyrosine	1.0	1.9	0.7
Phenylalanine	trace	2.1	0.7
Basic Amino Acids	5.5	42.3	12.2
Lysine	1.0	7.3	2.6
Histidine	2.5	25.3	6.8
Arginine	1.3	2.8	0.8
γ-amino butyric	0.6	6.0	1.8
Ornithine	0.9	2.1	0.3

* Since the alcohol extract was hydrolyzed for 12 hours with 6 N HCl quantitative data for the sulfur amino acids cannot be given. No peaks for cysteine or methionine were detected in unhydrolyzed samples.

partic acids and their amides (those components most closely related to the acids of the tricarboxylic acid cycle) while the formation of those amino acids requiring more complex synthetic pathways was slower than consumption in protein synthesis.

The Effect of Endosperm Preparations on Growth and Nitrogen Levels of the Excised Embryos. The synthesis of total organic nitrogen and protein was completely restored in the excised embryos when pieces of the separated endosperm were included in the incubation medium (table V). Glucose was however necessary for normal increases in dry weight. Water extracts were not effective in restoring protein synthesis in the excised embryos, but when the detached endosperms were incubated alone for 72 hours in sterile water, the resulting solution which will be designated "leachate" was just as effective in promoting protein synthesis as the endosperm itself. This activity was dialyzable and was absorbed by Dowex-50 (H^+) cation exchange resin. It was not absorbed by charcoal. When the cation fraction was hydrolyzed with Dowex-50 (H^+) resin (6) all the activity was recovered. Furthermore when this preparation was passed through a Dowex-1 (acetate) column all the activity moved with the neutral and basic amino acids. After hydrolysis there was a twofold increase in the total α-amino nitrogen, and a threefold increase in the neutral and basic amino acid fraction. No activity was recovered in the neutral and basic amino acid fraction when the unhydrolyzed sample was divided into acid, amide, and neutral plus basic amino acid fractions. Equally active leachates were obtained when the endosperms were removed from 0 to 72 hours after the beginning of germination (table VI). In addition the production of the active agent coincided with the loss of nitrogen from the endosperm pieces. Although the active agent of the leachate was heat stable, its production was prevented by boiling the endosperm pieces before the incubation (table V).

These results are most easily explained by supposing that amino acids, largely in the form of peptides, are derived by the enzymic hydrolysis of endosperm protein. Fractionation of the unhydrolyzed leachate would yield a neutral and basic amino acid

Table VI

Effect of Age of Endosperm on its Ability to Restore Protein Synthesis in Excised Embryos

Age (hr)	Endosperm nitrogen* mg N/endosperm		Activity of leachate mg N/embryo**
	Initial	Recovered from medium after 72 hr	
0	3.72	2.06	3.29
8	3.71	2.00	3.29
13	3.41	2.11	3.32
25	3.16	1.85	3.17
48	2.72	1.48	3.38
72	2.06	1.35	3.13
120	0.76	0.23	1.29

* The endosperm pieces were incubated in 10 ml H_2O for 72 hours. The solution (leachate) was quantitatively recovered, concentrated, and sterilized.

** The activity of the leachate was determined by measuring the N content of embryos excised at 40 hours and allowed to grow for an additional 72 hours in the leachates from an equal number of endosperms together with the usual concentrations of glucose and mineral salts. Excised embryos alone contained 1.26 mg N, and when incubated with endosperm pieces contained 3.17 mg N.

Table V

Characteristics of the Active Principle of the Endosperm

The experimental conditions were described in table I. The embryos were excised at 40 hours and allowed to grow for an additional 72 hours.

	Dry wt	Total nitrogen (mg/embryo)	Alcohol-insoluble nitrogen
Control	62.2	3.03	1.44
Excised	54.9	1.27	0.76
" + detached endosperm; no glucose	40.4	2.59	...
" + detached endosperm	59.5	3.02	1.44
" + boiled detached endosperm	57.5	1.15	...
" + water extract	53.0	1.17	...
" + leachate	61.1	3.09	1.56
" + boiled leachate	57.6	2.93	...
" + cation fraction of leachate			
total	61.6	2.91	...
acids	...	1.15	...
amides	...	1.10	...
remainder	...	1.11	...
" + hydrolyzed cation fraction of leachate			
total	59.8	3.14	1.50
acids	55.2	1.14	...
remainder	57.9	2.91	...

* The cation fraction of the leachate was hydrolyzed with Dowex-50H^+ resin (6) for 72 hours at 100° Hydrolysis with 6N HCl yielded inactive preparations.

fraction deficient in those amino acids combined in acid or amide peptide units. However the particular peptides normally present in the leachate are not in themselves essential for a normal increase in embryo protein.

If the amino acids derived from the endosperm protein were the active agent, an appropriate synthetic mixture of L-amino acids should restore the protein levels of the excised embryo. Preliminary experiments showed that an arbitrary mixture of amino acids caused only a moderate increase in the protein nitrogen content. The amino acid content of the hydrolyzed endosperm leachate was then determined. The results are presented in table VII. Glutamic and aspartic acids comprise only 25 % of the total α-amino nitrogen compared to the value of about 50 % for the embryo when the amides are included (table III). The absence of tryptophan and the relatively high concentrations of leucine and proline suggest that zein is the principal donor of amino acids. However the presence of lysine and glycine and the relatively higher concentrations of valine, histidine, and arginine show that other endosperm proteins also contribute. Those amino acids which are most severely affected by excision in the embryo (table IV) are the same ones which are supplied most abundantly by the endosperm (table VII).

A synthetic mixture of L-amino acids in concentrations corresponding to those of the hydrolyzed leachate completely restored the normal levels of total and protein nitrogen in the excised embryos (table VIII). The mixture was equally effective when glutamic and aspartic acids were omitted. When the other amino acids were omitted singly the total nitrogen content was consistently but only slightly less than in the control embryos. Alanine, glycine, and serine omissions gave results typical for each of the 15 amino acids of the neutral and basic amino acid fraction; proline was an exception. However as shown in table VIII with multiple deletions or alterations the nitrogen level was markedly reduced. When proline and leucine were reduced to one-third of the leachate levels the relative concentrations of the amino acids approach those of a casein hydrolysate and the mixture was not effective. In the zein

Table VII

Amino Acids in the Hydrolyzed Leachates Obtained from Excised Endosperms

A Technicon amino acid autoanalyzer was used to determine the individual amino acid concentrations of hydrolyzed leachates. No corrections were made for loss during hydrolyses.

	μmoles/ endosperm	Molar percentage Leachate	Zein*
Methionine sulfoxide	0.51	...	...
Aspartic acid	6.58	6.0	4.6
Threonine	3.74	3.4	2.7
Serine	7.46	6.8	7.9
Glutamic acid	21.40	19.3	20.4
Proline	12.35	11.2	9.8
Glycine	5.02	4.5	0
Alanine	12.01	10.9	13.9
Cystine	1.46	1.3	0.9
Valine	6.34	5.7	2.8
Methionine	1.20	1.5	1.7
Isoleucine	4.06	3.7	6.0
Leucine	17.28	15.6	19.7
Tyrosine	3.26	3.0	3.1
Phenylalanine	5.05	4.6	4.2
Lysine	1.34	1.0	0
Histidine	2.34	2.2	1.2
Arginine	1.94	1.8	1.1
Tryptophan	n.d.**	...	...

* The molar percentage of the individual amino acids in Zein were calculated from the values given in g amino acid/100 g (19).

** n.d. is not detected. This was determined spectrophotometrically on an unhydrolyzed sample.

Table VIII

The Effect of L-Amino Acids on the Protein Content of Excised Embryos

The experimental conditions were described in table I. The embryos were excised at 40 hours and allowed to grow for an additional 72 hours.

	Total nitrogen (mg/embryo)	Insoluble nitrogen
Excised embryo	1.27	0.83
" " + endosperm leachate	3.09	1.56
" + mixture of L-amino acids*	2.97	1.48
" " " — (glutamic +aspartic)	2.98	1.54
" " " — alanine	2.50	1.20
" " " — serine	2.45	1.17
" " " — glycine	2.21	1.16
" " " — (alanine+serine+glycine)	2.04	1.00
" " " — proline	2.01	0.96
" " casein mixture*	1.85	1.05
" " zein mixture*	1.93	1.04

* Synthetic mixture of L-amino acids resembling that of the endosperm leachate (table VII) was used. The concentration added for each embryo was equivalent to that produced per endosperm. In the casein mixture (19) the concentration of proline (4.12μmole) and leucine (5.76μmole) were altered. In the zein mixture (19) glycine and lysine were omitted and the concentrations of valine (3.12μmole), histidine (1.17μmole), and arginine (1.30 μmole) were altered.

mixture glycine and lysine were omitted, half the amounts of valine and histidine and two-thirds of the normal amount of arginine were added. Again protein synthesis was not restored. The reduction in the nitrogen content when alanine, glycine, and serine were omitted is surprising in view of their relatively easy biosynthesis. These results show that neutral and basic amino acids supplied by the endosperm are required by the embryo for a normal increase in protein nitrogen.

Discussion

The synthesis of protein may be roughly divided into the supply of amino acid precursors and the actual polymerization reactions. Growth, which is intimately associated with the synthesis of new protein, appears to be normal in the shoot and primary root meristems of the excised embryos. Hence in these regions the normal sequences leading to new protein are not affected by excision, and in fact nitrogen deficiencies in the meristematic region of the primary root (5 mm tip) were not detected (unpublished). If the polymerization reactions necessary for growth are not altered by excision, then the supply of the amino acid precursors must be rate limiting. This conclusion is supported by the fact that deficiencies in the soluble nitrogen fraction preceded those in the alcohol-insoluble residue. The complete restoration of normal levels of total and protein nitrogen when an appropriate mixture of L-amino acids were supplied to the excised embryo provides more conclusive evidence.

Normally the endosperm supplies the organic nitrogen required by the developing embryo. When the endosperm is removed, the scutellum (7) and perhaps the older regions of the embryo proper contribute a larger proportion of their organic nitrogen to the growing regions. The failure of the embryo to incorporate sufficient amounts of inorganic nitrogen from the medium results in the reduced net increase of total and protein nitrogen. After the removal of the endosperm, the soluble pools of the neutral and basic amino acids are severely reduced whereas the concentrations of glutamic and aspartic acids remain close to the control level. These results suggest that the synthesis of glutamic and aspartic acids from glucose and inorganic nitrogen is adequate in the excised embryo. Thus the rate-limiting sequence of reactions is to be found in the further conversion of these key amino acids.

Pioneer work by Kandler (14) showed that for every 3 moles of glucose taken up by the excised maize embryo 2 were incorporated into embryo materials. The results of the present investigation suggest that glucose in the medium is converted primarily to embryo carbohydrate, not to embryo protein. A similar situation pertains under normal conditions. In barley seedlings two-thirds of the carbohydrate lost from the endosperm is recovered in the embryo, principally as polysaccharides; one-third is respired (2). There is also an efficient transfer of amino acids derived from endosperm protein to protein in the growing parts in barley (10, 11). Superficially the conversion of carbohydrate carbon to protein carbon is not extensive under normal conditions. However when barley seedlings are treated with ammonia, protein synthesis is stimulated (4). There is a concomitant rise in glutamic and aspartic acids and their amides, but a striking decrease in the soluble pools of the more complicated amino acids. It therefore appears that under these conditions the synthesis of the secondary amino acids does not keep pace with that of glutamic and aspartic acids and thus protein synthesis results in a selective depletion of the more complex amino acids.

In bacteria the formation of amino acids derived from glutamic and aspartic acids is controlled by the concentration of the end-product (21, 22). This control is elicited either directly by limiting the rate of a specific reaction or indirectly by suppressing the formation of a specific enzyme. It is tempting to think in terms of similar control mechanisms during seedling development. Typically in wheat (23), barley (3, 10) and maize embryos the soluble amino acid pools are large. Moreover it is those amino acids whose synthesis is limited in the embryos which are supplied in large amounts by the endosperm. By analogy to the bacterial system the high soluble amino acid content could prevent the formation of those enzymes necessary for the synthesis of the 15 amino acids of the neutral and basic amino acid fraction. In support of this is the observation that during the initial 72 hours after excision, when the soluble amino acid levels are low, the embryo develops the ability to incorporate inorganic nitrogen and after this time achieves a normal net increase in organic nitrogen.

Summary

Although excised maize embryos showed normal increases in dry matter when cultured with glucose or sucrose, the accumulation of alcohol soluble and insoluble nitrogen was much inferior. With excision the soluble pools of the neutral and basic amino acids were the first components to fall below the control values. Subsequently the increase in protein nitrogen failed. Levels of glutamic and aspartic acids and their amides were close to the control values throughout the experimental period. Leachates obtained from detached endosperm pieces were effective in restoring protein synthesis in the excised embryos. Those amino acids which were most severely affected in the detached embryo were the same ones which were most abundantly supplied by the endosperm. In addition a synthetic mixture of L-amino acids with concentrations corresponding to those of the hydrolyzed leachate completely restored the normal levels of total and protein nitrogen. These results show that a limited supply of amino acids restricts the accumulation of alcohol-insoluble nitrogen during the 72 hours following the removal of the endosperm.

After this period, the limitation is overcome. It is suggested that the high levels of soluble amino acids normally arriving in the developing embryo from the endosperm restrict their synthesis within the embryo.

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Literature Cited

1. ANDRONESCU, D. I. 1919. Germination and further development of the embryo of *Zea mays* separated from the endosperm. Am. J. Botany 6: 443–52.
2. BARNELL, H. R. 1937. Analytical studies in plant respiration. VII. Aerobic respiration in barley seedlings and its relation to growth and carbohydrate supply. Proc. Roy. Soc. B, 123: 321–42.
3. BROWN, R. 1946. Studies on germination and seedling growth. III. Early growth in relation to certain aspects of nitrogen metabolism in the seedling of barley. Ann. of Botany (N.S.) X: 73–96.
4. COCKING, E. C. AND E. W. YEMM. 1961. Synthesis of amino acids and proteins in barley seedlings. New Phytologist 60: 103–16.
5. DANIELSON, C. E. 1951. The breakdown of high-molecular reserve proteins of peas during germination. Acta. Chem. Scand. 5: 551–54.
6. DIXON, A. ST.J. 1955. Studies on the use of sulfonated cation exchange resins for the hydrolysis of ovomucoid. Biochem. J. 60: 165–70.
7. DURE, L. S. 1960. Gross nutritional contributions of maize endosperm and scutellum to germination and growth of maize axis. Plant Physiol. 35: 919–25.
8. EDELMAN, J., S. I. SHIBKO, AND A. J. KEYS. 1959. The role of the scutellum of cereal seedlings in the synthesis and transport of sucrose. J. Exptl. Botany 10: 178–89.
9. FOLKES, B. F. 1959. The position of amino acids in the assimilation of nitrogen and the synthesis of proteins in plants. In: S. E. B. Symposia XIII: 126–47.
10. FOLKES, B. F. AND E. W. YEMM. 1958. The respiration of barley plants. X. Respiration and the metabolism of amino acids and proteins in germinating grain. New Phytologist 57: 106–31.
11. FOLKES, B. F., A. J. WILLIS, AND E. W. YEMM. 1952. The respiration of barley plants. VII. The metabolism of nitrogen and respiration in seedlings. New Phytologist 51: 317–41.
12. HARRIS, G. P. 1954. Amino acids as sources of nitrogen for the growth of isolated oat embryos. New Phytologist 55: 253–68.
13. HIRS, C. H. W., S. MOORE, AND W. H. STEIN. 1953. The chromatography of amino acids on ion exchange resins. Use of volatile acids for elution. J. Am. Chem. Soc. 76: 6063–65.
14. KANDLER, O. 1953. Über den Synthetischen Wirkungsgrad in vitro kultivierter Embryonen, Wurzeln und Sprosse. Z. Naturforsch. 8b: 109–17.
15. LONG, C. A. 1958. Simple microdetermination of kjeldahl nitrogen in biological materials. Analyt. Chem. 30: 1692–98.
16. MCKEE, H. S. 1958. Nitrogen metabolism in seedlings. In: Encyclopedia of Plant Physiology, Springer-Verlag VIII: 477–515.
17. MOORE, S. AND W. H. STEIN. 1951. Chromatography of amino acids on sulfonated polystyrene resins. J. Biol. Chem. 192: 663–81.
18. NASON, A. 1950. The distribution and biosynthesis of niacin in germinating corn. Am. J. Botany 37: 612–23.
19. NEURATH, H. AND K. BAILEY, ed. 1953. The Proteins: Chemistry, Biological Activity and Methods. vol II, Pt A, p 511 Academic Press, New York.
20. OAKS, A. AND H. BEEVERS. 1961. The nitrogen balance in excised maize embryos. Plant Physiol. 36: xviii.
21. ROBERTS, R. B., P. H. ABELSON, D. B. COWIE, E. T. BOLTON, AND R. J. BRITTEN. 1957. Studies of biosynthesis in *Escherichia coli*. Carnegie Institution of Washington. Publication 607. Washington, D. C. p 521.
22. UMBARGER, H. E. 1961. Feedback control by end product inhibition. In: Control Mechanisms in Cellular Processes. The Roland Press Co., New York. p. 67–86.
23. WEISSMAN, G. S. 1959. Influence of ammonia and nitrate on the proteins and free amino acids in shoots of wheat seedlings. Am. J. Botany 46: 339–46.
24. YEMM, E. W. AND E. C. COCKING. 1955. The determination of amino acids with ninhydrin. Analyst 80: 209–13.