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THE METABOLISM OF GLUTAMATE AND LEUCINE BY MAIZE TISSUES

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Abstract—Glutamate-U-^{14}C and leucine-U-^{14}C were rapidly absorbed and metabolized after incubation with maize embryonic axis, scutellum, and endosperm tissues. The embryonic axis of maize absorbed 85 per cent of the available glutamate and 50 per cent of the available leucine in 24 hr. Maize endosperm absorbed 15 per cent of the available amino acid in 6 hr and absorption remained at this level. In all tissues, 85 per cent of the glutamate carbon was metabolized to CO_2. Of the leucine absorbed, 85 per cent was unmetabolized or incorporated into the insoluble residue. In axis tissue nearly 50 per cent of the leucine appeared in the insoluble residue. The amount of leucine-^{14}C incorporated into the residue of scutellum (22%) and endosperm (6%) tissues suggested that protein synthesis was occurring at a rapid rate. Little radioactivity from either amino acid appeared in lipids, sugars, nucleic acids or amino acids other than the amino acid fed. These experiments showed that in maize tissues an extensive conversion of glutamate or leucine into other amino acids did not occur.

INTRODUCTION

THE germination of a seed and the subsequent growth of the resulting seedling produced marked changes in the nitrogenous constituents of a seed.[1] One of the most prominent was the breakdown of seed protein and the appearance of free amino acids.

Reserve proteins differ among plant species.[2] The distribution of amino acids found in these reserve proteins was vastly different from that found in the protoplasmic protein.[2–4] Many of the amino acids released from the reserve proteins during germination were involved in extensive metabolic transformations. Competition for the amino acids probably existed between the machinery for new protein synthesis and amino acid breakdown. The amino acid nitrogen may become involved in oxidative deaminations or transaminations.[5] The resulting degradation product then became involved in further catabolism or became the starting material for the synthesis of new amino acids.[6] Those carbon skeletons derived from amino acid catabolism have also been shown to be involved in respiration.[7] The actual amount of amino acid carbon diverted from the synthesis of new amino acids into respiration has not been determined.

Folkes and Yemm[8] have concluded that the major losses of glutamate, asparagine and proline in germinating barley could be accounted for in chlorophyll, other amino acids and other bases. However, Oaks[9] has reported that although many amino acids were deficient

[1] H. S. MCKEE, *Nitrogen Metabolism in Plants*. Clarendon Press, Oxford (1962).
[2] J. BONNER, *Plant Biochemistry*. Academic Press, New York (1950).
[3] D. BOULTER and J. T. BARBER, *New Phytologist* **62**, 301 (1963).
[4] B. F. FOLKS and E. W. YEMM, *Biochem J.* **62**, 4 (1956).
[5] E. W. YEMM and B. F. FOLKS, *Ann. Rev. Plant Physiol.* **9**, 245 (1958).
[6] J. INGLE, *Phytochem.* **2**, 353 (1963).
[7] E. W. YEMM, *New Phytologist* **48**, 315 (1949).
[8] B. F. FOLKES and E. W. YEMM, *New Phytologist* **57**, 106 (1958).
[9] A. OAKS, *Plant Physiol.* **41**, 173 (1966).

in maize roots, these deficiencies were not removed by other amino acids, and has suggested that the extensive interconversion of amino acids may be of minor importance.

The results of an investigation of the metabolism of glutamate-U-^{14}C and leucine-U-^{14}C by maize axis, scutellum and endosperm are presented in the present report. In all maize tissues, it was found that glutamate was metabolized principally to CO_2 while leucine was incorporated as leucine into insoluble residue. Less than 5 per cent of the label from the absorbed amino acids appeared in lipids, nucleic acids, organic acids, sugars or other amino acids.

RESULTS

Absorption of Glutamate and Leucine by Maize Tissues

The results of 24-hr glutamate or leucine absorption experiments are shown in Figs. 1 and 2. Over 98 per cent of the amino acid fed was accounted for. The embryonic axis of

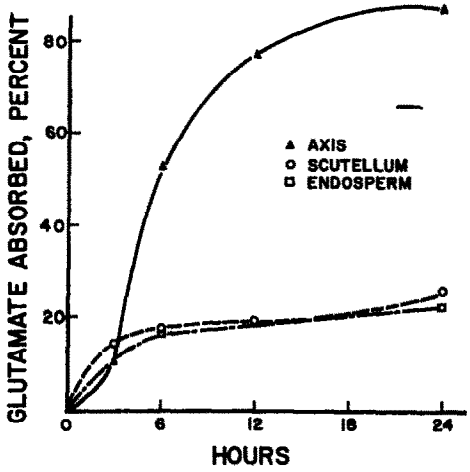

FIG. 1. ABSORPTION OF L-GLUTAMIC ACID-U-^{14}C WITH TIME BY VARIOUS MAIZE TISSUES.

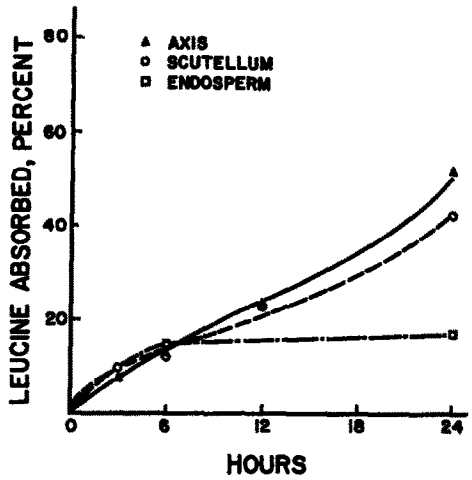

FIG. 2. ABSORPTION OF L-LEUCINE-U-^{14}C WITH TIME BY VARIOUS MAIZE TISSUES.

maize absorbed more leucine and glutamate than scutellum or endosperm tissues and absorbed considerably more glutamate than leucine. Maize endosperm absorbed about 15 per cent of the available amino acid in 6 hr and absorption remained at this level over an additional 18 hr.

Metabolism of Glutamate by Maize Tissues

The results of 24-hr feeding experiments of glutamate-U-^{14}C to maize embryonic axis, scutellum and endosperm are summarized in Tables 1–3.

TABLE 1. DISTRIBUTION OF ^{14}C FROM GLUTAMATE-U-^{14}C METABOLISM BY MAIZE EMBRYONIC AXIS

	Activity (cpm × 10^{-3})			
Fraction	3 hr	6 hr	12 hr	24 hr
Carbon Dioxide	47·1	390·8	621·8	775·6
Lipids	0·6	3·6	7·7	10·4
Organic Acids	25·8	48·4	97·3	50·9
Unmetabolized Glutamate	26·3	60·0	32·2	9·6
Aspartate	1·2	8·0	10·2	4·3
Insoluble Residue	2·6	6·5	9·5	13·5
All Others*	5·0	6·7	6·4	7·7
Total ^{14}C absorbed	108·6	524·0	785·1	872·0

* Sugars and nucleic acids which comprised less than 0·1 per cent of the total ^{14}C and neutral and basic amino acids.

TABLE 2. DISTRIBUTION OF ^{14}C FROM GLUTAMATE-U-^{14}C METABOLISM BY MAIZE SCUTELLUM

	Activity (cpm × 10^{-3})			
Fraction	3 hr	6 hr	12 hr	24 hr
Carbon Dioxide	47·6	91·7	127·0	196·0
Unmetabolized Glutamate	86·4	71·2	70·0	29·8
Aspartate	1·7	1·8	2·2	1·5
Insoluble Residue	0 1	1·3	2·3	9·0
All Others*	9·1	13·5	18·5	22·4
Total ^{14}C absorbed	144·9	179·5	220·0	258·7

* Sugars, organic acids, lipids and nucleic acids which comprised less than 4 per cent of the total ^{14}C and neutral and basic amino acids.

TABLE 3. DISTRIBUTION OF ^{14}C FROM GLUTAMATE-U-^{14}C METABOLISM BY MAIZE ENDOSPERM

	Activity (cpm × 10^{-3})	
Fraction	6 hr	24 hr
Carbon Dioxide	8·8	82·9
Unmetabolized Glutamate	174·5	140·8
All Others*	2·8	3·6
Total ^{14}C absorbed	186·1	227·3

* Comprised of sugars, organic acids, amino acids other than glutamate, nucleic acids, lipids and insoluble residue.

Unmetabolized glutamate-^{14}C and $^{14}CO_2$ accounted for the largest portion of the total ^{14}C. Up to 2 per cent of the label appeared as aspartate and smaller amounts were found in glycine, serine and arginine. In maize axis, 89 per cent of the glutamate-^{14}C absorbed in 24 hr was metabolized to $^{14}CO_2$. The corresponding figure for scutellum tissue was 76 per cent and endosperm tissue, 37 per cent.

In maize embryonic axis, there was a considerable incorporation of the label into organic acids. Small amounts of ^{14}C were found in this fraction in scutellum and endosperm tissue. Paper chromatography showed that these organic acid fractions were comprised principally of the citric acid cycle intermediates, malate and citrate.

A steady increase occurred in incorporation of label into lipids and insoluble residue in axis and scutellum tissue. Endosperm tissue incorporated less than 0·5 per cent of the available glutamate into the insoluble residue. Hydrolysis of the insoluble residue from all three tissues showed that glutamate contained all of the label in this fraction.

In maize axis and scutellum tissue, less than 10 per cent of the ^{14}C from glutamate-U-^{14}C spread to sugars, nucleic acids and amino acids other than glutamate. In endosperm tissue, the corresponding figure was less than 1 per cent. However, only 6 per cent of the glutamate absorbed by maize endosperm in 6 hr was metabolized, and respiration measurements showed that O_2 uptake by endosperm tissue was 30 μl/hr/g, considerably lower than axis tissue (525 μl/hr/g) or scutellum tissue (400 μl/hr/g). Respiration measurements between samples varied less than 5 per cent.

Metabolism of Leucine by Maize Tissues

The results of 24-hr feeding experiments of leucine-U-^{14}C to maize axis, scutellum and endosperm are summarized in Tables 4–6. In all tissues, the bulk of the ^{14}C from leucine-U-^{14}C appeared as unmetabolized leucine or leucine incorporated into the insoluble residue. Acid hydrolysis of the residue revealed that leucine was the only labeled compound incorporated.

Some $^{14}CO_2$ was produced in all tissues indicating that leucine-^{14}C was degraded. Analysis of the organic acid fraction showed that citric acid cycle intermediates were labeled. Little label occurred in lipids, sugars, nucleic acids or amino acids other than leucine.

Nearly half of the leucine-^{14}C absorbed by the axis tissue was incorporated into the insoluble residue. A steady increase in this fraction occurred with time. In 3 hr, half of the leucine-^{14}C absorbed by scutellum tissue was incorporated into the residue and although the total

TABLE 4. DISTRIBUTION OF ^{14}C FROM LEUCINE-U-^{14}C METABOLISM BY MAIZE EMBRYONIC AXIS

Fraction	Activity (cpm × 10^{-3})			
	3 hr	6 hr	12 hr	24 hr
Carbon Dioxide	0·8	3·1	8·3	18·0
Organic Acids	5·6	3·2	17·7	51·4
Unmetabolized Leucine	43·0	71·9	112·2	214·2
Insoluble Residue	35·6	55·8	96·8	227·6
All Others*	1·4	1·4	3·4	10·2
Total ^{14}C absorbed	86·4	135·4	238·4	521·4

* Comprised of lipids, sugars and amino acids other than leucine.

TABLE 5. DISTRIBUTION OF ^{14}C FROM LEUCINE-U-^{14}C METABOLISM BY MAIZE SCUTELLUM

Fraction	Activity (cpm × 10^{-3})			
	3 hr	6 hr	12 hr	24 hr
Carbon Dioxide	0·3	0·4	7·8	26·0
Organic Acids	1·4	3·0	20·2	47·0
Unmetabolized Leucine	34·2	82·8	157·6	249·8
Insoluble Residue	57·7	41·5	49·1	94·9
All Others*	1·4	1·4	4·0	9·4
Total ^{14}C absorbed	95·0	129·1	238·7	427·1

* Comprised of lipids and amino acids other than leucine.

TABLE 6. DISTRIBUTION OF ^{14}C FROM LEUCINE-U-^{14}C METABOLISM BY MAIZE ENDOSPERM

Fraction	Activity (cpm × 10^{-3})	
	6 hr	24 hr
Carbon Dioxide	0·9	1·4
Organic Acids	5·2	17·5
Unmetabolized Leucine	130·5	139·3
Insoluble Residue	9·1	10·1
Total ^{14}C absorbed	145·7	168·3

absorption of leucine increased four-fold in the next 21 hr (Fig. 2), incorporation into the residue increased only two-fold (Table 5). A small amount of leucine was incorporated into the residue by endosperm tissue.

About 15 per cent of the leucine absorbed in 24 hr was catabolized by all maize tissues, although the total amount of leucine absorbed by maize endosperm was considerably less than the other two tissues (Fig. 2).

DISCUSSION

Several facts stand out as a result of the feeding experiments described. One of the more obvious is the ease with which glutamate is metabolized to CO_2. Glutamate, by virtue of its metabolic conversion to α-ketoglutarate, could serve as an important link between the intermediate metabolism of proteins and of carbohydrates. Folkes and Yemm[8] have concluded that in germinating barley the major losses of glutamate, aspargine and proline could be accounted for in chlorophyll, other amino acids, and other bases. In all of the maize tissues studied however, less than 5 per cent of the radioactivity from glutamate-^{14}C or leucine-^{14}C was found in these fractions.

The labeling patterns from leucine-U-^{14}C are of particular interest. Previous studies with carrot tissues[10, 11] have shown that a maximum of 14 per cent of the leucine absorbed

[10] L. BRIT and F. HIRD, *Biochem. J.* **70**, 277 (1958).
[11] W. E. SPLITTSTOESSER, *Plant Physiol.* **41**, 755 (1966).

was degraded. In actively growing tissues such as maize used here, more leucine might be expected to be metabolized. As in storage tissues however, the bulk of the leucine was incorporated into protein or remained unmetabolized (Tables 4–6). Some leucine was degraded to CO_2 presumably via the citric acid cycle as intermediates of this cycle were labeled.

Maize axis tissue was the most metabolically active, metabolizing more of the amino acids to CO_2 and spreading the radioactivity into more compounds than either endosperm or scutellum tissues.

Although the protein and nucleic acid content of maize scutellum remain fairly constant during germination,[12] leucine was incorporated into scutellum protein in sizable amounts (Table 5). This indicates that considerable protein synthesis was occurring in this tissue. The glyoxylate cycle operates in maize scutellum[13] and Beevers[14] has shown that an increase in the enzymatic activity of this cycle occurs during germination. If a synthesis of glyoxylate cycle and protein degrading enzymes occurred, along with a simultaneous depletion of storage protein, this could produce little change in the total protein content for a limited time, while incorporating a sizable amount of leucine into protein.

In maize scutellum, the carbon from glutamate can become incorporated into sugars[15] and significant amounts of ^{14}C were found in sugars in these experiments. However, the carbon from leucine was not converted into sugars, emphasizing again the difference in metabolism of these two amino acids by maize tissues.

Maize endosperm absorbed 20 per cent and metabolized 20–40 per cent of the glutamate or leucine absorbed. The aelurone layer of barley endosperm has been shown to be very active.[16] Presumably the metabolism which occurred in maize endosperm, took place in the aelurone layer. As was the case in axis and scutellum tissue, a significant amount of the leucine absorbed by endosperm tissue was incorporated into the residue (Table 6) while glutamate was primarily degraded to CO_2 (Table 3).

These experiments show that in maize tissues an extensive conversion of glutamate or leucine into those amino acids which are limiting does not occur. Rather the amino acid carbon is used in respiration or used directly in protein synthesis. This is in agreement with Joy and Folkes[17] who showed that in excised barley embryos glutamine yielded appreciable quantities of respiratory CO_2 while little carbon was lost as CO_2 from leucine.

The extent to which glutamate and leucine undergo further metabolism appears to be related to the closeness of the amino acid to the pathways of carbohydrate catabolism. Thus the amount of label lost as respiratory CO_2 from glutamate was greater than that lost from leucine, as leucine is a greater number of reaction steps from the carbohydrate source. The nitrogen derived from the catabolism of these amino acids is probably used in the synthesis of new and different amino acids. Sims and Folkes[18] have shown that, in most cases, the majority of amino acids are formed by transamination rather than by interconversion of amino acids as such. It would appear, therefore, that a series of keto-acid interconversions must occur and that the excess keto-acids are catabolized further, with the limiting keto-acids being derived from sugar.

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[13] A. OAKS and H. BEEVERS, *Plant Physiol.* **39**, 431 (1964).
[14] H. BEEVERS, *Nature*, **191**, 433 (1961).
[15] W. E. SPLITTSTOESSER, *Plant Cell Physiol. (Tokyo)* **7**, 711 (1966).
[16] J. E. VARNER and G. R. CHANDRA, *Proc. Natl Acad. Sci. U.S.* **52**, 100 (1964).
[17] K. W. JOY and B. F. FOLKES, *J. Exptl Botany* **49**, 646 (1965).
[18] A. P. SIMS and B. F. FOLKES, *Proc. Roy. Soc. (London), Ser. B* **159**, 479 (1964).

EXPERIMENTAL

MATERIALS AND METHODS

Plant Material

Maize seeds (*Zea mays*) were germinated on moist filter paper for 3 days at 25°. The germinated seedlings were separated into the embryonic axis, scutellum and endosperm. The seed coat was removed from the endosperm. All tissues were rinsed in distilled water and lightly blotted dry.

Radioactive Materials

L-Leucine-U-^{14}C and L-glutamic acid-U-^{14}C were supplied by Nuclear Chicago or Calbiochem. The stock material was dissolved in distilled water to give a solution of 1 μmole of amino acid having 7·5 μc of ^{14}C in 0·05 ml. This gave 10^6 cpm with our detection equipment. Paper chromatography revealed no other labeled compounds in the samples.

Incubation Procedure

Duplicate samples of tissues (15 embryonic axis or scutellum, 10 endosperm) were placed in No. 15 medium fritted glass filter funnels containing 0·05 ml of the amino acid and 10 ml of 0·1 M potassium phosphate, pH 6·7 and 100 μg of tetracycline to prevent bacterial contamination.[19] After termination of the experiments, the incubation medium was filtered and no respiratory activity was noted on the filter discs. In addition, the filtered incubation medium was assayed both for respiration and the presence of compounds other than the amino acid fed. As the results of this were also negative, it was concluded that bacterial contamination did not play a significant role in the results presented. No difference in the metabolism of maize tissues was noted in the absence of the bacterostat.

Air was passed through a 50% KOH scrubber and then through the base of the filter funnels to aerate the tissue suspended in solution. Respired $^{14}CO_2$ was carried in the air stream and bubbled through 10 ml of 20% KOH in a 50 ml centrifuge tube. The absorbed CO_2 was converted to $BaCO_3$, filtered and the filter paper counted for radioactivity, by a Geiger-Muller tube. The counts were corrected for background and self-absorption.[20]

Analytical Methods

At predetermined times the tissues were removed, rinsed with deionized water to remove any non-absorbed amino acid and transferred into 50 ml of boiling 100% ethanol for 3 min. The ethanol was decanted and the tissues were ground with a mortar and pestle. The residues were successively extracted in boiling 80% ethanol, 50% ethanol and then again in 80% ethanol. The extracts were combined and taken to dryness at 35° under reduced pressure.

The dried ethanol extract was dissolved in water, extracted with ether (lipid fraction) and then fractionated sequentially on 1 × 6 cm columns of Dowex 50 (H+) and Dowex 1 (Formate) resins.[21] The amino acid fraction from the Dowex 50 (H+) column was further fractionated on a Dowex 1 (acetate) column into acidic amino acids, and neutral and basic amino acids.

The ethanol insoluble residue was extracted with hot TCA to remove nucleic acids as described by Ingle.[6] The remaining residue was hydrolyzed with 1 N HCl for 12 hr at 200° and then treated in the same manner as the ethanol extract. Aliquots of the fractions were taken and assayed for radioactivity.

The components of the fractions were separated by paper chromatography in butanol:propionic acid: water (623:310:437 v/v/v) and tert-butanol:methylethylketone:formic acid:water (40:30:15:15).[22] After chromatography, the radioactive components were located by use of a strip counter.

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