INCORPORATION OF RADIOACTIVE ACETATE AND SUCROSE INTO AMINO ACIDS AND PROTEIN OF EXCISED ORGANS OF RED KIDNEY BEAN[1,2]

BRUCE J. ROGERS[3]

KERCKHOFF LABORATORIES OF BIOLOGY, CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

Little information is available on rates of incorporation of C^{14}-labeled amino acids into plant protein. The first reported work was that of Arreguin, Bonner and Wood (1) in 1951 in which C^{14}-carboxyl-labeled acetate was supplied to guayule plants. The bulk of the radioactivity in the protein fraction was located in leucine, glutamate and aspartate. Boroughs and Bonner (4) incubated C^{14}-carboxyl-labeled leucine and glycine with corn and oat coleoptiles and recovered radioactive protein. More recently Webster (13) has carried out experiments on the incorporation of C^{14}-labeled glycine, glutamate, and aspartate into bean hypocotyl mitochondria. This work has been extended to show incorporation of glutamate into the proteins of several different plants (14). Racusen and Aronoff (11) supplied $C^{14}O_2$ to discs cut from soybean leaves and found considerable amounts of radioactivity in protein. In the present work the rates of incorporation of C^{14}-labeled amino acids into the protein of detached leaves, stems and roots of red kidney bean plants were studied. This is of interest for two reasons: 1) the relative rates of incorporation of amino acids into the protein of the three major organs of a particular plant have heretofore not been compared, and 2) it was desired to investigate further the factors connected with the rapid decrease in protein contents of leaves after excision (6).

In connection with the latter point, the incorporation of C^{14} into the protein-bound amino acids of the organs was studied. Both Paech (10) and Chibnall (6) have suggested that a generalized mass action relation may control the protein content of detached leaves, protein synthesis being dependent on the maintenance of a sufficiently high concentration of amino acids. Chibnall has suggested that the formation of alpha-keto derivatives of the amino acids may disturb this equilibrium. The results of Wood and Cruickshank (15) and of Kemble and Macpherson (7) tend to support Chibnall's suggestion. It was demonstrated by these workers that the concentrations of certain amino acids decrease in detached leaves even though these amino acids are being liberated by protein hydrolysis and the total amino acid nitrogen is increasing. It is implied that the mass action relation between protein and amino acids may be deranged by the high rate of catabolism of these amino acids (15). The data led Bonner to suggest that perhaps leaves may be unable to synthesize all of the required amino acids. Other organs—probably the roots—do synthesize all of the amino acids and supply them to the leaves in the intact plant (3). This study was designed to test the possibility that the synthesis of certain amino acids is localized in organs other than the leaves.

[1] Received June 22, 1955.

[2] Report of work supported in part by the Polychemicals Department, E. I. du Pont de Nemours and Company.

[3] Present address: Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana.

MATERIALS AND METHODS

Red kidney bean (*Phaseolus vulgaris*) plants were grown to a height of 30 to 45 cm in the Earhart Plant Research Laboratory at a day temperature of 23° C and a night temperature of 17° C. The plants were harvested and divided into still-expanding leaves and stems and roots. Twenty-gram portions of each organ were floated partially submerged in a solution containing either C^{14}-uniformly-labeled sucrose (initial activity 67,000 cpm/ml; final activity 30,000 cpm/ml) or C^{14}-carboxyl-labeled sodium acetate (initial activity 115,000 cpm/ml; final activity 70,000 cpm/ml) for different periods of time up to 12 hours. The specific activity of the labeled sucrose was 0.17 mc/millimole, and that of the labeled acetate was 1 mc/millimole. The experiment was carried out at room temperature at light intensities of about 10 fc. At the end of each incubation period the tissues were removed from the solution, rinsed two times in water, and quick-frozen in a mixture of solid CO_2 and acetone. Each portion of frozen material was homogenized in water in a Waring blendor and the homogenate filtered. The protein in the filtrate was precipitated by addition of trichloroacetic acid (TCA)

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN AMINO ACIDS OF RED KIDNEY BEAN AFTER 12 HRS INCUBATION OF EXCISED ORGANS WITH ACETATE-1-C^{14} AND SUCROSE-C^{14}

AMINO ACID	ACETATE-1-C^{14}		SUCROSE-C^{14}	
	LEAVES	ROOTS	LEAVES	ROOTS
	cpm × 10^{-3}/millimole of amino acid			
Alanine	320	30	100	1
α-Aminobutyric acid	1	1	...	..
Arginine	10	5	6	10
Aspartic acid	100	50	150	80
Cystine	1	1	1	10
Glutamic acid	100	100	400	60
Glycine	650	250	60	50
Leucine-Isoleucine	1	4	10	4
Lysine	3	4	10	2
Methionine	1	2	3	10
Phenylalanine	1	1	20	10
Serine	40	100	150	30
Threonine	110	100	100	3
Valine	10	100	10	10

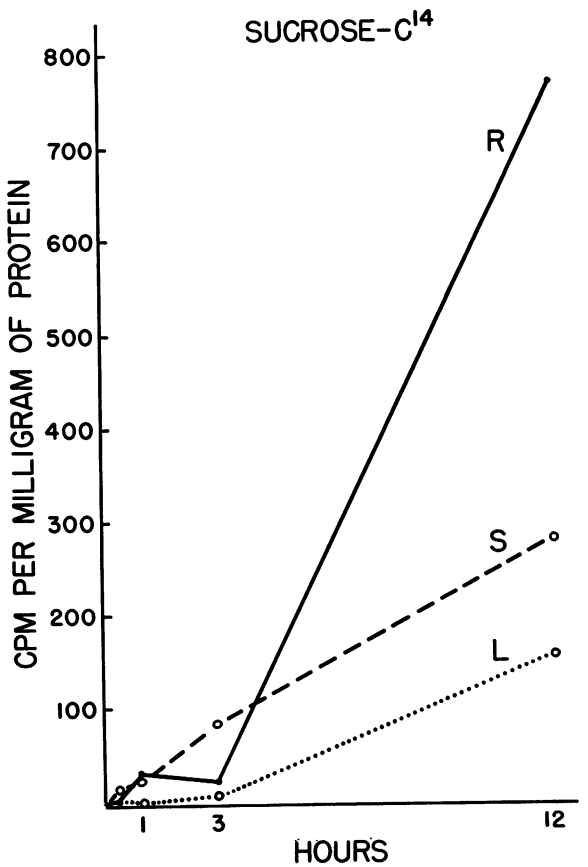

FIG. 1. The rate of incorporation of C^{14} of sucrose-C^{14} into leaf, stem and root protein of red kidney bean. R—roots; L—leaves; S—stems.

to a final concentration of 2 to 3 % and removed by low-speed centrifugation. The protein was washed in TCA, dissolved in alkali and re-precipitated by addition of TCA. This treatment was found adequate for the removal of adsorbed radioactive material. The protein was spread evenly on aluminum planchets and the incorporated radioactivity measured with a thin-window Geiger-Müller tube by standard techniques. A sufficient number of counts were taken to insure a standard error of not more than ± 5 %. Correction was made for self-absorption according to the recommendations of Calvin et al (5).

The supernatant solution was reduced in volume, placed on a Dowex-50 ion exchange column in the acid phase, and eluted by successive applications of water and HCl. The treatment sufficed to separate the free amino acids from sugars and organic acids almost entirely. The aliquots collected from the acid elution were combined and the amino acids were separated into groups by paper chromatography (8). The individual amino acids were isolated from the groups by a second series of paper chromatographs (2). This double system of chromatography served both to isolate the amino acids and further to separate them from contaminants. Each amino acid was eluted from the paper and divided into two equal aliquots. One portion was placed in a copper planchet for assay of radioactivity by means of a gas-flow counter to a probable error of ± 8 %. The amino acid concentration of the second portion was determined by the ninhydrin method of Moore and Stein (9).

RESULTS AND DISCUSSION

The results for the amino acids were calculated as cpm/millimole of amino acid and are given in table I for the 12-hour incubation period. Data are not available from the present work for all of the amino acids which are found in plants, but it is apparent that most of those found in plant protein which are measured here are synthesized by leaves. It does not seem probable, therefore, that protein loss in excised leaves can be attributed to a lack of amino acid synthesis in leaves.

The data obtained for the incorporation of amino acids into protein are shown in figures 1 and 2. A comparison may be made of the relative rates of incorporation of C^{14} into the protein of the 3 organs. After a lag period of some 3 hours large differences in the rate of incorporation of C^{14} into protein become apparent. In both the acetate and the sucrose experiments, the protein radioactivity rises much more rapidly in the root tissue than in either the stem

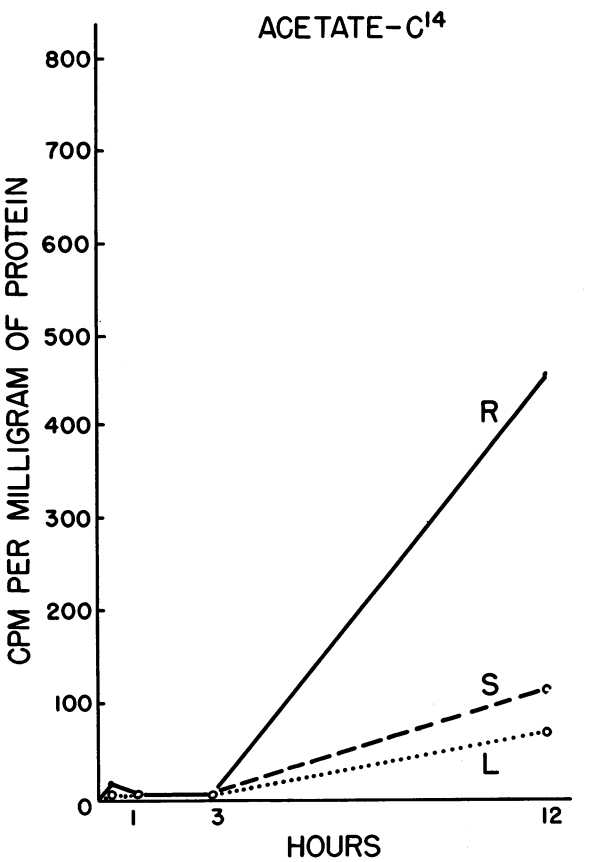

FIG. 2. The rate of incorporation of C^{14} of acetate-C^{14} into leaf, stem and root protein of red kidney bean. R—roots; L—leaves; S—Stems.

or the leaf tissue. The possibility that this might be due to higher specific activities for the amino acids of the root is ruled out from the studies on the amino acids.

The differences in rates of incorporation cannot definitely be attributed to excision on the basis of the present work. However, a comparison may be made with data on incorporation into the tissues of intact plants from the report of Vickery et al (12) on the incorporation of N^{15} ammonia into the protein of intact tobacco plants. Results obtained in the latter work show an incorporation of N^{15} into root protein which is twice that found for the incorporation of N^{15} into leaf protein. The isotope was applied to the roots, so a translocation factor may be involved. In the work reported on here, the ratio of incorporation into root protein to incorporation into leaf protein is 5 : 1 for the sucrose experiments and 6 : 1 for the acetate experiments after 12 hours. The extent to which the experiments with N^{15} and C^{14} are comparable is, of course, uncertain. Under the conditions of the experiment the data demonstrate, however, that incorporation of amino acids proceeds at a considerably greater rate into protein of excised roots than into protein of excised leaves. In so far as incorporation may be said to be synthesis, it seems possible that the decrease in protein content of detached leaves is due in part, at least, to a lower rate of protein synthesis.

The author wishes to acknowledge the advice and assistance of Professor James Bonner and Dr. G. C. Webster during the course of this work.

Literature Cited

1. Arreguin, B., Bonner, J., and Wood, B. J. Studies on the mechanism of rubber formation in the guayule. III. Experiments with isotopic carbon. Arch. Biochem. Biophys. 31: 234–247. 1951.
2. Berry, H. K., Sutton, H. E., Cain, L., and Berry, J. S. Biochem. Inst. Studies IV. II. Development of paper chromatography for use in the study of metabolic patterns. Univ. Texas Publ. No. 5109: 22–55. 1951.
3. Bonner, J. Plant Biochemistry. Pp. 1–537. Academic Press, Inc., New York. 1950.
4. Boroughs, H. and Bonner, J. Effects of indoleacetic acid on metabolic pathways. Arch. Biochem. Biophys. 46: 279–290. 1953.
5. Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. and Yankwich, P. F. Isotopic Carbon. Pp. 1–376. John Wiley & Sons, Inc., New York. 1949.
6. Chibnall, A. C. Protein Metabolism in the Plant. Pp. 1–306. Yale Univ. Press, New Haven, Connecticut. 1939.
7. Kemble, A. R. and Macpherson, H. T. Liberation of amino acids in perennial rye grass during wilting. Biochem. Jour. 58: 46–49. 1954.
8. Levy, A. L. and Chung, D. Two-dimensional chromatography of amino acids on buffered papers. Anal. Chem. 25: 396–399. 1953.
9. Moore, S. and Stein, W. H. Photometric ninhydrin method for use in the chromatography of amino acids. Jour. Biol. Chem. 176: 367–388. 1948.
10. Paech, K. Über die Regulation der Eiweissumsatzes und über den Zustand der proteolytischen Fermente in den Pflanzen. Planta 24: 78–129. 1935.
11. Racusen, D. W. and Aronoff, S. Metabolism of soybean leaves. VI. Exploratory studies in protein metabolism. Arch. Biochem. Biophys. 51: 68–78. 1954.
12. Vickery, H. B., Pucher, G. W., Schoenheimer, R., and Rittenberg, D. The assimilation of ammonia nitrogen by the tobacco plant, a preliminary study with isotopic nitrogen. Jour. Biol. Chem. 135: 531–539. 1940.
13. Webster, G. C. An energy dependent incorporation of amino acids into the protein of plant mitochondria. Plant Physiol. 29: 202–203. 1954.
14. Webster, G. C. Incorporation of radioactive glutamate into the proteins of higher plants. Plant Physiol. 29: 382–385. 1954.
15. Wood, J. G. and Cruickshank, D. H. The metabolism of starving leaves. 5. Changes in amounts of some amino acids during starvation of grass leaves, and their bearing on the nature of the relationship between proteins and amino acids. Australian Jour. Exptl. Biol. Med. Sci. 22: 111–123. 1944.