cytochrome c from different sources. Jour. Biol. Chem. 197: 701–708. 1952.
20. QUINLAN-WATSON, T. A. F. Aldolase activity in zinc-deficient plants. Nature 167: 1033–1034. 1951.
21. ROSENTHAL, O. and DRABKIN, D. L. Cytochrome c content and neoplastic mammalian epithelium and its correlation with body mass. Jour. Biol. Chem. 150: 131–141. 1943.
22. SCHNEIDER, W. C. and POTTER, V. R. The assay of animal tissues for respiratory enzymes. II. Succinic dehydrogenase and cytochrome oxidase. Jour. Biol. Chem. 149: 217–227. 1943.
23. SCHULTZE, M. O. The effect of deficiencies in copper and iron on the cytochrome oxidase of rat tissues. Jour. Biol. Chem. 129: 729–737. 1939.
24. SHEMIN, D. and WITTENBERG, J. The mechanism of porphyrin formation: the role of the tricarboxylic acid cycle. Jour. Biol. Chem. 192: 315–334. 1951.
25. SLONIMSKI, P. and EPHRUSSI, B. Action de l'acriflavin sur les levures. V. Le système des cytochromes des mutants "petite colonie." Ann. Inst. Pasteur 77: 47–63. 1949.
26. STEINBERG, R. A. A study of some factors influencing the stimulative action of zinc sulphate on the growth of *Aspergillus niger*. I. The effect of the presence of zinc in the cultural flasks. Mem. Torrey Bot. Club 17: 287–293. 1918.
27. STOTZ, E. The estimation and distribution of cytochrome oxidase and cytochrome c in rat tissues. Jour. Biol. Chem. 131: 555–565. 1939.
28. TIPTON, S. R. The relationship between certain vitamin B factors and the response to thyroid of succinoxidase and cytochrome oxidase of rat liver. Amer. Jour. Physiol. 161: 29–34. 1950.
29. WEISEL, PHYLLIS and ALLEN, P. J. Amer. Inst. Biol. Sci. Meetings. Minneapolis, Minnesota. September. 1951.
30. WILLIAMS, C. M. Biochemical mechanisms in insect growth and metamorphosis. Federation Proc. 10: 546–552. 1951.
31. YOSHIKAWA, H. Studien über die Bedeutung des Eisen-Porphyrins im Zellstoffwechsel. I. Eisenporphyringehalt und Atmungs-fähigkeit der verschieden genärten Hefen. Jour. Biochem. (Japan) 25: 627–655. 1937.

AUXIN PHYSIOLOGY IN BEAN LEAF STALKS[1]

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Auxin production, auxin transport (8), and auxin inactivation (12) in tissues with relatively high auxin concentration have been extensively investigated and reviewed. Little attention, however, has been given to the auxin physiology of tissues with low auxin concentration. This paper reports an investigation of conditions associated with the low auxin concentration found in bean leaf stalks, a concentration of 0.4 μg equivalents of IAA per kg fresh weight (10).

In the authors' opinion, the extension of the term "auxin" to include all plant hormones, as well as all exogenous plant regulators (Zimmerman & Hitchcock, 19), ignores known differences between hormones and known differences between hormones and exogenous plant regulators, and is therefore confusing rather than helpful. In this paper the term "auxin" will be restricted to compounds manufactured by the plant (endogenous) which produce curvature in the standard Avena coleoptile assay. Synthetic compounds (exogenous) will be specifically named; indoleacetic acid will be designated by its abbreviation, IAA. Inactivation is considered a chemical alteration of a compound so that it no longer produces its characteristic chemical or biological reactions. An inactivator is a substance or biochemical system capable of inactivating a specific compound. Inhibition is a reduction or retardation of a response. An inhibitor is a substance which inhibits.

The material used in this investigation consisted of stalks bearing trifoliate leaves of greenhouse-grown Black Valentine beans. The leaves were in all cases fully expanded and showed no sign of senescence. Auxin was determined by the standard Avena coleoptile curvature assay (18), and IAA equivalents were calculated by the formula of van Overbeek (16). The blocks used in all experiments were 1.5 % agar, $2.7 \times 2.7 \times 1.0$ mm. Twelve blocks were used in each part of each experiment and each experiment was repeated at least once. The results given are based on the average curvature of 12 coleoptiles. Other details of method are included in the description of the experiments where they were used.

EXPERIMENTS

AUXIN PRODUCTION: No change in auxin concentration from the time the leaf is fully expanded until it is senescent (10) has been found in previous studies with bean leaf stalks on the plant. But a change in auxin concentration in excised bean leaf stalks was found in experiments here reported. In an experiment using three 35-gm lots of freshly cut bean leaf stalks, one lot was frozen, lyophilized, and its auxin extracted with ether. A second lot was submerged in water. The third lot was placed in air in a moist chamber. At the end of 24 hours the second and third lots were treated as the first had been, and their auxin extracted. The details of the method by which these extracts were obtained have already been described (10). The Avena assays of these three lots, reported as microgram equivalent of IAA per kilogram fresh weight, showed at the start of the experiment (first lot) an auxin concentration of 0.4 μg/kg; after 24 hours in air (second lot) a concentration of

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0.0 μg/kg; and after 24 hours in water (third lot) a concentration of 1.5 μg/kg.

This accumulation of auxin in excised stalks under water suggests that stalks on the plant produce auxin but either inactivate it or transport it from the stalk. The fact that auxin accumulated in stalks submerged in water but did not accumulate in stalks in air suggests that an oxidative auxin inactivating mechanism is present in bean leaf stalks.

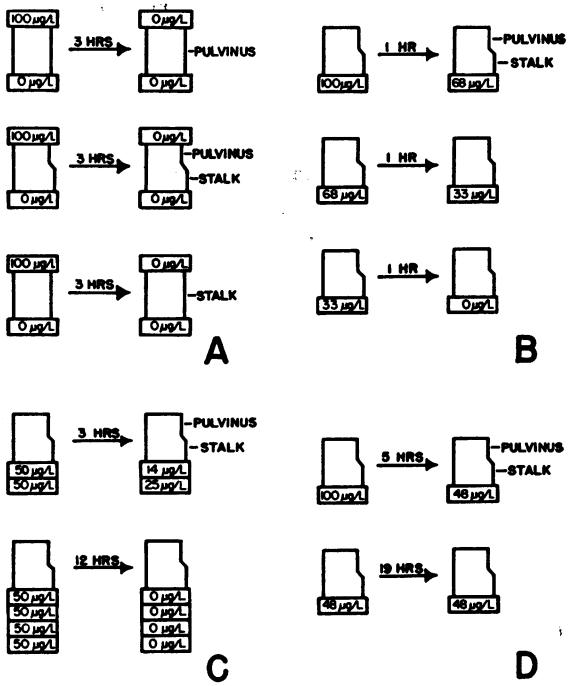

FIG. 1. A. Summary of a 3 hour experiment on transport of IAA by bean leaf stalk sections. A block of agar containing 100 μg/l of IAA was placed on the upper surface of the section and a block of plain agar was placed on the lower, basal, surface. IAA disappeared from the upper blocks but did not appear in the lower blocks. B. Summary of a 3 hour experiment showing three stages in the disappearance of IAA from agar blocks in contact with leaf stalk sections. C. Summaries of a 3 hour, and a 12 hour experiment, showing disappearance of IAA from agar blocks in contact with leaf stalk sections. D. Summary of a 24 hour experiment showing disappearance of IAA from agar blocks in contact with leaf stalk sections that had been boiled. About 50 % of the IAA disappeared in the first 5 hours, but there was no further disappearance in the subsequent 19 hours.

INDOLEACETIC ACID TRANSPORT: The following experiment was designed to test transport of IAA through the leaf stalk: From the leaf stalk subtending the terminal leaflet, 3-mm sections were cut from (a) the leaflet pulvinus, (b) the leaflet abscission zone (since the abscission zone is less than 1 mm long this section included tissues of the leaflet pulvinus and stalk proper), and (c) the stalk 3 cm proximal to the abscission zone. Each section was placed on a block of agar. A similar block containing 100 μg/l of IAA was placed on top of each section. At the end of 3 hours all the blocks were assayed on Avena coleoptiles. The experiment and its results are diagrammed in figure 1 A. None of the blocks produced any response from coleoptiles. Thus this experiment produced no evidence of transport of IAA, but did produce evidence of the presence of an IAA inactivating mechanism in the leaf stalk, since IAA disappeared from the upper blocks in contact with stalk sections for 3 hours.

A similar experiment was conducted with stalk tissues boiled in water for 5 minutes. As in the preceding experiment, after 3 hours no IAA was found in the lower blocks, but in contrast with the preceding experiment, at the end of the 3 hours the upper blocks on each of the 3 types of sections, pulvinus, abscission zone, and stalk, proper, contained respectively, 40, 18, and 14 μg/l of IAA.

This indicates that boiling interfered with the disappearance of IAA but did not completely prevent it.

DIFFUSION EXPERIMENTS: To learn more about the disappearance of IAA, diffusion experiments were performed. In the first of these experiments 3-mm sections of leaf stalk were placed on agar blocks containing 100 μg/l of IAA. In this and subsequent experiments the sections were taken at the abscission zone and therefore contained pulvinal tissue as well as tissue of the stalk proper. The basal, stalk surface was always placed in contact with the agar blocks. The blocks were assayed 3 times at hourly intervals. The results are presented in figure 1 B. They indicate that the disappearance of IAA proceeded at a uniform rate, about 33 μg/l per hour.

In the next experiment the sections were placed on columns of 2 and 4 agar blocks, the agar containing 50 μg/I of IAA. The columns of 2 blocks were assayed at the end of 3 hours and the columns of 4 blocks at the end of 12 hours. The experiment and its results are summarized in figure 1 C. The results indicate that an active diffusion was associated with the disappearance of the IAA.

To learn whether or not enzymes were involved in the disappearance of IAA, sections were boiled in water for 5 minutes and placed on agar containing 100 μg/l of IAA. The blocks were assayed at the end of 5 and 24 hours. The experiment and its results are summarized in figure 1 D. They show a disappearance of approximately 50 % of the IAA in the first 5 hours, with no further disappearance during the next 19 hours. These results indicate that a heat labile mechanism, probably enzymatic, is partly responsible for the disappearance.

To learn of the O_2 relations of the disappearance, sections were placed on agar blocks containing 100 μg/l of IAA and these were then placed in an atmosphere of N_2. The blocks were assayed 5 and 24 hours after being placed in the N_2. The experiment and its results are diagrammed in figure 2 A. After 5 hours, 44 % of the IAA had disappeared. No further loss had occurred at the end of 24 hours. These results

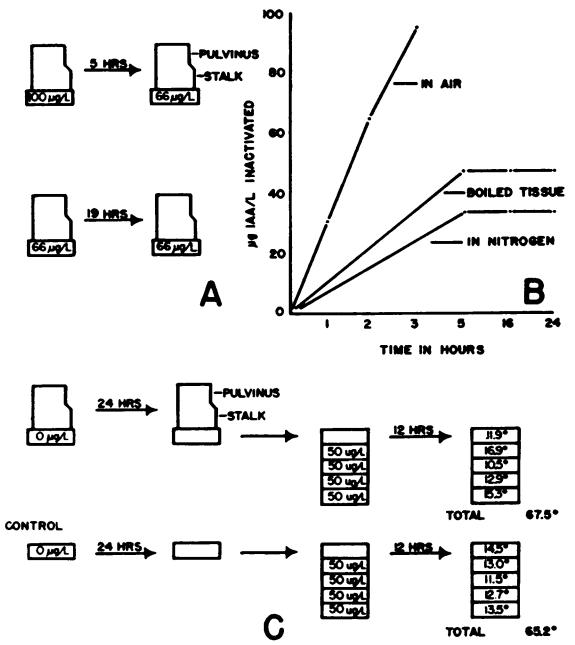

FIG. 2. A. Summary of a 24 hour experiment showing disappearance of IAA from agar blocks in contact with bean leaf stalk sections in an atmosphere of N_2. As with boiling, the disappearance was not complete. B. Summary of the rates of disappearance of IAA from agar blocks in contact with bean leaf stalk sections in air, after boiling, in N_2. C. Summary of an experiment to determine whether an IAA inactivator or inhibitor was diffusing from bean leaf stalk sections. The experiment gave no evidence of diffusion of inactivator or inhibitor.

suggest that O_2 is required for at least a part of the disappearance.

A comparison and summary of the rates of IAA inactivation in blocks in contact with fresh tissue in air, and in N_2, and in blocks in contact with boiled tissue, are shown in figure 2 B.

The disappearance of IAA from the agar blocks in contact with leaf stalks can be interpreted as the result of one, or a combination, of the following actions: a) IAA diffused into the tissue of the section and was inactivated or bound. b) An IAA inactivator diffused from the tissue into the agar and there reacted with IAA. c) An Avena curvature inhibitor diffused from the tissue into the agar. There may be other hypotheses, but the above three are the obvious ones in the light of our present knowledge of auxin physiology.

The effect of boiling in reducing the disappearance of IAA suggests that enzymatic reactions are involved. The similar effect of N_2 suggests that an oxidative reaction is required for the production of the inactivator or inhibitor.

A test for a diffusible inactivator or inhibitor was made in the following experiment. Sections of stalk were placed on blank agar blocks and the combinations kept in a moist chamber for 24 hours. At the end of this period the stalk sections were removed and each of the agar blocks was placed on a stack of 4 similar blocks each of which contained 50 μg/l of IAA. These stacks of 5 blocks each were kept in a moist chamber for 12 hours. All blocks were then assayed. A control was run in an identical manner except that no sections of stalk were used. The experiment and its results are summarized in figure 2 C. In both stacks curvatures from each of the 5 blocks were approximately equal. The total curvature was the same from experimental and control stacks: 67.5° and 65.2°, respectively. Thus this experiment gave no evidence that either an inactivator or an inhibitor diffused from stalk to agar.

Considered together, the results of the diffusion experiments indicated that the disappearance of IAA was due to its diffusion into the sections. Since under ordinary circumstances the disappearance was complete, the IAA was probably bound or chemically changed within the sections.

NATURE OF INACTIVATED INDOLEACETIC ACID: It was not within the scope of this investigation to determine the chemical reactions associated with the disappearance of IAA; however, the results of two simple tests are included here because they may be of interest to other investigators.

Thirty leaf stalks, each about 3 cm long, were placed in a sintered glass filter and covered with 50 ml of a solution of 10 mg/l of IAA in water. Pure O_2 was passed from below through the solution. The solution was tested at the start, after 12 hours, and after 24 hours, with the ferric chloride test for IAA and the Hopkins-Cole test for indole. The ferric chloride reagent was composed of 15 ml of 0.5 M $FeCl_3$, 500 ml distilled water, and 300 ml concentrated H_2SO_4, specific gravity 1.84 (14). Eight ml of this reagent and 2 ml of the IAA solution were placed in a test tube and the color was allowed to develop for 30 minutes. In the Hopkins-Cole test (6), 2 ml of the IAA solution were placed in a test tube and 2 drops of saturated $CuSO_4$ and 6 ml of concentrated H_2SO_4 were added in that order. The color was allowed to develop for 15 minutes. Measurements were made on an Evelyn photoelectric colorimeter using filter No. 515 for the ferric chloride test

TABLE I

EFFECT OF BEAN LEAF STALKS ON A SOLUTION OF IAA AS INDICATED BY THE FERRIC CHLORIDE AND HOPKINS-COLE TESTS

TEST	RESULTS AS MG/L OF IAA			PERCENT DISAPPEARANCE IN 24 HOURS *
	0 hr	*12 hr*	*24 hr*	
Ferric chloride (for IAA) ...	10	6	0	100
Hopkins-Cole (for indole) ..	10	9.5	9.5	5

* Tissueless controls showed no change after 24 hours.

and filter No. 765 for the Hopkins-Cole test. The results are presented in table I. At the end of the 24 hour period, the ferric chloride test for IAA was negative, the Hopkins-Cole test for indole was positive.

These results are consistent with those in the preceding section if they are interpreted as indicating that the IAA diffused into the stalks, was there inactivated, and the inactivated molecule diffused back to the solution.

INHIBITION OF COLEOPTILE CURVATURE: Inhibitors of the coleoptile curvature response have been found in ether extracts of plant tissues by a number of investigators (1, 3, 4, 5, 7, 9, 13). Since the type of inhibitor most frequently found has been non-acid, two experiments were conducted using the Boysen Jensen method which purifies the crude extract by the elimination of non-acid substances. If these eliminated non-acid substances include inhibitors, the resulting purified extract should give a curvature greater than that given by the crude extract. In neither experiment did the purified extract give a curvature greater than that given by the crude extract. Therefore it is assumed that non-acid inhibitors were probably not present in the ether extract of the bean leaf stalks, and the results of the described experiments were probably not due to inhibition of the coleoptile response.

DIRECT ENZYMATIC INACTIVATION OF INDOLEACETIC ACID: Tang and Bonner (14, 15) have described an IAA-inactivating enzyme from etiolated tissues. To determine whether such an enzyme was present in (green) bean leaf stalks, the following experiments were conducted. Stalks were ground in a Waring Blendor with a minimum amount of water. The resulting mixture was filtered through a coarse filter paper and the filtrate, buffered at pH 6.5 with MacIlvain's standard citrate-phosphate buffer, used as an enzyme preparation. Part of this preparation was kept in boiling water for 5 minutes, added to an equal volume of agar (mixture No. 2, table II), and assayed on Avena coleoptiles. A standard solution (200 μg/l) of IAA was buffered as above, part of it mixed with agar as above (mixture No. 1, table II)

TABLE II

THE EFFECT OF A PREPARATION OF BEAN LEAF STALK ENZYMES ON IAA

	MIXTURES	AVENA CURVATURE (DEGREES) WITH STANDARD DEVIATION	PERCENT INACTIVATION
No. 1	Buffer and IAA	9.1° ± 0.41	..
No. 2	Buffer and boiled enzyme preparation	0.0°	..
No. 3	Buffer, enzyme preparation and IAA		
	0 hr.	4.1° ± 0.46	53
	1 hr.	4.0° ± 0.41	54
	2 hr.	5.3° ± 0.56	39
	3 hr.	5.2° ± 0.43	43
	24 hr.	4.8° ± 0.67	47
	48 hr.	4.5° ± 0.57	50

and assayed. Another part of this buffered IAA was mixed with an equal quantity of the enzyme preparation (mixture No. 3, table II). This mixture was divided into 6 equal portions: one portion was immediately mixed with agar as above, and Avena assayed; the other 5 portions were incubated at 25° C for periods of 1, 2, 3, 24 and 48 hours, respectively. At the end of its period of incubation, each portion was kept in boiling water for 5 minutes, mixed with agar, and Avena assayed.

The results are presented in table II. They show that the unincubated portion of the mixture of enzyme preparation with IAA produced a curvature less than half as great as did the IAA alone, indicating that a large part of the IAA disappeared immediately upon the making of the mixture. The results also show that time and incubation produced no further disappearance since the incubated portions also produced curvatures half as great as did the buffered IAA alone.

The mixtures tested in this experiment had been kept in test tubes, in which only limited amounts of O_2 were available. In order to determine whether this limitation of O_2 affected the results, the experiment was repeated with O_2 bubbling through the mixtures. The results were the same. There was an initial inactivation, but no increase with time.

These results indicate, as did previous results, the presence of a limited amount of a heat stable substance which is either an IAA inactivator or an inhibitor of the coleoptile response. Since there was no loss of IAA during incubation, a functional IAA-inactivating enzyme was not present.

DISCUSSION

The results of this investigation have emphasized some important aspects of auxin physiology and have led to a tentative conclusion concerning the mechanism of auxin inactivation in bean leaf stalks. The investigation was necessarily conditioned by the methods at present available for the investigation of auxin physiology. These methods are so restricted that it is difficult if not impossible to reach unequivocal conclusions. There is also a serious difficulty in interpretation of Avena assays of ether extracts: the possibility of the presence of Avena coleoptile curvature inhibitors. Such inhibitors are far more widespread than once was realized (1, 3, 4, 5, 6, 9, 13). Some inhibitors have been identified chemically, but most have been described merely as "acid" or "non-acid." A few are characterized by the production of a positive curvature (instead of the usual negative curvature) in the Avena coleoptile. However, neither in this nor in a previous investigation (10) has any evidence of the presence of an inhibitor been found; no positive curvatures appeared, nor any other evidence of either a non-acid inhibitor or a diffusible inhibitor. This evidence indicates that the data obtained with the Avena assays of bean leaves are valid.

Auxin production is frequently assumed to be restricted to young tissues, although this assumption is not supported by the literature (18). In the guayule

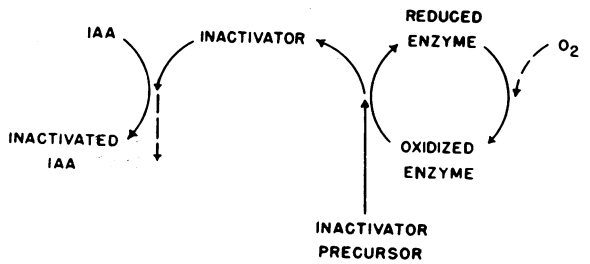

FIG. 3. Diagram of a tentative system of IAA inactivation in bean leaf stalks.

plant, the mature leaves were found to yield the highest concentration of auxin (Smith, 11). In our experiments evidence was obtained that the mature bean leaf stalks produce auxin. Excised bean leaf stalks kept under water showed an increased auxin concentration, whereas excised stalks kept in the air showed no auxin, at the end of the experiment.

The simplest explanation of this result assumes the presence in the bean leaf stalk of both auxin producing and auxin inactivating systems, the auxin producing system having a low O_2 requirement and the auxin inactivating system having a high O_2 requirement. Similar assumptions have been made by Van Raalte (17) to explain the increased diffusion of auxin from Vicia root tips under conditions unfavorable for respiration.

Auxin transport probably occurs in the bean leaf stalk although no evidence for it was obtained in this investigation. Retardation of abscission at the proximal end of a debladed bean leaf stalk has been found to follow the application of IAA to the distal end of the stalk (Addicott & Lynch, 2). This can be interpreted as evidence of the transport of IAA through the stalk. It is probable that failure to obtain transport in this investigation was due largely if not entirely to inactivation. In this connection it is of interest to note that in a recent paper on IAA transport in the bean hypocotyl the low recoveries were explained solely in terms of variations in polarity of IAA transport (Jacobs, 8). The low recoveries could have been explained equally well in terms of inactivation of IAA, or the release of an Avena inhibitor. Unfortunately the data presented do not permit a choice among these alternatives.

Inactivation appears to be the most reasonable explanation of the observed disappearance of auxin and of IAA. Systems for the oxidative inactivation of auxin and IAA are widespread (Steeves et al, 12). This investigation showed auxin disappearance from excised bean leaf stalks under aerobic conditions, and accumulation under anaerobic conditions. IAA was inactivated when in contact with bean leaf stalks and when added to a preparation of bean leaf stalk protoplasm.

Taken together, the evidence indicates that a thermostable inactivator, which can react with auxin and with IAA, is present in bean leaf stalks. The amount of this inactivator present at any one time appears to be limited, since only limited amounts can be demonstrated in tissue that has been boiled or kept in N_2; for continued inactivation, living tissue and O_2 are required. The simplest explanation of this requirement is that an oxidase is necessary for the production of the inactivator. A scheme of the mechanism which is suggested by this evidence is shown in figure 3. Study of this mechanism will be continued; further evidence may alter the present picture.

SUMMARY

Auxin production was found in excised bean leaf stalks when immersed in water. Auxin disappeared from excised bean leaf stalks when they were kept in moist air.

The low auxin concentrations found in previous Avena assays of bean leaf stalks appear to be real; no evidence was found of inhibitors modifying the response of the Avena coleoptile.

IAA transport through sections of bean leaf stalks was not obtained, although IAA disappeared from agar blocks in contact with leaf stalk sections. The disappearance was apparently due to the diffusion of IAA into the sections and its inactivation there.

Evidence of an IAA inactivating system in bean leaf stalks was obtained. The system appears to be composed of at least two parts. In the first part there is oxidative, enzymatic production of an IAA inactivator which is thermostable. In the second part there is a reaction of the inactivator and IAA to form a product which is inactive in the Avena assay. The product is negative to the ferric chloride test (for IAA) but is positive to the Hopkins-Cole test (for indole).

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LITERATURE CITED

1. ADDICOTT, F. T., DAWSON, V. D., and SEKERA, G. S. A growth inhibitor in cotton. Report presented at the Seventh Cotton Defoliation Conference. Memphis, Tennessee. 1953.
2. ADDICOTT, F. T. and LYNCH, R. S. Acceleration and retardation of abscission by indoleacetic acid. Science 114: 688–689. 1951.
3. BONDE, E. K. Growth inhibitors and auxin in leaves of cocklebur. Physiol. Plantarum 6: 234–239. 1953.
4. BOYSEN JENSEN, P. Quantitative Bestimmung der beschleunigenden Streckungswuchsstoffe in der saurer Fraktion der Atherextrakte aus höheren Pflanzen. Planta 31: 653–669. 1941.
5. GOODWIN, R. H. Evidence for the presence in certain ether extracts of substances partially masking the activity of auxin. Amer. Jour. Bot. 26: 130–135. 1939.
6. HAWK, P. B., OSER, B. L., and SUMMERSON, W. H. Practical Physiological Chemistry. The Blakiston Co., Philadelphia. 1949.
7. HEMBERG, T. The significance of the acid growth-inhibiting substances for the rest-period of the potato tuber. Physiol. Plantarum 5: 115–129. 1952.

8. JACOBS, W. P. Auxin transport in the hypocotyl of *Phaseolus vulgaris* L. Amer. Jour. Bot. 37: 248–254. 1950.
9. LEOPOLD, A. C., SCOTT, F. I., KLEIN, W. H., and RAMSTEAD, E. Chelidonic acid and its effect on plant growth. Physiol. Plantarum 5: 85–90. 1952.
10. SHOJI, K., ADDICOTT, F. T., and SWETS, W. A. Auxin in relation to leaf blade abscission. Plant Physiol. 26: 189–191. 1951.
11. SMITH, P. F. Auxin in leaves and its inhibitory effect on bud growth in guayule. Amer. Jour. Bot. 32: 270–276. 1945.
12. STEEVES, T. A., MOREL, G., and WETMORE, R. H. A technique for preventing inactivation at the cut surface in auxin diffusion studies. Amer. Jour. Bot. 40: 534–537. 1953.
13. STEWART, W. S. A plant growth inhibitor and plant growth inhibition. Bot. Gaz. 101: 91–108. 1939.
14. TANG, Y. W. and BONNER, J. The enzymatic inactivation of indoleacetic acid. I. Some characteristics of the enzyme contained in pea seedlings. Arch. Biochem. 13: 11–25. 1947.
15. TANG, Y. W. and BONNER, J. The enzymatic inactivation of indoleacetic acid. II. The physiology of the enzyme. Amer. Jour. Bot. 35: 570–578. 1948.
16. VAN OVERBEEK, J. A simplified method for auxin extraction. Proc. Nat. Acad. Sci. Wash. 24: 42–46. 1938.
17. VAN RAALTE, M. H. On factors determining the auxin content of the root tip. Rec. trav. bot. néerlandais 34: 278–332. 1937.
18. WENT, F. and THIMANN, K. V. Phytohormones. P. 69. The Macmillan Co., New York. 1937.
19. ZIMMERMAN, P. W. and HITCHCOCK, A. E. Plant hormones. Ann. Rev. Biochem. 17: 601–626. 1948.

INCORPORATION OF RADIOACTIVE GLUTAMIC ACID INTO THE PROTEINS OF HIGHER PLANTS

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It has now been demonstrated that enzymes associated with plant mitochondria are able to utilize the respiratory energy conserved by the mitochondria in adenosinetriphosphate (ATP) (3) for the incorporation of glutamic acid into the amide, glutamine (9), and the peptides, γ-glutamylcysteine (11) and glutathione (10). During the course of these investigations, it was noted that mitochondria incorporate radioactive amino acids into their own protein (12). The incorporation process resembles that of peptide bond synthesis in its dependence on ATP. This suggests that further understanding of protein turnover in plants might be obtained by systematic study of the incorporation of labeled amino acids into plant proteins. The only previously recorded observations on radioactive tracer incorporation into plant protein have been those of Arreguin et al (1), using C^{14}-acetate, Racusen and Aronoff (7) with $C^{14}O_2$, and Boroughs and Bonner (4) with C^{14}-leucine and -glycine. In the first two cases mentioned, the substances used were probably converted to amino acids before incorporation into protein. The present investigation was concerned chiefly with establishing some of the overall characteristics of the process of amino acid incorporation in plants. Glutamic acid was utilized for these initial studies as previous results (12) indicated that it is incorporated into protein at relatively high rates even in a cell-free system.

EXPERIMENTAL

The seedlings used in the present studies were germinated and grown in the dark at 25° C as described previously (10). Certain of the mature tissues employed were obtained from local markets. The sectioned tissues were incubated in 0.05 M potassium phosphate buffer (pH 6.8) at 25° C with 1 to 100 microcuries (μc) of either 0.01 M potassium glutamate-2-C^{14} or 0.01 M uniformly labeled potassium glutamate-C^{14}. The amount of radioactivity employed was varied according to the needs of the separate experiments. At the end of the incubation period, the tissue sections were washed free of external amino acid in running water and ground in 0.05 M potassium phosphate buffer (pH 7.5) containing 0.45 M sucrose. Total activity incorporated into the cellular constituents was determined directly from the specific activity of the homogenate. Total protein was precipitated by addition of an equal volume of 12 % trichloroacetic acid at 1° C for 3 hrs. Cellular fractions were separated by centrifugation of the sucrose-phosphate homogenate as described previously (9). Glutathione formation was determined by radioactive assay on the material isolated in the form of cuprous glutathione as earlier described (10). Glutamic acid, α-ketoglutaric acid, and γ-aminobutyric acid were separated chromatographically and the relative activities of the acids estimated directly from that of the glutamic acid.

Radioactive assays were carried out by standard techniques using a thin mica window Geiger-Müller tube and scaling circuit. Protein was washed with trichloroacetic acid, with dilute alkali, and with thioglycollic acid before radioactive assay in order to remove any amino acid either adsorbed or bound through sulfhydryl linkage. These treatments generally removed 10 to 15 % of the total activity. The remainder of the activity was released only by hydrolysis of the protein.

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