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# METABOLISM OF THE AXIS AND COTYLEDONS OF *PHASEOLUS VULGARIS* SEEDS DURING EARLY GERMINATION

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**Abstract**—The metabolism of the embryonic axis and cotyledons of *Phaseolus vulgaris* seeds during early germination has been investigated using tritiated water. Initially there are differences in the compounds labelled but the patterns of labelling become more complex and similar with time. It appears that the Kreb's cycle is probably not functioning before 30 min in the axis or before 60 min in the cotyledons on germination While sucrose is labelled at the same time in both axis and cotyledons it is always more intensely labelled in the axis. During the first 6 hr of germination it appears that the cotyledons are not degrading their storage materials for translocation to the axis. Rather the axis is metabolising its own storage compounds and apparently some of its storage carbohydrates are being respired through sucrose.

## INTRODUCTION

ALTHOUGH seed germination has been extensively studied[1,2] much of this work has been confined to changes in the whole seed. Since the axis (hypocotyl and plumule) and the cotyledons have different functions they may have different pathways of metabolism. Previous investigations of the metabolic differences and interrelationships of various parts of seeds have rarely been concerned with times shorter than 24–48 hr after imbibition.[3] Findings from such experiments can only reflect the metabolic states of the seeds many hours after imbibition. The present paper reports on the labelling of compounds that occur when parts of *Phaseolus vulgaris* seeds are imbibed in tritiated water and left for periods of from 3 min to 6 hr.

The use of tritiated water enables the metabolism of seeds to be studied at much earlier times than does the use of other methods. In this technique,[4] which has already been applied to whole seeds,[5,6] the only way a compound can become non-exchangeably tritiated is by a biochemical (or chemical) reaction. Thus any compound which becomes labelled must have been involved in metabolism.

In this paper the seeds were dissected before being imbibed in tritiated water. This has to be done to study the metabolism of *Phaseolus vulgaris* seeds at short times because the seed coat is hard and water is not absorbed evenly. Nor do all seeds begin absorbing at the same time. Because of this, when whole seeds are imbibed in tritiated water the labelling varies from one part of the seed to another. However experiments were carried out on intact seeds which were later dissected and it was found that the same compounds are labelled in the whole seed as in the dissected parts.

[1] W. CROCKER and L. V. BARTON, *Physiology of Seeds*, Chronica Botanica, Waltham, Mass. (1953).
[2] D. KOLLER, A. M. MAYER and A. POLJAKOFF-MAYBER, *Ann, Rev. Plant Physiol.* **13**, 437 (1962).
[3] A. A. ABDUL-BAKI, *Plant Physiol.* **44**, 733 (1969).
[4] A. T. WILSON, *J. N.Z. Inst. Chem.* **28**, 87 (1964).
[5] D. J. SPEDDING and A. T. WILSON, *Phytochem.* **7**, 897 (1967).
[6] A. W. MISSEN and A. T. WILSON, *Phytochem.* **9**, 1473 (1970).

## RESULTS AND DISCUSSION

At the earliest times the metabolism of the axis is quite different from that of the cotyledons (Table 1). After 3 min only amino acids are labelled in the axis, and the labelling in the cotyledons is very small, but at 5 min citric acid is labelled in the cotyledons but not

TABLE 1. COMPOUNDS LABELLED WITH TRITIUM WHEN *Phaseolus vulgaris* AXIS AND COTYLEDONS ARE IMBIBED IN TRITIATED WATER AND LEFT FOR VARIOUS TIME INTERVALS

| Compounds labelled | Time (min) 3 | 5 | 15 | 30 | 60 | 120 | 180 | 360 |
|---|---|---|---|---|---|---|---|---|
| (a) Axis | | | | | | | | |
| γ-Aminobutyric acid | | t | + | ++ | ++ | ++ | ++ | + |
| Aspartic acid | + | + | + | + | + | + | + | ++ |
| Glutamic acid | + | + | + | + | + | + | + | +++ |
| Alanine | + | + | + | + | ++ | ++ | ++ | ++ |
| Citric acid | | | + | + | t | ++ | ++ | +++ |
| Malic acid | | | | + | + | ++ | ++ | +++ |
| Succinic acid | | | | + | t | + | + | t |
| Lactic acid | | | | | ++ | t | ++ | t |
| Sucrose | | | | | | + | +++ | +++ |
| Glutamine | | | | | | + | ++ | ++ |
| Lipid? | | | | + | + | + | + | + |
| Sugar Phosphates ? | | | | t | t | + | ++ | +++ |
| (b) Cotyledon | | | | | | | | |
| γ-Aminobutyric acid | | t | + | ++ | ++ | ++ | ++ | ++ |
| Aspartic acid | | t | + | + | + | ++ | + | ++ |
| Glutamic acid | t | t | + | + | ++ | ++ | ++ | ++ |
| Alanine | | | + | + | ++ | ++ | ++ | t |
| Citric acid | t | + | + | ++ | ++ | ++ | ++ | ++ |
| Malic acid | | | | | + | ++ | ++ | ++ |
| Succinic acid | | | | | t | + | + | + |
| Lactic acid | | | | | | + | ++ | t |
| Sucrose | | | | | | t | + | ++ |
| Glutamine | | | | | | + | ++ | t |
| Lipid? | | | | | + | + | + | + |
| Sugar Phosphates ? | | | | | | t | t | + |

Key: +, ++, +++ = increasing relative intensity; t = trace.

alanine. Since compounds are labelled even as early as 3 min it appears that the addition of water immediately causes the seeds to metabolize. As time goes on the patterns of labelling become more similar and by 60 min the same compounds are labelled in both axis and cotyledon. Sucrose and glutamine appear at 2 hr in both axis and cotyledon and by 6 hr sucrose is strongly labelled in the axis. Succinic acid is labelled at 30 min in the axis and 60 min in the cotyledons. The amount of label in lactic acid varies widely and there is no clear pattern of increasing or decreasing label. It is probable that this reflects small variations in the amount of tritiated water in which the bean parts are immersed. Doireau and Duperon[7] showed that increases in the depth of water over germinating seeds could cause an increase in anaerobic respiration, presumably by restricting the availability of oxygen and Missen and Wilson[6] showed that when seeds are germinating in tritiated water under an anaerobic atmosphere large amounts of lactic acid are produced.

The compounds that were found to be labelled in *Phaseolus vulgaris* are the same as

[7] P. DOIREAU and R. DUPERON, *C.R. Soc. Biol.* **160,** 1926 (1966).

those labelled in *Sinapis alba* (white mustard);[5,6] although the order of labelling and the time at which different compounds become labelled are not the same. As pointed out in those papers, aspartic acid, glutamic acid and γ-aminobutyric acid are closely related to the Kreb's cycle and could become non-exchangeably labelled in the presence of either deaminases or transaminases. The corresponding oxo acids that would be produced by these enzymes form part of the Kreb's cycle or are intimately connected with it. As well as these amino acids, citric, malic and succinic acids are also labelled at 30 min in the axis but they are not all labelled until 60 min in the cotyledons. It would therefore appear that the Kreb's cycle is probably starting to operate in the respective tissues at these times.

As in the experiments with *Sinapis alba*, γ-aminobutyric acid was found to be one of the earliest compounds labelled in *Phaseolus vulgaris*. This is of interest because while γ-aminobutyric acid is virtually a universal constituent of plants it is not usually regarded as being an important metabolic intermediate.

The only other study of metabolism in the early stages of germination of *Phaseolus vulgaris* is that of Duperon, who fed $^{14}$C-labelled citric acid,[8] glucose,[9] and $CO_2$.[10] He concluded that the Kreb's cycle was operating 3 hr after imbibition and that transaminases and carboxylases were also present. However, he made no study of the differences in cotyledon and axis metabolism and does not mention finding $^{14}$C-labelled γ-aminobutyric acid.

The differences in labelling between axis and cotyledon at the earliest times is probably due to differences in water absorption. The axis absorbs water much faster than the cotyledons. It has a definite structure with inner and outer layers. A histochemical study of the axis of *Phaseolus vulgaris* by Sato[11] 20 hr after imbibition, showed that the mitochondria and succinic dehydrogenase are not distributed evenly through the axis. Conversely, cotyledon tissue absorbs water slower but is a more or less homogeneous mass of cells. It has been shown that different enzymes are activated by different amounts of water in wheat germ.[12] Since the cotyledons and axis of *Phaseolus vulgaris* have different structures and since they absorb water at different rates, enzymes might be activated in a different order in each tissue. This could explain the fact that the axis does not label the same compounds as the cotyledons at the earliest times. The very faint labelling of the cotyledons at 3 min and the increase in complexity of labelling with time in both axis and cotyledons are probably also due to the rate of water absorption.

At the 2–6 hr stage, glutamine and sucrose are labelled in both axis and cotyledons. However, both compounds are more heavily labelled in the axis. The small amount of labelling in the cotyledons may be due to the fact that there is little translocation in seeds from storage organs to axis during the first day of germination. So sucrose and glutamine might only be produced in large amounts when they are needed for translocation. A further possibility is that glucose is translocated. This would not be expected to become non-exchangeably tritiated from starch degradation.

In the bean *Vigna sesquipedalis* studied by Oota *et al.*[13] there is a large increase in the amount of starch in the plumule during the first days of germination. This is at the expense of the hypocotyl. Since starch cannot be transported as such they conclude that the plumule

[8] R. Duperon, *Compt. Rend.* **265**, 1698 (1967).
[9] R. Duperon, *Compt. Rend.* **258**, 5960 (1964).
[10] R. Duperon, *Compt. Rend.* **253**, 1488 (1961).
[11] S. Sato, *Bot. Mag. Tokyo* **69**, 137 (1956).
[12] P. Linko and M. Milner, *Plant Physiol.* **34**, 392 (1959).
[13] Y. Oota, R. Fujii and S. Osawa, *J. Biochem. Tokyo* **40**, 649 (1953).

must be synthesizing it from sugars acquired from the hypocotyl. However, in *Phaseolus vulgaris* it seems unlikely that sucrose is being formed in the hypocotyl and translocated to the plumule during early germination. This is because both parts produced labelled sucrose in experiments in which they were imbibed separately in tritiated water. A more likely explanation for this production of labelled sucrose in the axis is that endogenous starch of the hypocotyl and plumule is being degraded to sugars for respiration. A pathway by which starch is first converted to sucrose before further degradation has been suggested by Swain *et al.*[14] who studied the pea seedling. If this pathway was present in the axis of *Phaseolus vulgaris* labelled sucrose would be produced if the axis was imbibed in tritiated water.

The axis was more active in labelling lipids than the cotyledon tissue. *Phaseolus vulgaris* contains only small amounts of lipids in the dry seed.[15] These could have only a limited food storage function. Labelling probably occurs therefore during lipid synthesis which would be needed to make the cell membranes required for future growth of the seedling. This is supported by the results of Macey and Stumpf[16] who found that in the seed of the pea, which like *Phaseolus vulgaris*, is a legume with a low lipid content, there is active lipid synthesis during the first day of germination.

Previous studies on seeds using tritiated water[5,6] have shown that mustard and lettuce seeds have substantially the same patterns of labelling as *Phaseolus vulgaris*. The $^{14}CO_2$ work of Duperon[10] with *Phaseolus vulgaris* seeds gave the same labelled compounds as those found by Haber and Tolbert[17] in lettuce seeds and these results complement the investigations using tritiated water. It therefore appears that the metabolic processes occurring early in germination are common to the seeds of a number of species. This metabolism is characterized by an increase in complexity with time.

Previous studies on seeds have also shown that storage organs such as the cotyledons in beans and the endosperm in other seeds supply nutrients to the growing axis. It has often been assumed therefore that these storage organs have only simple degradative metabolic pathways while the axis has quite different metabolism. While this may be true where endosperm is the storage organ, the work described in this paper indicates that in the case of *Phaseolus vulgaris* where the cotyledons are the storage organs, the axis and cotyledons have common metabolic pathways, at least in the first hours of germination. There are differences between them in the relative amounts of labelling of some compounds and this could be due to differences in either the direction or relative importance of the various metabolic pathways involved.

## EXPERIMENTAL

*Imbibition.* Two seeds of *Phaseolus vulgaris* L. var. Seminole (dwarf French beans), having greater than 95% germination were dissected. The embryonic axes were removed, covered with tritiated $H_2O$ (5 Ci/ml, *ca.* 0·05 ml), in a conical tube and left for the required time at room temp. (21 ± 2°). Two small pieces of cotyledon were also dissected out and treated in the same way as the axes.

*Extraction.* After the required time the tissue was rinsed twice with $H_2O$, ground and extracted twice with EtOH and then extracted again with $H_2O$. The extracts were centrifuged and the two $H_2O$ and EtOH rinses were combined. The extracts were then evaporated to dryness under reduced pressure.

*Chromatography.* The extracts were chromatographed in two dimensions on 15 cm squares of Whatman

[14] R. R. SWAIN and E. E. DEKKER, *Biochim. Biophys. Acta* **122,** 87 (1966).
[15] H. ITO, *Bull. Agric. Chem. Soc. Japan* **15,** 135 (1939).
[16] M. J. K. MACEY and P. K. STUMPF, *Plant Physiol.* 1637 (1968).
[17] A. H. HABER and N. E. TOLBERT, *Plant Physiol.* **34,** 376 (1959).

No. 4 chromatography paper, first in phenol–$H_2O$ solvent and then in BuOH–propionic acid–$H_2O$.[18] Tritium labelled compounds were detected by scintillation autography.[19]

*Identification of tritium labelled compounds.* Tritium labelled carboxylic and amino acids were identified by two-dimensional co-chromatography in a number of different solvents. The ones most often used being; for amino acids *n*-BuOH–pyridine–$H_2O$ (1:1:1), and *n*-BuOH–acetone–diethylamine–$H_2O$ (10:10:2:5), and for carboxylic acids EtOH–0·880 $NH_4OH$–$H_2O$ (16:1:3) and *n*-propanol–eucalyptol–HCOOH (5:5:2, upper) saturated with $H_2O$ and used 2 days after preparation. Sucrose was identified by co-chromatography in one dimension with *n*-propanol–EtOAc–$H_2O$ (7:1:2). This solvent separates sucrose from all the other common sugars including fructose, glucose, galactose and maltose. Amino acids were detected by a solution of 0·2% ninhydrin in acetone, carboxylic acids by aniline-xylose[20] and sucrose by aniline hydrogen phthalate.[21] The co-chromatograms were dipped in one of these solutions after scintillation autography. Compounds which ran with high $R_f$ values in both phenol–$H_2O$ and BuOH–propionic acid–$H_2O$ were presumed to be lipids.

[18] A. T. WILSON and M. CALVIN, *J. Am. Chem. Soc.* **77**, 5950 (1955).
[19] A. T. WILSON, *Biochim. Biophys. Acta* **40**, 522 (1960).
[20] I. SMITH, *Chromatographic and Electrophoretic Techniques*, (3rd Edition), Vol. 1, p. 350, Heinemann, London (1969).
[21] S. M. PARTRIDGE, *Nature, Lond.* **164**, 443 (1949).

*Key Word Index—Phaseolus vulgaris*; Leguminosae; seed germination; amino acids; organic acids; sucrose.