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UNEXPECTED LABELLING PATTERNS FROM RADIOACTIVE SUGARS FED TO PLANTS CONTAINING MANNITOL

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Abstract—Several acyclic polyols found in plant tissues, including the widely distributed mannitol, have symmetric molecules or are optically inactive. During the metabolism of specifically labelled sugars, intermediate synthesis and oxidation of any of these compounds will lead to unexpected labelling patterns in products and errors in calculations of the pathways of carbohydrate catabolism.

INTRODUCTION

EXPERIMENTS, in which the incorporation of radioactivity from specifically labelled sugars (usually [1-^{14}C] and [6-^{14}C]glucose) into storage compounds or CO_2 is measured, have provided much information about the carbohydrate metabolism of plant tissues. However, to our knowledge, no consideration has previously been given to the possibility that the intermediate metabolism of certain acyclic polyols could lead to significant changes in the molecular distribution of radioactivity in related sugars.

DISCUSSION

The Occurrence and Metabolism of Mannitol in Plants

D-Mannitol is a major soluble carbohydrate in most higher fungi, in representatives of over fifty families of the angiosperms, and in many genera of the red and brown algae, lichens and leafy liverworts.[1–3] Furthermore, experiments using ^{14}C with species known to contain large amounts of mannitol have generally shown that the hexitol is a primary product of heterotrophic carbon assimilation or photosynthetic CO_2 fixation.

Many ascomycetes (but not brewing and baking yeasts), basidiomycetes and fungi imperfecti are able to synthesize mannitol from a wide range of carbon sources, including sugars, other acyclic polyols, *myo*-inositol, acetate, intermediates of the tricarboxylic acid cycle and amino acids.[4] Under favourable growth conditions 30% or more of the substrate

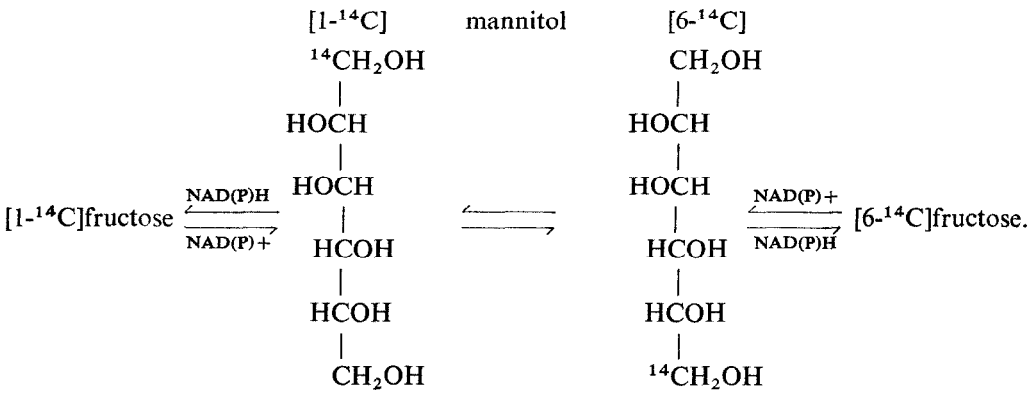
[1] D. H. LEWIS and D. C. SMITH, *New Phytol.* **66**, 143 (1967).
[2] D. H. LEWIS, *Trans. Br. Bryol. Soc.* **6**, 108 (1970).
[3] D. H. LEWIS, *Trans. Br. Bryol. Soc.* **6**, 391 (1971).
[4] P. M. HOLLIGAN and D. H. JENNINGS, *New Phytol.* **71**, 583 (1972).

may be converted to the hexitol.[5–7] Factors that stimulate mannitol accumulation in the mycelium include low levels of phosphate and organic nitrogen (or a high carbon to nitrogen ratio) in the medium and moderate aeration of the culture.[1] During glucose assimilation by *Dendryphiella salina*, there is a complete turnover of mannitol within 6 hr,[8] and it is possible that a labile mannitol pool is characteristic of many fungi (though this may depend on the mode of mannitol synthesis).

Information on the enzymology of mannitol metabolism has come mainly from studies with bacteria and fungi. Mannitol and mannitol-1-phosphate dehydrogenases, which reduce fructose and fructose-6-phosphate respectively in the presence of NADH or NADPH, have been isolated and characterized, and similar enzymes detected in the few photosynthetic plants investigated.[1] Also a mannitol acetylphosphate phosphotransferase has been extracted from an *Aspergillus* species.[9] However, there have been no reports of enzymes that lead to mannitol synthesis through the reduction of D-mannose or the epimerization of other polyols.

Symmetry of the Mannitol Molecule and its Effect on the Distribution of Isotopes

Since the mannitol molecule is symmetric with respect to the arrangement of the hydroxyl groups, the following type of reaction sequence is possible during the metabolism of [1-^{14}C]-fructose in the presence of mannitol dehydrogenase:



Overall reaction: [1-^{14}C]fructose ⇌ [6-^{14}C]fructose.

The redistribution of labelled atoms in the mannitol pool results, on oxidation, in two forms of labelled fructose (or fructose-6-phosphate in the presence of a mannitol-1-phosphate dehydrogenase). Although this transformation will not alter the subsequent fate of the ^{14}C during catabolism via the glycolytic sequence (due to the formation of two identical triose-phosphate molecules), the labelling patterns of polysaccharides and products of the

[5] W. H. LEE, *Appl. Microbiol.* **15**, 1206 (1967).
[6] H. ONISHI and T. SUZUKI, *Appl. Microbiol.* **16**, 1847 (1968).
[7] H. ONISHI and T. SUZUKI, *Biotechnol. Bioeng.* **12**, 912 (1970).
[8] P. M. HOLLIGAN and D. H. JENNINGS, *New Phytol.* **71**, 1119 (1972).
[9] W. H. LEE, *Biochem. Biophys. Res. Commun.* **29**, 237 (1967).

pentose-phosphate pathway will be affected. For example:

$$2\ [1\text{-}^{14}C]\text{hexose} \rightarrow 2\ [^{14}C]\text{mannitol} \rightarrow \begin{matrix} [1\text{-}^{14}C]\text{hexose (-P)} \rightarrow {}^{14}CO_2 + \text{pentose-P} \\ + \\ [6\text{-}^{14}C]\text{hexose (-P)} \rightarrow CO_2 + [5\text{-}^{14}C]\text{pentose-P.} \end{matrix}$$

In this way, radioactivity from [1-^{14}C]hexose can be incorporated into pentoses and pentitols via the oxidative, glucose-6-phosphate and 6-phosphogluconate dehydrogenase, reactions of the pentose-phosphate pathway. Similar effects will also occur during the metabolism of hexoses specifically labelled with tritium, though in these instances the transference of radioactivity to NAD(P)$^+$ instead of CO_2 must be considered.

As shown above, yields of $^{14}CO_2$ from the oxidation of [1-^{14}C]hexose in the pentose-phosphate pathway may be as low as half of the expected values for tissues containing mannitol, and yields from [6-^{14}C]hexose proportionately higher than expected. Thus, when mannitol is an intermediate in hexose-phosphate synthesis, methods based on the relative incorporation of radioactivity into CO_2 or triose-phosphate derivatives from [1-^{14}C] and [6-^{14}C]glucose[10] are likely to lead to an underestimation of the pentose-phosphate pathway. The magnitude of the error caused by label redistribution will be related to the proportion of hexose-phosphate derived from the mannitol pool and to the degree of equilibration between fructose-6-phosphate and glucose-6-phosphate. When glucose-6-phosphate is synthesized equally from substrate glucose and from intermediate mannitol via fructose-6-phosphate, values for the activity of the pentose-phosphate pathway will be underestimated by as much as 50%. However, if the hexose-phosphates are not in complete equilibrium so that the formation of glucose-6-phosphate from mannitol is restricted, errors in the calculations could be much smaller. In some fungi the synthesis of storage carbohydrates from glucose and fructose differs—glucose being preferentially converted to trehalose and fructose to mannitol[1,11]—and this may be partly caused by an incomplete equilibration between glucose- and fructose-6-phosphates in the cell.

During photosynthesis and gluconeogenesis, hexoses (and hexitols) are synthesized through the condensation of two triose-phosphate molecules. Incorporation of radioactivity, either by $^{14}CO_2$ fixation or by assimilation of labelled intermediates of the tricarboxylic acid cycle, will be equivalent into the two halves of hexose molecule. For this reason, any subsequent synthesis and oxidation of mannitol will not alter the distribution of label unless the hexose units are first cycled through the pentose-phosphate pathway to give an asymmetric labelling pattern.

Observed Labelling Patterns and Values for the Pathways of Hexose Catabolism in Plants that Contain Mannitol

Studies on the assimilation of specifically labelled sugars by plants that contain mannitol have been confined almost entirely to the fungi. Experiments with the mannitol-producing fungi *Melampsora lini*[12] and *Dendryphiella salina*[8] have shown that radioactivity is incorporated into the pentitol, arabitol, from both [1-^{14}C] and [6-^{14}C]glucose. Strong evidence for a cyclic pentose-phosphate pathway in *Dendryphiella* indicates that the redistribution of

[10] H. G. Wood, J. Katz and B. R. Landau, *Biochem. Z.* **338**, 809 (1963).
[11] D. Smith, L. Muscatine and D. H. Lewis, *Biol. Rev.* **44**, 17 (1969).
[12] D. Mitchell and M. Shaw, *Can. J. Bot.* **46**, 453 (1968).

label during intermediate mannitol metabolism is the most likely cause of this apparently anomalous labelling pattern. By contrast, in an osmophilic yeast which did not contain mannitol, the labelling of carbon atoms in the arabitol molecule could be explained only in terms of pentose-phosphate synthesis via a combination of the oxidative and non-oxidative reactions of the pentose-phosphate pathway.[13] In a recent paper on polyol metabolism in *Aspergillus clavatus*,[14] the source of reduced coenzyme for ribitol (?arabitol) synthesis was ascribed to fatty acid oxidation. However, there is an alternative explanation, namely that tritium incorporated into the pentitol from [6-^{3}H]glucose originated from NADP^{3}H formed in the pentose-phosphate pathway after the oxidation of ^{3}H mannitol and [1-^{3}H]-glucose-6-phosphate.

TABLE 1. FORMULAE OF SYMMETRIC AND OPTICALLY INACTIVE ACYCLIC POLYOLS FOUND IN PLANT TISSUES

meso-Erythritol	D-Threitol	Ribitol	Xylitol	D-Mannitol
CH_2OH | HCOH | HCOH | CH_2OH	CH_2OH | HOCH | HCOH | CH_2OH	CH_2OH | HCOH | HCOH | HCOH | CH_2OH	CH_2OH | HCOH | HOCH | HCOH | CH_2OH	CH_2OH | HOCH | HOCH | HCOH | HCOH | CH_2OH

Galactitol	Allitol	L-Iditol	*meso*-Glycero-*ido*-heptitol
CH_2OH | HCOH | HOCH | HOCH | HCOH | CH_2OH	CH_2OH | HCOH | HCOH | HCOH | HCOH | CH_2OH	CH_2OH | HCOH | HOCH | HCOH | HOCH | CH_2OH	CH_2OH | HCOH | HOCH | HCOH | HOCH | HCOH | CH_2OH

Determinations of the activity of the pentose-phosphate pathway in higher fungi[15] have never taken into account the possible effects of mannitol metabolism and the participation of this pathway in hexose catabolism has almost certainly been considerably underestimated.

[13] J. F. T. SPENCER, A. C. NEISH, A. C. BLACKWOOD and H. R. SALLANS, *Can. J. Biochem. Physiol.* **34**, 495 (1956).
[14] D. L. CORINA and K. A. MUNDAY, *J. Gen. Microbiol.* **69**, 221 (1971).
[15] H. J. BLUMENTHAL, *The Fungi—I. The Fungal Cell* (edited by G. C. AINSWORTH and A. S. SUSSMAN), p. 229, Academic Press, New York (1965).

The same criticism applies to studies on the respiration of higher plant tissues infected by fungi.[16] Doubt must also remain about the interpretation of data on glucose catabolism by the phosphofructokinase-deficient yeast, *Rhodotorula gracilis*,[17] until it is shown that mannitol synthesis does not occur.

Other Symmetric Polyols

As shown in Table 1, the four carbon threitol and six carbon iditol also have symmetric molecules, and erythritol, xylitol, galactitol, allitol, and *meso*-glycero-*ido*-heptitol are all optically inactive. Each of these polyols, although relatively uncommon in plant tissues compared with mannitol,[1] could produce unexpected labelling patterns if involved in the metabolism of specifically labelled substrates. However, the tetritols and pentitols are generally considered to be formed from the sugar-phosphates of the pentose-phosphate pathway and, as suggested for data on arabitol synthesis by *Dendryphiella*,[4] may be only slowly oxidized during carbohydrate assimilation. Very little is known about the metabolism of hexitols, apart from mannitol, and heptitols in plants.

CONCLUSIONS

During the assimilation of specifically labelled sugars by cells and tissues, any subsequent synthesis and oxidation of symmetric or optically inactive acyclic polyols will lead to hitherto unexpected labelling patterns in other carbohydrates. For fungi with a labile mannitol pool, in particular, the rate of turnover of the hexitol and the degree of equilibration between glucose- and fructose-6-phosphates must be taken into account before attempting to estimate the pathways for pentose-phosphate synthesis or hexose catabolism.

Acknowledgements—We are most grateful to Dr. D. H. Lewis for advice during the preparation of this paper.

[16] R. K. S. Wood, *Physiological Plant Pathology*, p. 375, Blackwell, Oxford (1967).
[17] M. Hofer, K. Brand, K. Deckner and J. Becker, *Biochem. J.* **123**, 855 (1971).