THE EFFECT OF TEMPERATURE ON THE CARBOHYDRATE METABOLISM OF POTATO TUBERS*

By P. J. WALSH† and K. S. ROWAN†

Abstract

The increase in rate of synthesis of starch in potato tubers transferred from 0°C to 18°C is probably not due to increasing rate of utilization of glucose 1-phosphate in a phosphorylase or a pyrophosphorylase reaction.

Introduction

Many workers have described the synthesis of sugars from starch in potato tubers transferred from high to low storage temperature and have shown that the overall process is reversible. Analysis of the respiration rate of tubers when they are returned to high temperature (Barker 1965) shows that respiration accounts for only a fraction of the decrease in sugar and it is assumed that most is reconverted to starch. Pathways suggested for conversion of sucrose may not involve hexose phosphates as intermediates, since glucose 1-phosphate (G1P) will not be involved if uridine diphosphate glucose (UDPG) formed in the reaction catalysed by sucrose synthetase is the intermediate:

$$\text{Sucrose} + \text{UDP} \rightleftharpoons \text{fructose} + \text{UDPG} \quad (1)$$

$$\text{UDPG} \rightarrow \text{starch} + \text{UDP} \quad (2)$$

Although adenosine diphosphate glucose (ADPG) is a more effective substrate for starch synthesis (Recondo and Leloir 1961), the concentration of UDPG in plant tissue is usually much higher than ADPG, thus compensating for the lower activity (Leloir 1964). In maturing pea seed, the concentration of hexose monophosphates (HMP) decreases (Rowan and Turner 1957) when the rate of synthesis of starch increases (Turner and Turner 1957), coinciding with increased activity of amylose phosphorylase (Turner and Turner 1957), and ADPG- and UDPG-pyrophosphorylase (Turner 1969*a*, 1969*b*). The action of invertase could be the first step in the conversion of sucrose to starch, although Pressey and Shaw (1966) and Pressey (1969) state that activity of this enzyme is low in mature potato tubers.

We have analysed changes in concentration of reducing sugars, sucrose, and HMP so that we can decide whether increasing activity of an enzyme utilizing G1P regulates synthesis of starch from sucrose in potato tubers transferred from low to high storage temperature. Since the concentration of G1P is low in potato tubers (Barker 1968*a*, 1968*b*), we have assumed that the hexosephosphate isomerase reaction is in equilibrium in the tissue and that the concentration of G1P is proportional to glucose 6-phosphate (G6P) (Barker 1968*b*).

* Manuscript received 2 February 1973.

† Botany School, University of Melbourne, Parkville, Vic. 3052.

Materials and Methods

Freshly harvested tubers of *Solanum tuberosum* L. (cv. Sequoia) were stored at 1 °C in darkness. As the concentration of sugars varied considerably between tubers, we carried out temperature treatments on half tubers and expressed results of analyses as a percentage of the value for the control half tuber retained at 1 °C. Since starch content is higher in the heel than in the rose end (Burton 1966), we cut tubers along an axis of symmetry through the apical bud and stem scar. The cut surfaces were sealed with a mixture of paraffin wax and petroleum jelly held between 55 and 65 °C. Halves were then stored in darkness either at 1 or 18 °C. Temperature equilibrium after transfer to 18 °C took 4 hr.

Since metabolic changes at the cut surface are restricted to about 1·5 mm (Laties 1957), the tissue 5 mm below the cut surface was discarded before extraction, and the peel removed from the remaining surface. Sugars were extracted by macerating 40 g of tissue in 144 ml absolute ethanol in a Waring Blendor for 1 min and the supernatant was recovered by centrifugation at 7000 *g* for 15 min. Acid-soluble phosphates were extracted by blending 45 g of tissue in 45 ml 10% (v/v) $HClO_4$ in a M.S.E. homogenizer for 1 min. The macerate was filtered on No. 541 Whatman paper in a Buchner funnel. A measured volume of filtrate was adjusted to pH 7 with 8M KOH (Rowan 1958) and $KClO_4$ removed by centrifugation at 18,000 *g* for 30 min. All operations for both extractions were made at 1 °C, and all extracts were stored at −20 °C before analysis.

Fructose and glucose were estimated separately by the method of Furuholmen *et al.* (1964). Sucrose was estimated by the same method following hydrolysis of 1-ml samples with 2% (v/v) H_2SO_4 by boiling under reflux for 1 hr. Standard samples of both reducing sugars and sucrose were recovered completely when added to samples of extracts before the analysis.

G6P and fructose 6-phosphate (F6P) were estimated by enzymic analysis (Hohorst 1963), measuring fluorescence of NADPH at 460 nm in an Aminco-Bowman spectrophotofluorometer. Orthophosphate was measured by the method of Everson and Hinde (Everson 1964): samples of neutralized extract (0·20 ml) were added to 1·0 ml ammonium molybdate (5% w/v) in 2M H_2SO_4 plus 2·80 ml water in a glass-stoppered test tube. After mixing, 5·0 ml butan-2-ol–petroleum spirit (b.p. 100–110 °C) (1:1 v/v) was added from a burette and the tube shaken briskly for 15 s. The hypophase was removed by pipette and the epiphase containing the phosphomolybdate was dehydrated with 0·5 g anhydrous Na_2SO_4. The extinction of the epiphase was measured at 380 nm and the concentration of the extract calculated by reference to a standard curve.

Results

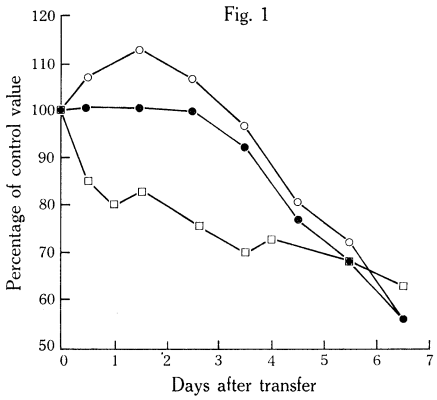
For 60 hr after tubers were transferred from 1 to 18 °C the amount of total sugar did not decrease, but sucrose was converted to reducing sugars. Paper chromatography in butan-1-ol–acetic acid–water (4:1:1, by vol.) demonstrated that the only reducing sugars present in significant amount were glucose and fructose. The concentration of sucrose, initially 0·5–1·0% of fresh weight, decreased throughout the experiment (Fig. 1) and was approximately one-quarter of the initial value after 12 days. Reducing sugars, initially 1·4–2·0% of fresh weight, after increasing for 36 hr, decreased to approximately half by 7 days (Fig. 1), finally levelling at 40% at 12 days.

The initial concentration of G6P (10–14 μmoles/100 g fresh weight) and F6P (3–4 μmoles/100 g fresh weight) decreased rapidly within 24 hr after transferring tubers from 1 to 18 °C; the concentration remained low for 3 days, then returned almost to the original level by 7 days (Fig. 2).

The initial concentration of orthophosphate in the tubers was high (1–2 mmoles/100 g fresh weight), decreasing by about 10% after 1 day at 18 °C, and thereafter fluctuated about the control level (Fig. 2).

Discussion

When tubers were transferred from low to high storage temperature the concentration of HMP decreased rapidly, though rising again almost to the control level after 3 days. This curve (Fig. 2) is a reciprocal of the rate of respiration reported by Barker (1965) and we conclude that the concentration of HMP reflects the rate of carbon flux in the process of respiration rather than in starch synthesis, since, judging by the decrease in concentration of sucrose and reducing sugars (Fig. 1), starch synthesis does not begin until 2 days after transfer, at which time the concentration of HMP does not alter for another day. Comparison of this lack of change in concentration of HMP with the decrease occurring in pea seed when starch synthesis increases (Rowan and Turner 1957) suggests that the increase in rate of starch synthesis in potato is not due to increasing utilization of G1P in a pyrophosphorylase or a phosphorylase reaction, although simultaneous increase in the rate of synthesis and utilization of G1P cannot be excluded entirely. However, this hypothesis is consistent with the absence of detectable ADPG-pyrophosphorylase in potato tuber



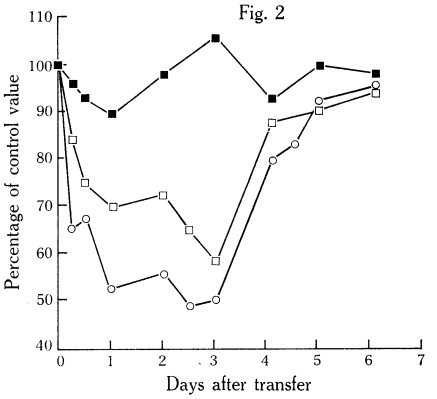


Fig. 1.—Changes in concentration of total sugars (●), sucrose (□), and reducing sugars (○) in half tubers after transfer from 1 to 18°C expressed as a percentage (w/w) of the value in the control.
Fig. 2.—Changes in concentration of glucose 6-phosphate (○), fructose 6-phosphate (□), and orthophosphate (■) in half tubers after transfer from 1 to 18°C expressed as a percentage (w/w) of the value in the control.

(Espada 1962), and synthesis of starch from ADPG or UDPG produced by direct reaction of sucrose with ADP or UDP appears a more probable pathway (equations 1 and 2). Since the concentration of orthophosphate is 2000 times the probable concentration of G1P, the synthesis of starch by amylose phosphorylase is unlikely (Hanes and Maskell 1942).

Pressey (1969) assigns a possible role to sucrose synthetase (equation 1) in starch synthesis in immature potato tubers. Although he found that activity decreased after harvest and remained constant during high or low temperature storage for 8 weeks, he did not follow activity of the enzyme in tubers transferred from cold to warm storage. The activity and substrate specificity of starch synthetase and the concentrations of ADPG and UDPG in mature tubers transferred between high and low temperature storage require further investigation.

The transitory increase in concentration of reducing sugars after transfer of tubers to 18°C (Fig. 1) probably reflects the activity of invertase before the activity of an invertase inhibitor increases significantly (Pressey and Shaw 1966). Since starch synthesis does not begin until 3 days after transfer, invertase does not appear important in starch synthesis under these circumstances.

Acknowledgments

Potato tubers were supplied by the Potato Research Station, Department of Agriculture, Toolangi, Victoria. P. J. Walsh held a Research Scholarship from the University of Melbourne.

References

BARKER, J. (1965).—Studies in the respiratory and carbohydrate metabolism of plant tissues. XVIII. The effect of oxygen on starch formation and dissolution in potatoes. *New Phytol.* **64,** 201–9.

BARKER, J. (1968*a*).—Studies in the respiratory metabolism of plant tissue. XXIV. The influence of a decrease in temperature on the contents of certain phosphate esters in plant tissues. *New Phytol.* **67,** 487–93.

BARKER, J. (1968*b*).—Studies in the respiratory metabolism of plant tissues. XXV. Changes in rate of CO_2 output and in content of various phosphate compounds in potatoes. *New Phytol.* **67,** 495–503.

BURTON, W. G. (1966).—"The Potato—A Survey of its History and of Factors Influencing its Yield, Nutritive Value, Quality and Storage." (2nd Ed.) (Veenman and Zonen: Wageningen, Holland.)

ESPADA, J. (1962).—Enzymic synthesis of adenosine diphosphate glucose from glucose 1-phosphate and adenosine triphosphate. *J. biol. Chem.* **237,** 3577–81.

EVERSON, R. G. (1964).—Studies in the physiology of plant cells. Ph.D. Thesis, University of Melbourne.

FURUHOLMEN, A. M., WINEFORDNER, J. D., KNAPP, F. W., and DENNISON, R. A. (1964).—The quantitative analysis of glucose and fructose in potatoes. *J. agric. Fd Chem.* **12,** 109–12.

HANES, C. S., and MASKELL, E. J. (1942).—The influence of hydrogen ion concentration upon the equilibrium state in phosphorylase systems. *Biochem. J.* **36,** 76–9.

HOHORST, H. J. (1963).—D-Glucose-6-phosphate and D-fructose-6-phosphate. Determination with glucose-6-phosphate dehydrogenase and phosphoglucose isomerase. In "Methods of Enzymatic Analysis". (Ed. H-U. Bergmeyer.) pp. 134–8. (Verlag-Chemie, Academic Press: New York.)

LATIES, G. G. (1957).—Respiration and cellular work and the regulation of the respiration rate in plants. *Survey biol. Prog.* **3,** 215–99.

LELOIR, L. F. (1964).—Nucleoside diphosphate sugars and saccharide synthesis. *Biochem. J.* **91,** 1–8.

PRESSEY, R. (1969).—Potato sucrose synthetase: Purification, properties, and changes in activity associated with maturation. *Pl. Physiol., Lancaster* **44,** 759–64.

PRESSEY, R., and SHAW, R. (1966).—Effect of temperature on invertase, invertase inhibitor, and sugars in potato tubers. *Pl. Physiol., Lancaster* **41,** 1657–61.

RECONDO, E., and LELOIR, L. F. (1961).—Adenosine diphosphate glucose and starch synthesis. *Biochem. biophys. Res. Commun.* **6,** 85–8.

ROWAN, K. S. (1958).—Phosphorylated compounds in plants. II. The estimation of hexose phosphates and adenosine pyrophosphates in plant tissue by the method of Slater. *J. exp. Bot.* **9,** 436–45.

ROWAN, K. S., and TURNER, D. H. (1957).—Physiology of pea fruits. V. Phosphate compounds in the developing seed. *Aust. J. biol. Sci.* **10,** 414–25.

TURNER, D. H., and TURNER, J. F. (1957).—Physiology of pea fruits. III. Changes in starch and starch phosphorylase in the developing pea seed. *Aust. J. biol. Sci.* **10,** 302–9.

TURNER, J. F. (1969*a*).—Physiology of pea fruits. VI. Changes in uridine diphosphate glucose pyrophosphorylase and adenosine diphosphate glucose pyrophosphorylase in the developing seed. *Aust. J. biol. Sci.* **22,** 1145–51.

TURNER, J. F. (1969*b*).—Starch synthesis and changes in uridine diphosphate glucose pyrophosphorylase and adenosine diphosphate glucose pyrophosphorylase in the developing wheat grain. *Aust. J. biol. Sci.* **22,** 1321–7.