Phytochemistry, 1973, Vol. 12, pp. 2579 to 2591. Pergamon Press. Printed in England.

STARCH–SUGAR INTERCONVERSION IN *SOLANUM TUBEROSUM*

FREDERICK A. ISHERWOOD

A.R.C. Food Research Institute, Colney Lane, Norwich NOR 70F

(*Received* 19 *February* 1973. *Accepted* 15 *May* 1973)

Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuber; starch–sugar interconversion; energy requirements.

Abstract—The changes in starch, sugars, and respiration of both immature and mature potato tubers (variety King Edward) caused by transfer from +10° to +2° and back to +10°, were followed throughout. At each storage temperature the tubers were allowed to reach a steady state before transfer to another temperature. In potatoes transferred from +10° to +2°, the sugar at first rose rapidly and then reached a constant value after 30 days. The respiration showed a characteristic pattern, for the first 5–8 days being below the initial value, then rising to a maximum at 14 days and finally returning to the initial value at 28 days. In potatoes transferred from +2° to +10° the sugar declined steadily, the respiration reaching a maximum after 10 days and then slowly falling to a value slightly above the initial value. Quantitative analysis of the results showed that the sum of starch + sugar + CO_2 expressed in equivalent anhydrohexose units did not change throughout the various changes in temperature. This work provided a quantitative experimental basis for what had hitherto been an assumption. Starch was the only polysaccharide involved in these carbohydrate changes. No change in the amylose/amylopectin ratio was detected either during the breakdown of starch (temperature change +10° to +2°) or during its synthesis (+2° to +10°). The increased respiration which accompanied any change in temperature was related quantitatively to the formation of sucrose from starch (+10° to +2°) and starch from sugar (+2° to +10°). The ATP equivalent of the extra CO_2 output was of the same order as that predicted on the basis of known biochemical pathways linking starch and sugar.

INTRODUCTION

THE CONVERSION of starch into sugar in plant tissues held at low temperatures is well recognized but the mechanism of the sweetening process is not known.[1–4] Both sucrose and reducing sugars accumulate during low temperature storage and in the case of potatoes held at +2° the increase in sugar can rise to over 2% of the fresh weight. If these potatoes are subsequently held for a few weeks at a temperature above +10°, the accumulated sugar is lost, presumably by recondensation to starch. In the past, most workers have assumed that these changes can be summarized by the equation starch $\rightleftharpoons$ sugars with an allowance for the sugar lost in respiration, but at no stage has a quantitative experimental basis been provided for this assumption. The difficulty lies in finding a sufficiently reliable and sensitive analytical method for the estimation of the starch present in plant tissue, since the maximum change in the starch content on a fresh weight basis is not likely to be more than 3% and the amount of starch present in mature tubers is of the order of 20%. Previous workers have estimated the loss of sugar due to respiration and the changes in the concentrations of the sugars present but in general have not attempted to measure the changes in the starch content. If other polysaccharides such as polyfructosans were involved this would not have been apparent.

[1] MULLER-THURGAU, H. (1882) *Landw. Jbr.* **11**, 751.
[2] ARREGUIN-LOZANO, B. and BONNER, J. (1949) *Plant Physiol.* **24**, 720.
[3] BARKER, J. (1965) *New Phytologist* **64**, 201.
[4] BARKER, J. (1968) *New Phytologist* **67**, 487; (1968) *New Phytologist* **67**, 495.

The present study had as its immediate object a complete balance sheet of the equivalent anhydrohexose units involved in the starch–sugar respiration changes in potatoes either during the degradation of starch to sugar at low temperatures or during the formation of starch from sugars at high temperatures. The equivalent anhydrohexose was chosen rather than the carbon content as a basis for comparison because of its more obvious relation to carbohydrates. This was a necessary preliminary to a detailed study of the changes in phosphate esters and other metabolites in the tissue during the 'sweetening' and 'de-sweetening' processes. The starch occurs in amyloplasts which are a specialized form of plastid, and it is clear that this study of the starch–sugar interconversion has a wide significance. The same interconversion probably occurs in the chloroplasts of leaves.[5] Potato tubers are a very useful plant material in which to study the interconversion of starch and sugars caused by a simple change in environment because during the period of the experiment they are in a dormant state. This implies that most other cellular processes are not likely to interfere seriously in the interpretation of the results.

The experiments covered periods of 28 days at each temperature and since samples were taken at intervals of 3–10 days, short-term variations which are known to occur immediately the temperature of storage is changed are not indicated. Such variations, however, do not affect the long-term overall changes in starch and sugars which were the principal concern of this study.

Apart from the theoretical implications of the low temperature sweetening of potatoes, the phenomenon has considerable economic importance. Storage of potatoes under refrigeration at $+2°$ to $+4°$ has many advantages.[6] Sprout growth and senescent sweetening are largely prevented and most storage rots are inhibited and, if the low temperature sweetening could be controlled, storage of potatoes under these conditions would become a practical possibility.

RESULTS

Three experiments are described, the first of which was a preliminary exercise and only respiration and sugar figures are given. The starch figures were similar to those described in the second experiment but were less reliable and have not been included. Subsequently, minor difficulties mainly concerning the hydrolysis of the starch which were experienced in the application of the method for starch to potato tissue in the first experiment were overcome and the precision improved. The results for the second and third experiments were obtained with the improved method. A further experiment which was started on 12 November 1971 (unpublished), confirmed the results obtained for mature potatoes in the experiment of 29 October 1970.

First experiment

Mature potatoes were lifted 16 September 1969. The experiment started 2 December 1969, potatoes being transferred from $+10°$ to $+2°$ on 26 January 1970 and then from $+2°$ to $+10°$ on 28 February 1970. The changes in the respiration and in the sugars are shown in Figs. 1 and 2. The respiration and sugars show a characteristic pattern of change similar to those described by Barker.[7]

[5] PORTER, H. K. (1953) *Biochem. Soc. Symp.* **11,** 27.
[6] BURTON, W. G. (1969) *European Potato J.* **12,** 81.
[7] BARKER, J. (1936) *Proc. R. Soc.* **119B,** 453.

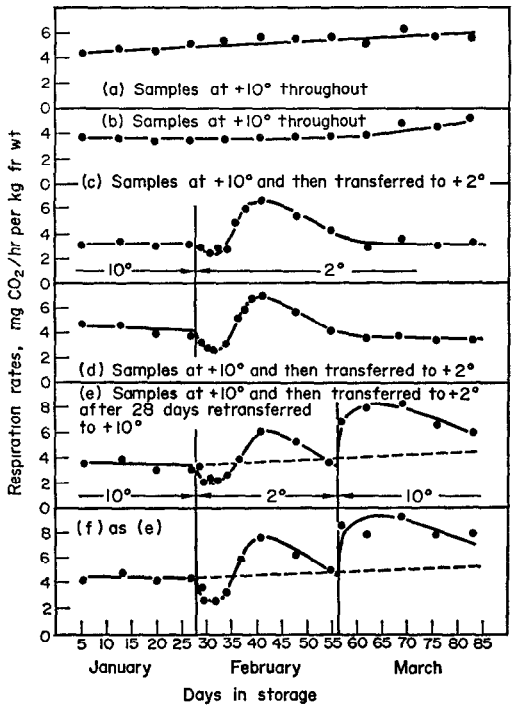


FIG. 1. EFFECT ON CO_2 PRODUCTION OF EXPOSURE TO DIFFERENT TEMPERATURES.
Potatoes harvested 16 September 1969. Vertical lines indicate times at which potatoes were transferred.

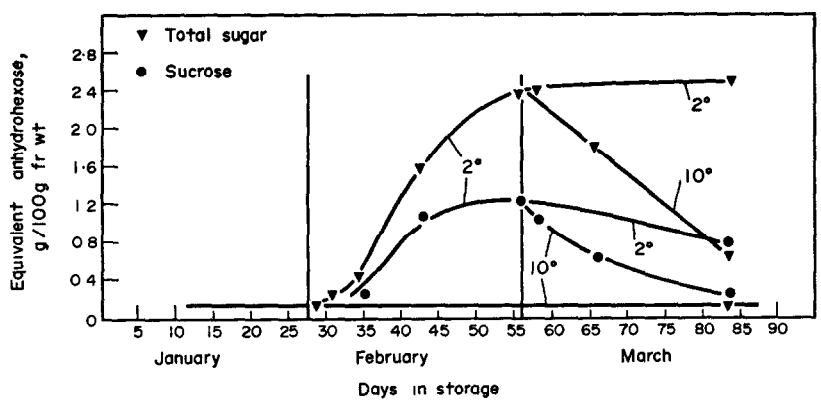


FIG. 2. EFFECT ON SUGARS (CALCULATED IN TERMS OF EQUIVALENT ANHYDROHEXOSE UNITS) OT EXPOSURE TO DIFFERENT TEMPERATURES.
See legend for Fig. 1 for details of temperature changes.

Second experiment

Mature potatoes were lifted 6 October 1970. The experiment started 29 October 1970, potatoes being transferred from +10° to +2° on 23 November 1970 and then from +2° to +10° on 21 December 1970. The respiration figures were very similar to those in Fig. 1 and have not been given in detail since they are included in the general summary of the

changes in the starch, sugars and CO_2 evolved shown in Fig. 3. The results are expressed in anhydro-hexose equivalents and it is clear from Fig. 3(d) that the sum of starch + sugar + CO_2 does not change throughout the various changes in temperature.

Third Experiment

Immature potatoes were lifted 28 July 1970. The experiment started 10 September 1970, potatoes being transferred from +10° to +2° on 21 September 1970 and then from +2° to +10° on 19 October 1970. The changes in starch, sugar and CO_2 evolved are shown in Fig. 4. It is again clear that the sum of starch + sugar + CO_2 does not change [Fig. 4(d)].

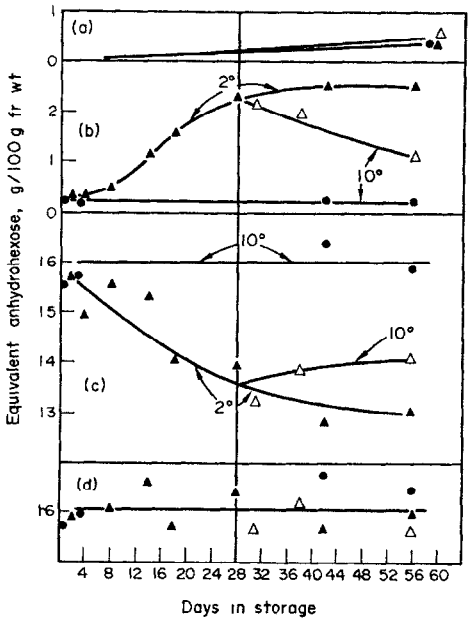


FIG. 3. EFFECT ON CO_2 PRODUCTION, SUGAR AND STARCH (ALL AS EQUIVALENT ANHYDROHEXOSE UNITS) OF EXPOSURE TO DIFFERENT TEMPERATURES (EXPERIMENT 2).
Mature potatoes lifted 6 October 1970. Vertical line indicates time at which potatoes were transferred.

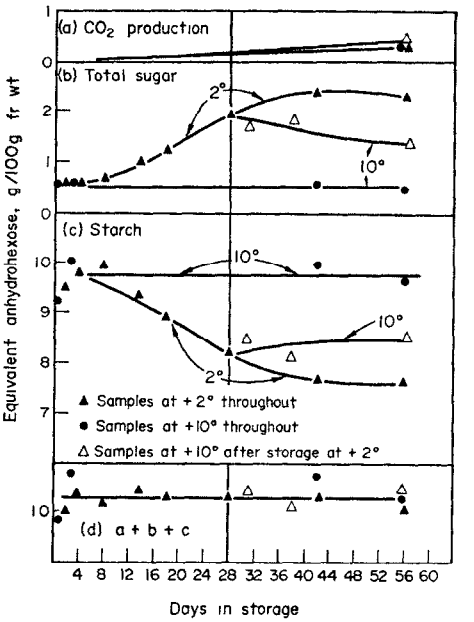


FIG. 4. EFFECT ON CO_2 PRODUCTION, SUGAR AND STARCH ON EXPOSURE TO DIFFERENT TEMPERATURES. SYMBOLS AND LETTERS AS IN FIG. 3.
Immature potatoes lifted 28 July 1970

Sugar Changes in the Second and Third Experiment

The detailed results are given in Fig. 5. It is noticeable that in mature potatoes the main sugar produced when the temperature changes from +10° to +2° is sucrose.

Changes in the Amylose—Amylopectin Ratio in the Starch in the Second and Third Experiments

This is given as the 'Blue Value'[8] for both mature and immature potatoes (experiments 2 and 3) in Fig. 6. It is noticeable that the 'Blue Value' does not alter during the various temperature changes.

[8] GILBERT, G. A. and SPRAGG, S. (1964) *Methods in Carbohydrate* Chemistry (WHISTLER, R. L., SMITH, R. J., BEMILLER, J. N. and WOLFRAM, M. L., eds), Vol. IV, p. 168, Academic Press, New York.

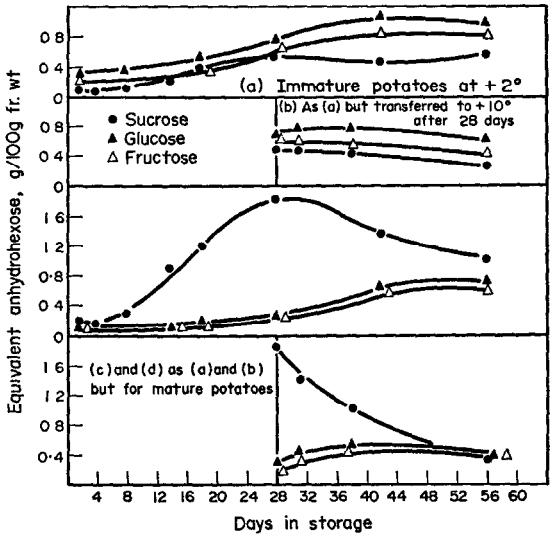


FIG. 5. CHANGES IN INDIVIDUAL SUGARS (AS EQUIVALENT ANHYDROHEXOSE UNITS) FOR EXPERIMENTS SUMMARIZED IN FIGS. 3 AND 4.

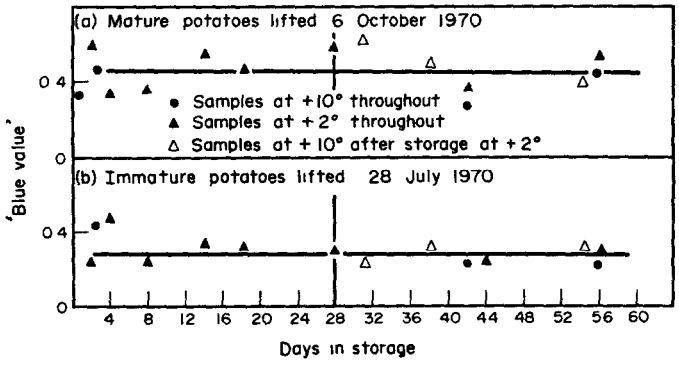


FIG. 6. 'BLUE VALUE' OF THE STARCH IN IMMATURE AND MATURE POTATOES DURING STORAGE AT +10° AND +2°.
Vertical line indicates time at which appropriate samples were moved from +2° to +10°.

DISCUSSION

In preliminary experiments (1969–70 season) the potatoes were selected from a large stock on the basis of uniformity of size (tubers about 100 g in weight) and freedom from blemishes. A random sample of 30 tubers was taken for each experiment. Since the main object of the study was to investigate the changes in the starch $\rightleftharpoons$ sugar relationship and the maximum change expected represented only 10–15% of the total starch present, it was important that variations in the measurement of the starch content should be as small as possible. In general, the observed variations between duplicate samples for respiration, sugar and starch contents were similar but the variations in the starch content had a much greater weighting in any conclusions to be drawn from an analysis of the data. The changes in the sugar content involved almost the entire amount present and accordingly the variations were less important.

In the next season (1970–71), potatoes selected as described above were then graded on the basis of their specific gravity. This was introduced in an attempt to obtain a sample of potatoes with a more uniform starch content for it has been shown[9] that there is a direct relationship between the starch content of a tuber and its specific gravity. However, the specific gravity is affected by the volume of the intercellular space and it is clear that any variation in this will cause the exact relationship to break down. The maximum error likely to be introduced from this cause is about 2% in the content of starch and in the present study the potatoes were graded within a specific gravity range covering 0·01 which is equivalent to a variation of about this amount.[10] Analysis of the results obtained on two stocks of mature potatoes (1969–70; 1970–71) and one immature (1970–71) showed that the maximum variation between triplicate estimations of the starch was not greater than $\pm 5\%$ in each stock and half the results were within $\pm 2\%$ of the mean. The corresponding figures for the insoluble residue (which is mainly starch) were ± 2 and $\pm 0{\cdot}5\%$ respectively. The results on these three stocks of potatoes indicate that selecting the tubers on the basis of their specific gravity did not improve the precision because the variation is practically the same with each stock and only those studied in 1970–71 were selected on the basis of specific gravity. It is interesting to note that though not shown in the Results, a graph of the amount of insoluble residue against the time in storage at $+10°$ and $+2°$, gave an exactly similar picture to that shown in Figs. 3 and 4 for starch in both mature and immature potatoes.

The main reason for choosing enzymic methods for starch and sugar estimations was that in general they were much more specific than chemical ones and avoid any ambiguity as to the nature of the particular carbohydrate fraction estimated. For starch analysis, an amylo-α-1,4-α-1,6 glucosidase (E.C. 3.2.1.-) enzyme and acid were separately used for hydrolysis to glucose. The glucose produced was measured using glucose oxidase (E.C. 1.1.3.4). Acid hydrolysis was found to be more convenient because with the enzyme method[9] the digest of insoluble residue and enzyme had to be incubated at 60° overnight.

Apart from starch, which is insoluble in cold aqueous TCA used for extraction, other polysaccharides may be present which could conceivably be concerned with the starch $\rightleftharpoons$ sugar interconversion. These are a soluble α-glucan called phytoglycogen (found in sweet corn[11]) together with polysaccharides analogous to the dextrans[12] and fructosans[13] known to be produced from sucrose by various bacteria. To investigate this possibility, the extract, after removal of the insoluble residue by centrifugation or filtration, was subjected to ultrafiltration. Material retained on the filter was examined to determine whether acid hydrolysis released either fructose or glucose. No evidence was found for the presence of these polysaccharides and it was concluded that the only reserve polysaccharide connected with the sugars in potato tubers was starch.

Interconversion of Starch and Sugars

Inspection of the data summarized in Figs. 3 and 4 for mature and immature potatoes indicates that when the temperature of storage is changed from $+10°$ to $+2°$, the total

[9] VON SCHEELE, C., SVENSSON, G. and RASMUSSEN, J. (1937) *Landw. VerStnen* **127** 67.
[10] BURTON, W. G. (1966) *The Potato*, Veenman & Zoner, Wageningen, Holland.
[11] WHELAN, W. J. (1958) *Encyclopedia of Plant Physiology* (RUHLAND, W., ed.), Vol. VI, p. 154, Springer, Berlin.
[12] JEANES, A. (1965) *Methods in Carbohydrate Chemistry* (WHISTLER, R. L., BEMILLER, J. N. and WOLFRAM, M. L., eds.), Vol. V, p. 118, Academic Press, New York.
[13] AVIGAD, G. (1965) *Methods in Carbohydrate Chemistry* (WHISTLER, R. L., BEMILLER, J. N. and WOLFRAM, M. L., eds.), Vol. V, p. 161, Academic Press, New York

anhydrohexose equivalent remains constant. This means that the overall change can be described exactly by the equation starch $\rightleftharpoons$ sugars when allowance is made for the small amount of sugar lost by oxidation to carbon dioxide. The data cover a period of about 3 months (Jan.–Mar.) during which the tubers were in a dormant state. In the case of mature potatoes, the total sugar increased from 0·3 to 2·5% during the sweetening phase and then fell to 1·0% during the desweetening phase. Immature potatoes showed similar changes. This work provided a quantitative experimental basis for what had hitherto been an assumption that the changes in the carbohydrate in the potato due to a change in the temperature of storage were almost entirely due to the interconversion of starch and sugars. It thus provided a firmer basis for speculations on the biochemical pathways linking the two carbohydrate groups.

Amylose–amylopectin Ratio in the Starch Grain

One such speculation concerns the relationship of amylose and amylopectin in the starch grain. A regulatory mechanism must exist which determines how much of each is synthesized. Separate pathways have been suggested for the synthesis of amylopectin and amylose, amylopectin by the combined action of phosphorylase and a branching enzyme (*Q* enzyme forming α (1–6) linkages) and amylose by transfer of glucose from a nucleotide diphosphate glucose. However, it seems more likely by comparison with the biosynthesis of glycogen that both are synthesized from a nucleotide diphosphate glucose and most recent theories assume some sequential order of events in the formation of the two polysaccharides. It is of interest that in short-term radiochemical feeding experiments in wheat plants (sucrose-^{14}C), amylose had a higher specific activity than amylopectin and would appear to be formed preferentially.[14,15] In this connection, the present study provided an opportunity of testing whether the ratio of amylose to amylopectin changed significantly during the degradation and synthesis of starch as a result of temperature changes. The maximum changes observed in starch content were 22 and 10% respectively for immature potatoes and 16 and 8% respectively for mature potatoes. If we assume that the whole change in the starch is amylose then these figures correspond to changes of 0·22, 0·1, 0·16 and 0·08 respectively in Blue Values (assuming amylose to have a Blue Value of 1). Inspection of the results for the Blue Value of the starch throughout the storage experiments on both mature and immature potatoes (Fig. 6) shows no obvious changes, and it would appear that metabolic control of the two polysaccharides gives a nearly constant product. It is possible that on a short-term basis (24 hr) there may be a difference in the ratio, but the present method was not sufficiently sensitive to detect it.

Sugars in the Potato

In the case of mature potatoes it seems clear from the results in Fig. 5 that during the loss of starch at low temperature, sucrose is produced first and the other sugars later. In fact, sucrose represents the major part of the increase in sugar. At +10° the process is reversed and during synthesis of starch, sucrose is the main sugar to be lost. The position with immature potatoes is somewhat different in that while sucrose may be affected more than the hexoses, all tend to change in a parallel manner. It appears that with mature potatoes the sucrose and the hexoses are largely independent, possibly in different compartments between which there is only a slow exchange, whereas with immature potatoes the sugars are

[14] McConnell, W. B., Mitra, A. K. and Perlin, A. S. (1958) *Can J. Biochem. Physiol.* **36**, 985.
[15] Whistler, R. L. and Young, J. R. (1960) *Cereal Chem.* **37**, 204.

more closely linked. A point of particular interest is that the level of sucrose rose immediately following the change of temperature from +10° to +2° whereas the levels of glucose and fructose either did not change or fell slightly. Observations made in the course of a recent experiment (unpublished), have shown that even within 6 hr of the change in temperature (the centres of the tubers do not reach +2° until at least this time has elapsed) the level of sucrose has risen (5%) whereas the hexoses have fallen (10–20%). The sucrose continues to rise but the hexoses do not begin to rise until 1–2 days after the change in temperature and even then show only a small change up to 8 days. These observations are consistent with the view that the sucrose is formed from the starch within the amyloplast and is then transported into the cytoplasm where it is hydrolysed to the hexoses.

In the discussion which follows, the general assumption has been made that the conversion of starch to sucrose occurs primarily in the amyloplast. The reverse process in which sucrose and the hexoses are converted to starch possibly involves the transport of various phosphate esters, rather than the sucrose, into the amyloplast.

Speculations on the Energy Requirements of the Starch–Sugar Interconversion

The interconversion of starch and sugar requires energy because it is known that excluding oxygen retards both the conversion of starch to sugar at low temperature and the conversion of sugar to starch at high temperature.[3,5] A substantial breakdown of polysaccharide occurs in the absence of oxygen[3] but, instead of sugar, lactic acid is produced. This suggests that starch can be degraded as far as the sugar phosphates and that the oxygen dependent step occurs between the sugar phosphates and sucrose. It was of interest, therefore, to calculate from the respiration data the amount of ATP required for the conversion of starch to sucrose (hexoses) and also for the reverse process, and to compare these figures with those predicted on the basis of our present knowledge of the biochemical pathways linking starch and the sugars. In the discussion which follows, the results on mature potatoes (season 1969–70) which were in a dormant state throughout the period of the experiments have been analysed in detail. The fact that the potatoes were in a dormant state meant that any changes in respiration were likely to be closely linked to the interconversion of starch and sugars. The contributions of other processes such as the turnover of protein or organic acids were assumed to be low and relatively constant. The amount of protein present is about 1% of the fresh weight but any changes which occur during storage are small and not consistent with the type of storage.[6] In slowly growing carrot explants (the nearest analogous tissue for which figures are available) the turnover of protein was only about 0·5% of the total protein/day[16] and, assuming the same held for potato protein, this is equivalent to the consumption of 0·05 mmol ATP/100 g/24 hr (assuming the average MW of the amino acid to be 100 and that each required 1 mol ATP for incorporation into protein). This figure is small in relation to the calculated equivalence (see below) of the basal respiration, 1·4 mmol ATP/100 g/24 hr. It was also assumed that throughout the dormant period the respiratory quotient was 1.[7] There is a considerable amount of evidence to support this assumption for, even when the respiration changes markedly due to a variety of causes such as a change in the temperature of storage[17] or administration of poisons such as HCN,[23] the respiratory quotient remains close to 1. This implies that the substrate for respiration is carbohydrate in origin.

[16] BIDWELL, R. G. S., BARR, R. A. and STEWARD, F. C. (1964) *Nature* **203,** 367
[17] CRAFT, C. C. (1963) *Am. Potato J.* **40,** 288.

Though the overall change between starch and sugar is reversible, the pathway from starch to sugar is unlikely to be the same as from sugar to starch. Storage at low temperature is mainly concerned with the former and storage after sweetening with the latter. So the experimental results obtained under the two storage conditions have been considered separately.

Temperature of Storage Changed from $+10°$ *to* $+2°$

Examination of the respiration data in Fig. 1 shows that in all cases (c, d, e, f) there was a preliminary decrease in the output of CO_2 to about half that at $+10°$, but that afterwards the rate increased to a maximum of about twice the initial rate at $+10°$ within 14 days. After 28 days at $+2°$ (c and d only) the output of CO_2 had returned to the initial rate at $+10°$ and remained at this level for a further 28 days. This pattern of change was similar to that described by Barker[4,7] for a temperature change of $+10°$ to $+1°$.

In calculating the extra output of CO_2 concomitant with the conversion of starch to sucrose two factors have been taken into account.

First, the basal respiration changes during the course of the experiment for, on returning the potatoes stored at $+2°$ back to $+10°$, the final respiration at $+10°$ does not return to the original rate observed before storage at $+2°$ but remains somewhat higher. To allow for this it has been assumed that the overall basal rate changes in a linear fashion from the initial figure at $+10°$ to the final figure at $+10°$ after storage at $+2°$. This is indicated in Fig. 1 by the broken line.

Second, an allowance has been made for the preliminary decrease in output over the first 7 days. This could be due either to the greater solubility of CO_2 in the sap at the lower temperature or it could be due to a temporary slowing down in the utilization of substrate. If the former, then an approximate calculation made on the basis of the figures given by Burton[10] suggests that in potatoes in equilibrium with the atmosphere 19 μl of CO_2/ml of sap are present at $+10°$ and 33·6 μl/ml at $+5°$. The difference, 14·6 μl/ml is equivalent to 29 mg/kg of potatoes. Calculation of the CO_2 retained in solution from the area of the curves (c, d, e, f) below the steady state level at $+10°$ for the first 7 days indicated that between 80 and 90 mg were held back. This is of the same order when allowance is made for the increased solubility at $+2°$. Since the apparent output of CO_2 will be less than the true figure because the tissue has first to be saturated with CO_2, this amount of CO_2 must be included in the total output occasioned by the conversion of starch to sugar; such calculations are referred to as made by method A.

If the second possibility mentioned above is true, that the fall in output is due to a temporary drop in the oxidation of substrate and not to an increased solubility of CO_2 in the cell sap, then the calculation of the total output becomes more uncertain. However, in order to obtain some measure of the CO_2 output it has been assumed that the basal rate changes in a linear fashion from the depressed figure observed after 1–2 days to the final one at 28 days. After correction for the overall change in the basal respiration, the extra output of CO_2 in excess of this is the amount involved with the conversion of starch to sugar. Calculations made on this basis are referred to as made by method B.

Based on the assumptions described above the increased output of CO_2 for 4 separate experiments (Fig. 1, c, d, e, f) is as follows: Method A: 0·95, 0·64, 0·64 and 0·91 g/kg, respectively (mean 0·79), and Method B: 1·2, 0·9, 0·9 and 1·3 g/kg, respectively (mean 1·07).

To calculate the amount of ATP which this increase in respiration represents it is necessary to know the proportion of the respiration which is actually coupled to phosphorylation.

(For the present purpose, since the RQ is close to unity, the O_2 absorbed is equivalent to the CO_2 evolved.) Two electron transport pathways are known to be present in potatoes which means that, of the O_2 absorbed, it is possible for only part to be linked to phosphorylation. Two methods have been used to measure the relative contributions of each pathway.

The first method is based on the assumption that the electron transport pathway coupled to phosphorylation is sensitive to cyanide whereas the other electron transport system which is not coupled to phosphorylation is insensitive.[18] For example, the respiration[19] of freshly cut slices of potato tuber is inhibited about 70% by 5×10^{-4} M cyanide which indicates that only 70% of the respiration is coupled to ATP production. Comparison with intact tubers is difficult because it is known that the respiration of freshly cut slices is 2- to 3-fold greater than that of intact tubers. A figure of 70% is probably an upper limit for the proportion of the respiration coupled to ATP production. Another example is provided by an experiment in which potato tubers were transferred from storage at 0° to 25°.[17] These show a 3- to 4-fold increase in respiration in air but in the presence of cyanide the respiration is reduced to about 50% of its usual figure; at the same time cyanide also stimulates the cyanide-insensitive respiration which means that the inhibition of the phosphorylative pathway is probably greater than the 50% observed.

The second method is based on a study of the affinity of the terminal oxidases for oxygen since cytochrome oxidase which is the terminal oxidase of the phosphorylative pathway has a high affinity for oxygen. An analysis[20] of the relation between the respiration of tubers stored in atmospheres containing different concentrations of oxygen and the oxygen concentration indicated that in air approximately 70% of the respiration passed over cytochrome oxidase.

Based on the examples given above, it has been assumed in the present study that 70% of the respiration is coupled to the formation of ATP. It has also been assumed that since the basic mechanisms of plant and animal mitochondria are very similar[18] the oxidation of 1 mol of hexose gives 6 mol of CO_2 and is equivalent to 38 mol of ATP.[21]

The mean figures for the increased CO_2 due to a change of temperature from +10 to +2° (0·79 and 1·07 g/kg) correspond therefore to 0·08 and 0·105 mol ATP/kg. The change in total sugars (2·4% as anhydrohexose including the small contribution from the sugar which is oxidized) corresponds to 0·15 mol/kg. Since the first sugar produced is almost certainly sucrose (two anhydrohexose units) these figures suggest that 0·08 mol ATP are required for the synthesis of 0·075 mol sucrose, a ratio of 1·06. Calculations from the second figure for the ATP produced (0·105 mol) give a ratio of 1·3 but since it is very likely that the solubility of CO_2 in the cell sap accounts for the major part of the fall in the output of CO_2 over the first 7 days, this ratio has not been used in subsequent discussion.

Temperature of Storage Changed from +2° to +10°

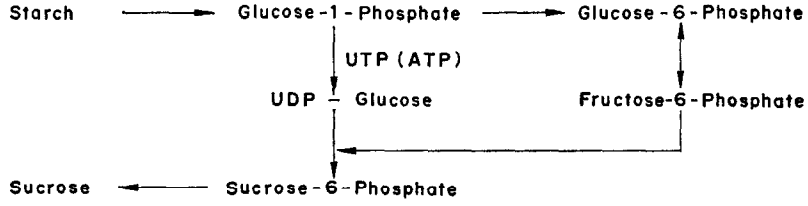
Calculation of the amount of ATP involved was made in a similar manner to that described above except that with method A the extra CO_2 accumulated in the tissue at low temperature was assumed to be lost at +10° thus increasing the apparent respiration. This quantity was therefore subtracted from the total when calculation was made in this way.

[18] IKUMA, H (1972) *Ann. Rev Plant Physiol.* **23,** 419.
[19] HACKETT, D. P., HAAS, D. W., GRIFFITHS, S. K. and NIEDERPRUEM, D. J. (1960) *Plant Physiol.* **35,** 8.
[20] MAPSON, L W. and BURTON, W. G. (1962) *Biochem J* **82,** 19.
[21] INGRAHAM, L L and PARDEE, A. B. (1967) *Metabolic Pathways* (GREENBERG, D. M., ed.), Vol. I, p. 2, Academic Press, New York.

The respiration curves (Fig. 1) were not continued beyond the 28th day at +10° because the final analyses of sugars and starch were made at this time. The respiration eventually fell to a new level somewhat higher than that observed initially at +10°. In Fig. 1 the broken line connects the initial level at +10° with the point at which the respiration curve reaches the new level at +10°. The outputs of CO_2 for the examples in Fig. 1 (e and f) were 1·96 and 1·90 g/kg respectively, mean 1·93 (Method A) and 1·93 and 1·96 g/kg respectively, mean 1·95 (Method B). These figures correspond to 0·196 mol ATP/kg, the equivalent figure for the sugar being 0·10 (total change of sugar as anhydrohexose 1·6%). The ratio is 1·96.

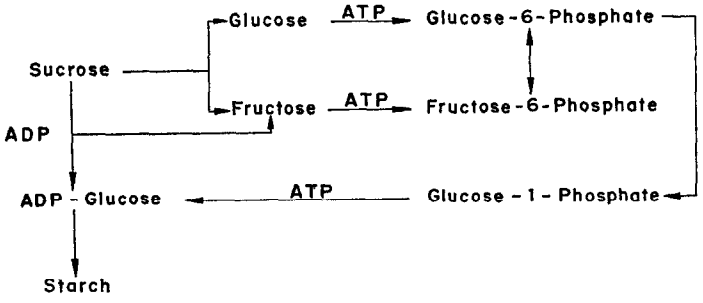
Relation of Energy Requirements to Possible Biosynthetic Pathways

The conversion of starch to sucrose is assumed to occur as shown in outline in Scheme 1.



SCHEME 1. CONVERSION OF STARCH TO SUCROSE.

The only known step involving ATP (as UTP) is the conversion of glucose-1-phosphate to UDP-glucose. This would indicate a ratio of 1 mole of ATP to each mole of sucrose formed. The observed ratio was just over 1 which is in excellent agreement. In both Scheme 1 and Scheme 2, which is shown below, no attempt has been made to describe the pathways in detail but only to indicate the overall relation between the number of anhydrohexose units involved and the ATP required.



SCHEME 2. CONVERSION OF SUGARS TO STARCH.

The first reaction sequence involves the formation of ADP-glucose by transglycosylation from sucrose to ADP. The fructose formed is converted into ADP-glucose in the same way as the free hexoses. The second reaction sequence starts with the hexoses and this pathway requires 2 mol of ATP per mol of anhydrohexose. A similar scheme can be suggested in which uridine nucleotides replace adenine but since the stoichiometry is the same, this has not been described.

Assuming that both pathways contribute to the formation of starch but that no hydrolysis of sucrose to hexoses occurs, the ratio, based on the known composition of the sugar mixture at the start of the storage at +10°, is 1·3. If the sucrose is first hydrolysed to the

hexoses and then these are converted to starch, the ratio is 2. The experimentally observed ratio was 1·96, which suggests that a large part of the starch is synthesized from the hexoses produced by hydrolysis of the sucrose.

Regulation of Starch Breakdown and Synthesis

The hypothesis described above suggests that both the formation of sucrose from starch when the temperature is changed from +10° to +2° and of starch from sugar when the temperature is changed from +2° to +10° can be related to the increased respiration of the tubers under these conditions. An extension of this idea suggests that even under constant storage conditions some of the starch is continuously being broken down and resynthesized. The pathway of breakdown is probably different from that of synthesis and the whole cyclic process requires the provision of energy in the form of ATP derived from the oxidation of carbohydrate to CO_2. At +10°, the basal respiration is about 4 mg CO_2/kg/hr, which is equivalent to 1·4 mmol ATP/100 g/24 hr. From the calculations described above for the interconversion of starch and sucrose, at least 3 mol of ATP would be required for each cycle of breakdown and synthesis and this means that the observed respiration would be equivalent to a turnover of 0·5 mmol anhydrohexose/100 g/24 hr. The initial rate of production of sucrose in a recent experiment (unpublished) was equivalent to 0·01% anhydrohexose/24 hr which corresponds to 0·06 mmol/100 g/24 hr. This is only a small fraction of the calculated turnover figure and it is clear that if the change in temperature from +10° to +2° only partially altered the synthesis of starch relative to the synthesis of sucrose, then the increased formation of sucrose would be easily explained. This picture would suggest that the control of the starch-sugar balance is linked to the complex of enzymes governing the carbohydrate interchange. Temperature does not appear to affect the activity of the isolated insoluble starch synthetase[22] which is as active at 0–4° as at 45°.

EXPERIMENTAL

Potatoes. The potatoes (var. King Edward) were obtained from the Norfolk Agricultural Station, Morley, Norfolk. From a batch of mature potatoes harvested 16 September 1969, those between 80 and 160 g were selected for the first set of experiments. Those harvested 6 October 1970 were selected in the same way but were then regraded on the basis of their specific gravity by flotation in solutions of common salt.[9] Those with a specific gravity between 1·08 and 1·09 were used for the second set of experiments. The immature potatoes harvested 28 July 1970 were first selected on the basis of their wt (50–70 g) and then on the basis of their specific gravity, 1·053–1·064.

Respiration. The method used was essentially the same as that described by Hanes and Barker.[23] Separate samples each containing about 30 tubers were placed in sealed 5 l. flasks through which passed a current of CO_2-free air at the rate of about 2 l./hr. This air was at the same temp. as the room in which the potatoes were stored and in addition was saturated with H_2O before being passed into the 5 l. flasks. At appropriate times the flasks were changed from storage at +10° to +2° and back again.

Extraction procedure. As the experiment proceeded, samples of the potatoes (each flask represents one sample) were taken after various periods of time for analysis. The potatoes (3 kg) were cut into thin slices (2 mm thick) as rapidly as possible and dropped into liq. N_2. The frozen slices, still under liq. N_2, were ground to a flour in a stainless steel beaker (4 l.) using an Ultra Turrax blender (Model T45, Janke & Kunkel, K. G. Stauffen B.R.S.G.). The slurry of potato powder and liq N_2 was thoroughly mixed and stored in a room at −40°. For analysis (in triplicate) 50 g of the powder (free from liq N_2) was weighed at −40° and blended with 200 ml of a mixture containing 10% (w/v) TCA, 30% (w/v) MeOH and 0·15% (w/v) 8-hydroxyquinoline in H_2O using a high speed blender. After 4 min the homogenate was centrifuged and the supernatant filtered through a Whatman 541 paper under suction. The residue was reblended with 200 ml of 5% (w/v) TCA for 2 min and filtered as before, the combined filtrates being set aside for estimation of sugars The residue was washed with H_2O on the filter paper until the washings were free from acid and then dried at +50° *in vacuo* for 4 hr.

[22] FRYDMAN, R. B. and CARDINI, C. E. (1967) *J. Biol. Chem.* **242,** 312.
[23] HANES, C. S. and BARKER, J. (1931) *Proc. R. Soc.* **108B,** 95.

Estimation of starch. Two methods were used for the estimation of starch in the residue. The first method depended on the hydrolysis of the starch to glucose using an amylo-α-1,4-α-1,6-glucosidase (E.C. 3.2.1.-) followed by a colorimetric determination of the glucose using glucose oxidase (E.C. 1.1.3.4), peroxidase (E.C. 1.11.1.7) and *o*-toluidine.[24,25] In the second method the starch was hydrolysed with dilute sulphuric acid[26] and the glucose estimated as before. The residue contained up to 20% H_2O which was determined separately.

Estimation of sugars. To an aliquot of the combined filtrates equivalent to 10 g of potato powder was added 2·5 ml of 70% (w/w) $HClO_4$ and the solution cooled to 0°. It was then extracted in the cold with an equal vol. of a mixture of Et_2O and light petrol. (40–60°) (2:3 by vol.) until the Et_2O layer was free from TCA. This usually required five extractions. A further three extractions ensured that all the TCA had been removed. The solution was neutralized to pH 7 using 20% KOH and then left for 1 hr at +1° to allow $KClO_4$ to precipitate. The mixture was centrifuged and the supernatant evaporated to small vol. to remove the MeOH. The sugars were estimated enzymically.[27]

Removal of soluble polysaccharides. The filtrate after removal of the starch was subjected to ultrafiltration through a Millipore Pellicon membrane using a standard ultra-filtration cell (membrane PSED 14 205 with a nominal MW cut-off of 25 000, apparatus XX42 142; Millipore Corp., Bedford, Mass., U.S.A.). Superimposed on the Pellicon membrane was a prefilter disc (Type AP25; Milliport Corp.) which consists of pure glass fibres with an acrylic binder. The prefilter removes material which would otherwise clog the Pellicon membrane. The material left on the filter and the prefilter was examined for glucosans or fructosans.

Estimation of amylose. The amount in the potato residues was measured from the blue colour produced in an aqueous solution upon the addition of the tri-iodide ion.[13] From the known amount of starch present the proportion of amylose in the starch was calculated.

[24] MACRAE, J. C. and ARMSTRONG, D. G. (1968) *J. Sci. Food Agric.* **19,** 578.
[25] MARKS, V. (1959) *Clinica. Chim. Acta* **4,** 395.
[26] PIRT, S. J. and WHELAN, W. J. (1951) *J. Sci. Food Agric.* **2,** 224.
[27] BERGMEYER, H. U. (1963) *Methods of Enzymatic Analysis,* Academic Press, New York.