STUDIES ON THE STARCH-SUGAR EQUILIBRIUM IN POTATO TUBERS

by

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TABLE OF CONTENTS

Table of Contents cont.

LIST OF TABLES

INTRODUCTION AND REVIEW OF THE LITERATURE

Storage Behavior of Irish Potato Tubers

Numerous investigations of the storage behavior of potato tubers have shown a conversion of starch to sugars during low temperature storage (31°-50°F.) and a reconversion of sugars to starch upon subsequent storage at higher temperatures (60°-80°F.). As early as 1882 Müller-Thurgau (66) reported that at temperatures of 0°-6°C. sugars accumulated and starch was broken down while, if the temperature was raised to 8°-10°C., the sugars disappeared and starch was formed again. Similar studies conducted by later investigators (5,6,21,101) have confirmed and extended these results. It has also been reported by Appleman (7) and Curtis (20) that storage of potato tubers at temperatures of 30°-40° C. causes a conversion of starch to sugar.

The magnitude of the sugar accumulation in potato tubers during storage is principally dependent upon temperature and duration. There is normally little sugar in potatoes at harvest; however, upon transfer to lower temperatures the sugar content gradually increases, the concentration attained being higher the lower the temperature. Thus, the concentration of total sugars in the potato tuber can be altered from a typical harvest or 60°-70°F. storage value of 0.2% on a fresh weight basis to a value of over 7% by storage at -1°C. for 50-60 days. The maximum concentration of total sugars attained at -1°C. is

roughly twice that attained at 1°C. and six times that attained at 3°C. (9). During storage below 45°F. the total sugars gradually increase for the first 40-60 days until a maximum is reached which remains fairly constant for storage periods of 6 months or longer (102). When potato tubers containing appreciable amounts of sugar are transferred to temperatures of 60°-80°F., the sugar content decreases consistently and in proportion to the increase in temperature. The rate of the conversion of sugars to starch at the higher temperatures is more rapid than the rate of accumulation of sugars at the lower temperatures, which is in accord with the general effect of temperature upon enzymatic reactions.

The predominant sugars formed during low temperature storage of potato tubers are glucose, fructose and sucrose. The relative proportions of the individual sugars are primarily dependent upon the temperature and duration of storage and to a lesser degree upon the maturity and temperature-history of the sample. In potato tubers that contain only moderate amounts of sugars, such as normally found at the time of harvest or during storage between 55°-80°F., reducing sugars and sucrose are present in roughly equal amounts and the reducing sugar fraction is made up of approximately equal amounts of glucose and fructose. When potato tubers are placed at low temperatures (45°-31°F.) and moderately high temperatures (85°-100°F.), there is a rapid increase in sucrose without a corresponding increase in reducing sugars during the first 10-20 days of storage. During subsequent storage, the low

temperature samples accumulate reducing sugars so that after 60-90 days reducing sugars predominate over sucrose, especially at 40°F. storage. This condition persists throughout subsequent storage up to six months. At 32°F., however, sucrose often continues to increase after its initial rise so that in many varieties sucrose may equal or exceed the reducing sugars after 60 days of storage. A comparable study cannot be made on the high temperature (90°-100°F.) samples since the tubers soon decay.

Arreguin-Lozano and Bonner (8) have reported that fructose increased in concentration nearly ninefold at 0° and twofold at 9°C. but remained approximately constant in concentration at 16° and 25°C storage for 2 weeks. This is in contrast to results reported by Barker (10,11,13) which indicate that the fructose/glucose quotients depart little from unity over the range from -1° to 20°C. Much of this discrepancy is probably due to the lack of a really quantitative test for fructose. Arreguin-Lozano and Bonner used the method of Roe (79) which is not only variable but requires a correction for the sucrose present, while Barker determined the reducing value after destruction of the glucose with periodate.

When potato tubers are transferred from temperatures below 45°F. to temperatures of 60°-80°F. both reducing sugars and sucrose decrease. Denny and Thornton (21) have reported that in tubers placed at 27°C. following low temperature storage there was a marked decrease in reducing sugars without a parallel decrease in sucrose.

Barker (12) found that the maturity of the potato tuber affected the proportions of the individual sugars and to some extent the magnitude of accumulation during storage at 10°C. He reported that potatoes dug in July accumulated hexoses to a much greater extent than comparable tubers dug later in the season. Sucrose, however, underwent a prolonged decline in samples from all harvesting dates. It has also been observed that early crop potatoes are more sensitive in their response to low temperatures than mature, or fall crop, potatoes (12). Barker (11) has also reported that the sensitivity of the sugar accumulating mechanism may be reduced by adaption to intermediate lower temperatures prior to transfer to the final low temperature; and, conversely, the sensitivity may be increased by prior exposure to higher temperatures but that this adaptive response depends to a large extent on the exact stage of maturity of the potato tubers. Denny and Thornton (21) reported that the time after harvest at which potato tubers were placed in cold storage was an important factor in the subsequent rate of sugar development. Early storage induced the formation of high amounts of reducing sugars while a late storage favored an accumulation of sucrose. In later investigations (22,23), these workers used samples in which this effect of date of storage was not nearly so pronounced. Wright, et al. (103) stored several varieties of potatoes at 60°F. for 3 weeks previous to storage at 32° and 40°F., and found that the effects upon sugar accumulation were inconsistent and probably of no great significance.

Potato tubers of different varieties differ markedly both in the extent to which they respond to temperatures below 55°F. and the manner in which they respond to storage at higher temperatures (60°-80°F.) following low temperature storage. Some varieties such as Bliss Triumph and Green Mountain accumulate relatively large amounts of sugar at temperatures as high as 55°F., while other varieties such as Russet Burbank do not accumulate appreciable amounts of sugar until placed in storage below 40°F. Denny and Thornton (21), in a study of 25 varieties, found considerable variation in the sugar-forming characteristics of the varieties. In one study the highest reducing sugar value was five times the lowest value with intermediate values throughout. The order of varieties for increasing amounts of reducing sugars was not the same as that for increasing amounts of sucrose. The correlation in rank for 11 varieties that were studied for two years was highly significant which indicates that certain varieties consistently produce high amounts of reducing sugars in low temperature storage. The authors also reported that tubers of the same variety grown under different soil conditions and in two different localities showed no important differences in the amounts of reducing sugars accumulated during cold storage. Other workers (103), however, have found variation in response to storage below 45°F. of tubers of the same variety grown in the same locality on different soils, in the same variety grown in different localities in the same year and in the same variety grown in the same locality in different

years. The magnitude of these variations, however, is not as large as that caused by differences in variety.

It is apparent from the foregoing considerations that the response of potato tubers to low and high temperature storage is a biological response and experimental attempts at its elucidation will be attendant with all the complexity associated with similar phenomena. There is no fixed pattern of response for every given sample of potatoes and considerable variation both quantitatively and qualitatively will be found among samples from the same variety and particularly among samples of different varieties. It appears that the response is initiated by protoplasmic factors that are to some extent under genetic control. Little advance has been made in the study of such factors and suitable techniques for their study are not available. The enzymatic mechanisms, however, by which the various starch-sugar equilibria are achieved as well as the physicochemical conditions leading to the equilibria should prove subject to the advances in allied biochemical fields and inferences that may be drawn from studies in comparative biochemistry.

Carbohydrate Transformations in Plants

It is now generally agreed that reversible carbohydrate transformations in plants take place through the fermentation pathway in which inorganic phosphate is enzymatically introduced at the maltosidic linkage of starch and the subsequent transformations involve phosphoric esters of the sugars. At

present, this is the only known path between simple sugars and starch. According to this view hydrolytic enzymes such as amylase are only of importance in special cases since the main products of amylase activity, dextrins and maltose, are not normally found in plant cells and the system is not reversible under ordinary physiological conditions.

Formation of glucose and fructose from starch: Glucose and fructose may be formed directly from starch by means of the fermentation pathway or indirectly by the hydrolysis of sucrose which is in some manner first formed from starch. The initial reaction in the formation of the simple sugars from starch is thought to be the production of glucose-1-phosphate as follows:

amylose + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate.

This reaction is catalyzed by the enzyme phosphorylase and is freely reversible since the bond energy of the C-O-P linkage in glucose-1-phosphate is approximately the same as the glycosidic linkage in starch. The equilibrium, in vitro, is not significantly affected by wide variations in starch concentration. Hanes and Maskell (35) found that when the pH value is varied from 5.0 to 7.0 the values of the ratio of inorganic phosphate to ester phosphate decrease progressively from about 10.8 to 3.1. If this could be extended to in vivo conditions, it would follow that a decrease in hydrogen-ion concentration in the living cell would cause a decrease in the ratio of inorganic phosphate to glucose-1-phosphate which would favor starch synthesis.

Glucose-1-phosphate does not accumulate in living tissue since in the presence of the enzyme phosphoglucomutase it is rapidly and reversibly converted to glucose-6-phosphate. According to the present state of our knowledge, glucose would result from the action of a phosphatase on glucose-6-phosphate but there is little specific information on this reaction and nothing is known of the conditions in the cell which would determine how much of the glucose-6-phosphate would be converted to glucose and how much would be converted to fructose-6-phosphate, the next compound in the fermentation scheme. However, it is known that the reaction

glucose-6-phosphate $\longrightarrow$ glucose + PO_4

is practically irreversible due to the energy liberated as heat upon hydrolysis of the phosphate bond.

Glucose-6-phosphate may be reversibly converted to fructose-6-phosphate by means of the enzyme phosphohexoisomerase. Fructose is thought to become available by the action of a phosphatase on fructose-6-phosphate. As is the case with glucose, few details of this reaction are known and, similarly, the reaction should prove to be practically irreversible.

Formation of sucrose from starch: The exact mechanism for the formation of sucrose in higher plants is at present obscure. Sucrose hydrolysis by invertase is practically irreversible. Hassid, et al. (45) have found that the dried cells of Pseudomonas saccharophilia contain an enzyme which catalyzes the following reversible reaction:

fructose + glucose-1-phosphate $\rightleftharpoons$ sucrose + PO_4.

The equilibrium of this reaction is as follows:

$$K = \frac{(\text{sucrose})(\text{inorganic phosphate})}{(\text{fructose})(\text{glucose-1-phosphate})} = .053 \text{ at pH } 6.6 \text{ and } 30^{\circ}\text{C.}$$

An enzyme similar to this sucrose phosphorylase has not been isolated from higher plants. Even so, it would be of doubtful consequence since, with even equal amounts of glucose-1-phosphate and inorganic phosphate, extremely large concentrations of fructose would be required to form even a portion of the sucrose found in some plants. This, of course, assumes that the equilibrium constant would be the same *in vivo* as *in vitro* and that reactants and products are not somehow selectively separated.

Other workers (47,49) have demonstrated bacterial enzymes that are capable of transferring the glycosidic linkage of sucrose for those of a polysaccharide. Sucrose may be reversibly converted to a dextran, a levulan or an amylopectin. Since the glycosidic linkage in sucrose is at a higher energy level than that of the polysaccharide, these reactions do not appear as probable mechanisms for sucrose synthesis. However, the possibility is raised for the direct conversion of sucrose into starch in higher plants. The reverse of this process would require metabolic energy. Further consideration of this possibility is warranted since glucose-1-phosphate has never been isolated in appreciable quantities from intact higher plants. Also, the principal product of starch breakdown in intact plants is sucrose. Thus, although sucrose utilization and respiration may occur in

leaves and other organs that form no starch, starch respiration does not occur in the absence of sucrose (54).

Recent work (18) on the products formed in photosynthesis has led to a proposed mechanism for sucrose formation in which fructose-6-phosphate and glucose-1-phosphate combine in some manner to yield sucrose. This is based on the finding that after 30 seconds of photosynthesis with $C^{14}O_2$, sucrose from Chlorella cells was found to have a higher labeling in the fructose portion than in the glucose but after 90 seconds both moieties were equally labeled. This is in accord with the concept that the formation of sucrose in photosynthesis occurs as a result of the reversal of fermentation. Fructose-6-phosphate would be labeled first and initially react with non-labeled glucose-1-phosphate. Calvin and Benson (18) as well as Wood and Burr (100) and Gibbs (27) have reported that sucrose is the first free sugar formed in photosynthesis. Stepka (87) studied the effects of 1.5×10^{-4}M iodoacetamide on the assimilation of $C^{14}O_2$ by Chlorella during photosynthesis and found that 3.5 times as much C^{14} was incorporated into sucrose in the presence of iodoacetamide. It was concluded that this was the result of a blocking of other paths of carbon utilization which led to a more rapid sucrose synthesis or, alternatively, sucrose was synthesized by a path not involving triose phosphate. Quellet and Benson (73) reported that when the pH of the medium in which Chlorella was photosynthesizing passed from ph 1.6 to 11.4 the percentage of malic acid

increased from 5 to 25% while the percentage of sucrose decreased from 7 to 0%.

Formation of starch from glucose, fructose and sucrose: In order for free glucose or fructose to be transformed to starch it is first necessary to raise the energy levels of these compounds to that of the hexose phosphate esters. The primary mechanism, according to present knowledge, is a reaction, catalyzed by either specific or non-specific hexokinases, in which ATP transfers a phosphate group to the free hexose as follows:

(1) glucose + ATP → glucose-6-PO_4 + ADP

(2) fructose + ATP → fructose-6-PO_4 + ADP

Both reactions as given are practically irreversible since the bond energy of the phosphate group in ATP is at a higher energy level than that in the hexose phosphates. Since aerobic oxidation is necessary for the formation of sizeable amounts of ATP, it can be stated that the conversion of glucose or fructose to a hexose phosphate will be an aerobic process and will require actively metabolizing tissue. Once the hexose phosphate esters are formed, the most likely path to starch, according to present information, is through glucose-1-phosphate to starch which is a reversal of the fermentation path. Another possible route, however, is the formation of sucrose from the hexose phosphates and the direct condensation of sucrose to starch through some unknown mechanism. Recent evidence (67) has indicated that the phosphatase reaction might prove to be reversible to

some extent, but even so an expenditure of metabolic energy would be required.

Little is known of the conversion of sucrose to starch in the higher plants. It might be expected that a reversal of the mechanism for sucrose synthesis would lead to the phosphorylated esters of glucose and fructose which could then proceed to starch through a reversal of fermentation. A reaction of this type should be accomplished with little expenditure of metabolic energy. However, there is no proof of such a mechanism in higher plants.

Identification of the Enzymes of Fermentation and Phosphorylated Intermediates in the Potato Tuber

With the exception of hexokinase and a specific enzyme for the synthesis of sucrose, the enzymes and phosphorylated intermediates required for the various transformations of the carbohydrates via the fermentative path have been demonstrated in the potato tuber.

Phosphorylase was first demonstrated in potato tubers by Hanes (34). It was further characterized by Green and Stumpf (28). Potato phosphorylase, along with phosphorylase from other sources, freed from traces of starch, cannot initiate the synthesis of starch from pure glucose-1-phosphate although this enzyme is capable of causing the phosphorylytic breakdown of starch to glucose-1-phosphate in the presence of a suitable concentration of inorganic phosphate. Potato phosphorylase requires the presence of some preformed poly-

saccharide for the synthesis of starch. The activation is optimal with amylopectin but the priming action can also be achieved by linear amylaceous materials, including natural and synthetic amyloses as well as the products of partial acid and α-amylase hydrolysis of these materials (50). Potato phosphorylase does not have the ability to catalyze either the complete formation or breakdown of starch (43) since only linear chains of 1,4 linked α- D-glucose units are synthesized or degraded. The formation and disruption of the branched structures of amylopectin which contain 1,6 linkages at the point of branching evidently requires the presence of an additional enzyme. Peat and his collaborators (72,73,74) have obtained an enzyme from potato tubers that is capable of converting linear amylose into branched amylopectin. This enzyme, which has been designated as the Q enzyme, functions as a non-phosphorolytic transglucosidase. Thus, the synthesis of native starch in potato tubers can be accounted for by the action of phosphorylase in collaboration with the Q enzyme, but the manner of formation of starch into insoluble starch grains in the plastids of the plant cell as well as the significance of this localization are not yet explained.

The phosphorylated intermediate glucose-1-phosphate which is essential for the synthesis or degradation of starch by the fermentative path has not been detected as a normal constituent of potato tubers although Arreguin-Lozano and Bonner (8) have reported it present in small concentrations in potatoes stored at temperatures of 0° and 9°C. Albaum (3) lists the occurrence of glucose-1-phosphate in potato tubers as reported by several foreign workers.

The enzyme systems, phosphoglucomutase and phosphohexose

isomerase, responsible for the interconversion of the hexose phosphates, have not been isolated from potato tubers although the activity of these systems may be readily demonstrated in vivo. Glucose-6-phosphate and fructose-6-phosphate, however, are present as normal constituents of potato tubers (8).

There are no reports of phosphohexokinase in potato tubers although McCready (64) has reported an accumulation of fructose 1,6 diphosphate during the process of starch conversion into sucrose. Aldolase (91) and triosephosphate (4) have been reported by several workers.

Indirect evidence of the presence of hexokinase in potato slices has been presented by Guzman-Barron, et al. (30). Bonner (16) also states that evidence of hexokinase activity has been found in the potato.

Numerous acid, alkaline as well as specific phosphatases have been reported in potato tubers. Kalckar (55) and Krishman (57) reported on an enzyme preparation from potatoes catalyzing the hydrolysis of the acid-labile phosphates in ATP. Hassid (43) has reported a potato phosphatase that readily splits off the phosphate from the reducing monophosphates but only to a very small extent from glucose-1-phosphate. Various other types of phosphatases have been reported by Pfankuch (75) and Naganna (68).

Transformation of Carbohydrates in Intact Plant Tissue

It is possible to shift the starch-sugar equilibrium in plants by means other than temperature changes. In the

leaves of many plants starch accumulates during periods of illumination and disappears during periods of darkness when sugars increase in amount. Starch rapidly disappears and sugar increases in leaves or other plant parts in which a water loss is occurring. In addition, starch may be formed in leaves or other plant parts which are being artificially fed a solution of glucose, fructose or sucrose. Under these same conditions glucose will form fructose and sucrose, fructose will form glucose and sucrose and sucrose will form glucose and fructose. It is apparent that intact tissues from many plants are capable of interconverting glucose, fructose, sucrose and starch. However, investigators have not reported such an activity in homogenates.

Spoehr and Milner (86), in a study of starch dissolution and amylolytic activity in leaves, reported that in certain leaves starch dissolution occurs in an atmosphere free of oxygen although the rate of loss under anaerobic conditions was roughly one-half that of the aerobic rate. The rate of loss was further decreased by the presence of CO_2 in concentrations above 5% under both aerobic and anaerobic conditions. These workers also report that starch dissolution does not occur in leaves which have been killed by toluene, chloroform or freezing even though such agents do not destroy the enzymes. The results of these workers are difficult to interpret since analyses of the products of starch dissolution are not given. Their results would seem to indicate that the necessity of intact tissue for car-

bohydrate transformations depends upon protoplasmic organization rather than a system capable of generating metabolic energy in appreciable amounts. Other workers (39,65) report that aerobic conditions are universally essential for sugar formation in plants. The effect of CO_2 is also difficult to interpret since there is no way of determining whether its effect was due to a narcotic effect on the protoplasm or to a diverting of the energy-yielding reactions to CO_2 fixation rather than sugar formation.

Winkler (97) studied the conditions under which starch is produced in leaves artificially fed sucrose. For most species a 10% solution of sucrose was found to be optimal. The minimum concentration of sucrose which caused starch formation was 0.2%. The minimum temperature at which this occurred was 6°-8°C. The presence of oxygen was indispensable for the conversion of sucrose to starch.

Nelson and Auchincloss (69) reported that potato slices dipped in solutions of glucose and fructose show an increase in sucrose and that sucrose did not form in the absence of oxygen.

Vertanen and Nordlund (93) have shown that sucrose is readily formed from artificially fed glucose and fructose in wheat and red clover leaves, and that there is some conversion of fructose into glucose, and vice-versa.

Nurmia (71) reported that the conversion of fructose into glucose and the synthesis of sucrose from the hexoses in living plant tissue are not directly affected by a 0.05

molar KCN solution.

Leonard (60,61) found that when a 6% glucose solution was supplied to corn leaf blades sucrose increased markedly during the 26-hour feeding period, while glucose and fructose increased only slightly. Essentially the same results were obtained with sorghum and cotton blades. Sorghum leaf blades fed glucose in an atmosphere of CO_2 or H_2 lost rather than gained in sucrose. Iodoacetic acid, at a concentration of 0.1% or higher, prevented the synthesis of sucrose from glucose but not its hydrolysis, whereas various concentrations of NaCN, KCN, KSCN or invertase had little effect on sucrose synthesis. Sucrose synthesis from glucose or fructose decreased with senescence in cotton blades. In corn blades fed 6% glucose, sucrose was synthesized less readily at 8°C. than at higher temperatures (30°-50°C.)

McCready and Hassid (65) reported that leaves infiltrated with glucose or fructose accumulated sucrose to a level of 6% of their dry weight. Earlier McCready (64) reported that this conversion would not take place in the absence of oxygen or in the presence of cyanide and that, during the process of starch conversion into sucrose, hexosemonophosphate and fructose 1,6 diphosphate accumulated. From this he assumed that fructose 1,6 diphosphate is probably an essential component for sucrose formation.

Hartt (36,37,38,39,40,41,42), in an extended study of sucrose synthesis in detached sugar cane blades, reached conclusions similar to those just reported. She found that

the interconversion of glucose and fructose did not take place at 6°C. but that at 20°C. the two hexoses were interconverted as rapidly as sucrose was formed, and that at 30°-40°C. hexose interconversion took place more rapidly than sucrose formation. She reported that aeration increased the absorption of glucose and was absolutely essential for the conversion of glucose into fructose and for the synthesis of sucrose. She concluded that without exception whenever the interconversion of glucose and fructose is inhibited, the formation of sucrose is also inhibited. In a study of the effect of metabolic inhibitors upon sucrose synthesis, she found that various growth regulators, bone phosphatase, iodoacetate, arsenite, selenite, fluoride and 2,4-dinitrophenol prevented or decreased the interconversion of glucose and fructose and the synthesis of sucrose whereas pyrophosphate, azide, 8-hydroxyquinoline, malonate and cyanide had little or no effect. In a later paper (42), Hartt reported that malic acid has a specific role in increasing the formation of sucrose from glucose even to the extent of partially overcoming the inhibition of iodoacetate, fluoride and brilliant alizarin blue but not of selenite. Hartt also concluded that fructose 1,6 diphosphate was an intermediate in the formation of sucrose by the sugar cane plant.

Arreguin-Lozano and Bonner (3) reported that discs from potato tubers stored at 0°C., upon being artificially fed glucose and fructose solutions, appear to take up and inter-

convert more glucose and fructose and to synthesize more sucrose irrespective of the temperature of incubation than discs from tubers stored at 25°C. In addition, they reported that discs from tubers stored at 0°C. synthesize more sucrose when incubated at 0°C. than when incubated at 25°C., and that discs from potato tubers stored at 25°C. form more sucrose when given fructose than when given glucose.

Krotkov and Bennett (58) studied the synthesis and hydrolysis of sucrose following vacuum infiltration into wheat leaves. They reported that 0.02M NaF, 0.01M cyanide, 0.005M iodoacetate and 0.45% dinitrophenol inhibited sucrose synthesis and increased sucrose hydrolysis. They also reported that glucose-1-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate, when infiltrated with a hexose sugar, strongly inhibit sucrose synthesis. The concentrations of inhibitors used in this experiment are considerably higher than those usually employed. Furthermore, other workers (30) have shown that phosphorylated sugars are not able to penetrate the cell membrane.

Many workers have found that the drying of leaves, tubers and other plant organs at room temperature causes the conversion of starch into sugars. Waterman (94) expressed the opinion that starch is changed to sucrose in some direct way when potato slices are dried at temperatures of 30°-50°C. Other investigators (82) working with starch-bearing leaves concluded that on drying the starch was converted into sucrose and that the process was indepen-

dent of the concentrations of hexose sugars present in the leaves.

Wolff (98,99) believed that the accumulation of sugar by potato tubers placed at temperatures of 30°-50°C. was similar to the accumulation of sugar in drying discs. He studied the effect of different experimental conditions on the kinetics of the conversion of starch to sucrose in drying discs and reported that the sucrose content increased more or less independently of the manner of drying. The temperature for drying had little effect on the equilibrium but accelerated the velocity about 1.5 times for each rise of 10°C. The magnitude of the accumulation rapidly dropped off at temperatures from 50°-70°C. and the conversion did not occur in potato brei or when the dry matter increased beyond 53 per cent. At most temperatures there was also a small increase in reducing sugars. When partially dried potato slices, whose sucrose content had come to equilibrium, were allowed to absorb water, the sucrose content decreased but not to the original value. Wolff also looked upon this reaction as one independent of the reducing sugars present.

Nelson and Auchincloss (69) found that when potato slices were dried in an atmosphere of N_2, either no increase, or at most, a very slight increase in sucrose occurred.

Leonard (61) found that the drying of corn leaf blades resulted in a slight increase in sucrose and fructose, while under the same conditions cabbage leaves synthesized large quantities of sucrose from reducing sugars. This synthesis

in cabbage leaves continued until the blades had a moisture content of 8%. This synthesis could be prevented by heating to 72°C.

Spoehr and Milner (86) found that, upon losing moisture, sunflower leaves showed a large decrease in starch over the controls in water. The effect of the decrease in water content on the loss of starch was much more pronounced at 22°C. than at 5°C. during the 18 hours of the experiment.

Careful work by a number of investigators (76,77) has established that the dominant feature of crassulacean metabolism is the reversible transformation of malic acid to starch. With illumination during the day the organic acids decrease while starch increases. During the night the organic acids increase while starch decreases. The largest share in the changes in organic acids is taken by malic acid. Citric acid varies in the same direction as malic acid but to a smaller extent. Leaves cultured in the dark also synthesized starch. When excised leaves were exposed to different temperatures it was found that at 9°C. the total organic acids increased during the first day more rapidly and to a greater extent than at 20°C., while at 1°C. the increase in organic acids was somewhat slower than at 9°C. but was almost equally as extensive. This response to temperature is somewhat similar to the conversions of sugars in the potato tuber but in none of the above experiments were there any substantial changes in the soluble carbohydrates and what trends there were involved

glucose rather than sucrose.

Discussion of the Problem

In a study of the physiology and starch-sugar transformations of potato tubers during storage, the following problems are pertinent:

1. In what manner does low temperature (32°-45°F.) and high temperature (90°-100°F.) alter the physiology of the potato tuber so as to lead to a conversion of starch to sugar?
2. Do temperature and desiccation operate through similar physiological shifts in metabolism?
3. What is the pathway of the starch-sugar transformations?
4. What specific chemical components cause a shift in the starch-sugar equilibrium?
5. Is sucrose synthesized by similar mechanisms at low and high temperatures, under desiccation, and with artificially fed glucose and fructose?
6. Why does sucrose accumulate first?
7. Why are aerobic conditions essential for sucrose synthesis and the interconversion of the hexoses?
8. What determines the ratio of reducing sugars to sucrose as well as the ratio of glucose to fructose at the different storage temperatures?
9. What factors are responsible for varietal and sample differences in response?

With the limited information now available on the control of metabolism in intact cells it is difficult to formu-

late even a systematic experimental attack on the above problems. It is known that the mechanisms involved in carbohydrate transformations are enzymatic in nature. In the living cell these enzymes are in a continuous interplay. The actual metabolic pathway of carbohydrates in living cells is determined by the multiple factors which constitute the regulatory mechanisms of cellular metabolism. Some possible regulatory mechanisms would be: (1) the presence, amount, activity and topographical location in a cell of a given enzyme, (2) the ratios of the important reactants such as ATP, ADP and inorganic phosphate as well as the availability of phosphate acceptors and (3) changes in the permeability of the various cellular membranes which may either bring enzyme and substrate together or determine the availability of an essential reactant.

The amount of enzyme present may control the course of metabolism since if two alternative metabolic pathways are available, the pathway followed will depend in part upon the relative amounts of the respective enzymes. Enzyme location is important as a primary mechanism for the control of metabolism. A given enzyme may be localized in the cell membrane, which is a highly complex part of the cell machinery participating both in the control of cell metabolism and transport of substances (80), it may be localized in the soluble fraction of cytoplasm or it may be restricted to the particulate matter. There is little evidence, however, that the topographical location of an enzyme will change since

it is fixed by the genetic constitution of the cell. The activity of an enzyme in a cell is controlled by the physico-chemical environment of the cell and is affected by such factors as (1) pH (2) substrate concentration (3) concentrations of necessary metallic elements and coenzymes and (4) presence or absence of various inhibitors.

It is difficult to postulate possible sites at which variations in amount of enzyme or activity with temperature could alter the starch-sugar equilibrium in potato tubers since the exact pathways of the transformations are not known. It would appear that variations in amount or activity of enzymes could only affect practically irreversible reactions such as the phosphorylation of glucose. Variations in amount of enzyme or activity should have little effect on the equilibria of reversible reactions unless there is present a competing enzyme at one temperature and not at another.

It is possible that cellular metabolism may be regulated by variations in the amounts or ratios of the reactants present in the system. The three most important reactants involved in fermentation and respiration are ADP, ATP and inorganic phosphate. Since the equilibrium point of fermentation corresponds to far higher inorganic phosphate concentrations and lower ATP/ADP ratios than the equilibrium point of aerobic oxidation, in the presence of oxidative mechanisms the level of inorganic phosphate will be lower. A greater proportion of inorganic phosphate will be combined

in ATP, and the initial reaction of fermentation will proceed toward starch synthesis rather than the formation of glucose-1-phosphate. However, this general picture can be considerably modified by the presence of suitable phosphate acceptors such as glucose and by the presence and activity of adenylpyrophosphatase which liberates inorganic phosphate from ATP. The manner in which the above concept may possibly play a part in regulating the starch-sugar equilibrium in potato tubers will be discussed later.

Cellular metabolism may also be regulated by variations in membrane permeability. The vacuolar membrane and the particulate boundaries would be more involved in this concept than the cell surface. Changes in the permeability of these membranes could more or less regulate the extent to which substrate and enzyme were brought together or determine the availability of an essential reactant. For example, organic acids which are localized in the vacuole would cause an increase in reactivity when released to particulates or reactive centers in the cytoplasm. Similarly, the release of ATP from the particulates would stimulate synthetic reactions. Physiologists have entertained this idea for many years. Coville (19) in 1920 suggested that the starch grains in plant cells are at first separated by living active cell membranes from the enzymes which hydrolyze starch but that when the cell is chilled the membranes undergo a change in permeability so that the enzyme "leaks" out and hydrolysis results. Experimental evidence for the

functioning of the mechanism of permeability changes in the membrane is largely lacking but this can be attributed to the difficulties encountered in studying the organization of the living cell rather than to any weakness in the concept.

MATERIALS AND METHODS

Irish Potatoes

For the work in 1951 potatoes of the varieties Russet Burbank from Washington, Green Mountain from Long Island, N.Y., Chippewa from Maine and Triumph from North Dakota were used. Previous investigations had shown that these varieties differed markedly in starch content and in the ability to accumulate sugars in low temperature storage. The potatoes, which were mature, unwashed U.S. No. 1's of the fall crop, arrived at Beltsville, Md. during the first week of November. For the work in 1952 and 1953, involving the behavior of potato discs, potatoes were obtained from numerous sources.

Storage

Upon arrival the potatoes were randomized and lots of each variety were stored at 31°, 40° and 55°F. in large storage rooms controlled within ± 1°F. In addition, samples consisting of 10 tubers from each variety were placed in an oven at 95°F. Duplicate samples from each of the storage temperatures were removed and analyzed after 21 days. Duplicate samples from the 31°, 40° and 55° storage rooms, representing the 4 varieties, were also analyzed after 4 months. In addition, duplicate samples from each variety, after 4 months storage at 40°F., were placed in a large

storage room at 70°F. for reconditioning (conversion of sugars into starch) studies.

Determination of Carbohydrate Fractions

The individual tubers from each sample were washed, dried and split longitudinally into quarters, i.e. from terminal bud to stem end. A quarter from each tuber was passed through a food grinder and the composite mixture from the quarters was blended in a Waring blendor for approximately one minute. Twenty grams of this blend were weighed to 0.01 g. into a 200 ml. Kohlrausch flask and approximately 100 ml. of hot 85% ethanol were added. The sample was boiled for approximately 2 minutes and, after cooling, was made to volume with 85% ethanol. The sample was set aside for a month or more after which time it was used for the determination of glucose, fructose, sucrose and starch. The alcohol sample was filtered and an aliquot, either 50 or 100 ml. depending upon the storage temperature of the tubers, was evaporated on a steam bath, cleared with neutral lead acetate, deleaded with potassium oxalate, filtered and made to 250 ml. Reducing and total sugars were determined by the Shaffer-Somogyi method as modified by Heinze and Murneek (48). Sucrose was calculated by difference. Fructose was determined as described by Roe (79) but the method was found unreliable when the correction for sucrose was large. Starch was determined by grinding the residue to pass an 80 mesh screen, and employing the pro-

cedure outlined by Heinze and Murneek (48).

Determination of Phosphorus Fractions and Phosphorylated Intermediates

The potato tubers used for the determination of the phosphorus fractions and phosphorylated intermediates were washed and frozen overnight at 0°F. Quarters of frozen tubers were passed through a food grinder. The material, which was still frozen and in a powdery state, was mixed and weighed and samples were ground in cold 7.5% trichloroacetic acid. The mixture was centrifuged and the residue reextracted with trichloroacetic acid. The combined supernatants were made to volume and used for the determinations.

Inorganic and total phosphorus were determined colorimetrically by the method of Fiske and Subbarow (26). However, due to difficulty in obtaining consistent results for total phosphorus, the method of Waygood (95) was used for the later samples. Organic phosphorus was calculated by difference between total and inorganic phosphorus values. Seven-minute, 60-minute and 180-minute hydrolyzable phosphorus fractions were determined according to Waygood (95).

Alkali-labile phosphorus was determined by adding 2 ml. of a 2N NaOH solution to a 2 ml. aliquot of the sample, allowing the solution to stand for 20 minutes at room temperature, neutralizing and treating as for inorganic phosphorus.

The phosphorylated intermediates were determined essentially by the method devised by Umbreit, et al. (92)

and Albaum (1).

Enzyme Assay

Two 50 g. portions of the frozen mix were used to obtain the enzyme preparations. Each sample was transferred to a cold blendor and 100 ml. of cold water was added. The material was blended for exactly 2 minutes and filtered into centrifuge tubes containing a small amount of toluene. Both tubes were then centrifuged at 3500xG for 10 minutes. Twenty-five ml. of the supernatant in each tube was pipetted into 50 ml. flasks and made to volume. This was the enzyme preparation used for the enzyme assay. Enzyme assay was carried out immediately after extraction.

Phosphorylase: The standard digest for phosphorylase assay was 3 ml. of maleate buffer (39) at pH 6.0, 5 ml. of glucose-1-phosphate (3 mg. of dipotassium salt per ml.), 1 ml. of a 1% starch solution and 5 ml. of the enzyme preparation. The enzyme preparation was pipetted in at zero time and 2 ml. aliquots were transferred to centrifuge tubes containing 1 ml. of 15% trichloroacetic acid after 15 and 30 minutes. Similar aliquots were transferred to colorimeter tubes containing 1 ml. of 0.1 N I_2-KI solution. The activity of phosphorylase was determined both by the increase in inorganic phosphate and by the increase in the iodine coloration of starch. Activity was determined at 25°C.

Phosphoglucomutase: The standard digest for phosphoglucomutase assay was 3 ml. of veronal buffer of pH 8.4, 5 ml. of glucose-1-phosphate (3 mg. of dipotassium salt per

ml.), 1 ml. of H_2O and 5 ml. of enzyme preparation. The enzyme preparation was pipetted in at zero time and 2 ml. aliquots were transferred after 10 and 20 minutes to centrifuge tubes containing 1 ml. of 15% trichloroacetic acid. Seven-minute hydrolyzable and inorganic phosphorus were determined at zero time and after 10 and 20 minutes of incubation at 25°C.

Amylase activity: The standard digest for amylase assay was 3 ml. of maleate buffer at pH 6.0, 5 ml. of water, 1 ml. of a 1% starch solution and 5 ml. of enzyme preparation. The enzyme preparation was pipetted in at zero time and, after 15 and 30 minutes, a 2 ml. aliquot was transferred to a 25 ml. flask containing 1 ml. of 0.1N I_2-KI solution. The decrease in the coloration of the iodine-starch complex between 15 and 30 minutes was taken as a measure of the amylase activity. Activity was determined at 25°C.

Aldolase activity: The standard digest for aldolase assay was 3 ml. of veronal buffer at pH 8.5, 2 ml. of 0.25M KCN adjusted to pH 8.5, 2 ml. of enzyme preparation and 2 ml. of 0.1M fructose-1,6-diphosphate. The fructose-1,6-diphosphate was pipetted in at zero time and the reaction was stopped after 30 minutes by adding 5 ml. of 10% trichloroacetic acid. The mixture was centrifuged and 2 ml. aliquots of the supernatant were used to determine inorganic phosphorus present before and after exposure to 2 ml. of 2N NaOH for 20 minutes. Aldolase activity was studied at 25°C.

Phosphatase activity: Acid and alkaline phosphatase were estimated colorimetrically as described by Seligman,

et al. (83). The standard digest for the study of the liberation of inorganic phosphorus from the phosphorylated sugars by potato phosphatases was 5 ml. of a 0.1M solution of the phosphorylated sugar, 5 ml. of enzyme preparation and 5 ml. of veronal buffer at pH 8.0. Inorganic phosphorus was determined at zero time and after 1 hour of incubation at 25°C.

Preparation and Sampling of the Potato Discs

Cylinders of potato tissue were cut from the potato tubers my means of a cork borer 1 cm. in diameter. Discs of 1-2 mm. thick were cut from the medullary area of the potato cylinders. The discs were washed and randomized for 20 minutes. In some experiments the discs were used after this preliminary washing, but for most of the work they were subjected to 24 hours of washing by tap water. After washing, the discs were spread out on a filter paper and blotted dry before weighing.

After the experimental treatments the discs were washed in tap water for 4 minutes and dropped into hot 80% alcohol. Sugars were then determined as described previously.

The partially desiccated discs were either dropped directly into alcohol without washing or first ground with sand and alcohol to insure complete extraction. Checks on the extraction procedure showed that extraction was essentially complete whether the material was ground or not.

Other procedures involved in the study of the potato discs are given in the text.

EXPERIMENTAL RESULTS

Experimental Studies on Intact Tubers

The data in Table 1 show the changes in the principal carbohydrate fractions of the four varieties of potato tubers after 21 days of storage at 31°, 40°, 55°, and 95°F. A large increase in sucrose occurred at 31° and 95°F. and a somewhat smaller increase occurred at 40°F. The decline of the starch percentages at these temperatures is a consequence of sucrose formation. Tubers stored at 55°F. have undergone practically no change during the 21 days. Attention is drawn to the similarity of response of the tubers stored at 31° and 95°F. since many of the earlier investigators were either unaware of or attached little significance to this similarity.

The data in Table 2 give the principal carbohydrate fractions after four months of storage at 31°, 40° and 55°F. Storage at 95°F. for four months is not possible since the tubers decay. In general, at 31° and 40°F. sucrose shows an increase over the values for 21 days. However, it is the reducing sugars, glucose and fructose, that show the major increase. Since sucrose is always first to accumulate it would appear probable that the reducing sugars have been formed from sucrose. The starch values have undergone corresponding decreases at 31° and 40°F. The composition of the tubers stored at 55°F. have changed little since the time

Table 1. Changes in carbohydrates in potato tubers during 21 days of storage.

Variety and (source)	Storage Temperature	Per cent on a fresh weight basis[1]			
		Moisture	Starch	Reducing Sugars	Sucrose
Triumph (North Dakota)	Harvest	80.4	13.6	0.31	.06
	31°F.	80.5	11.8	.74	2.04
	40°	80.6	13.0	.42	.82
	55°	80.4	13.4	.31	.04
	95°	80.6	12.1	.41	1.33
Green Mountain (Long Island)	Harvest	80.2	13.9	.24	.05
	31°	80.5	12.6	.30	1.63
	40°	80.5	13.8	.32	.87
	55°	80.4	14.2	.20	.07
	95°	80.7	12.5	.38	1.85
Chippewa (Maine)	Harvest	82.7	11.9	.29	.08
	31°	82.4	10.2	.91	1.43
	40°	82.2	11.4	.28	.51
	55°	82.4	11.4	.19	.05
	95°	82.4	11.0	.42	.81
Russet Burbank (Washington)	Harvest	74.8	19.6	.10	.05
	31°	74.6	17.8	.39	1.34
	40°	74.7	18.8	.22	.57
	55°	74.2	19.1	.07	.07
	95°	74.8	18.6	0.29	.72

1. Average of duplicate determinations

Table 2. Changes in carbohydrates in potato tubers during four months of storage.

Variety and (source)	Storage Tempera-ture	Per cent on a fresh weight basis[1]			
		Moisture	Starch	Reducing Sugars	Sucrose
Triumph (North Dakota)	Harvest	80.4	13.6	.31	.06
	31°F.	80.8	9.2	2.43	2.80
	40°	80.4	11.9	.98	.58
	55°	81.0	12.8	.40	.21
Green Mountain (Long Island)	Harvest	80.2	13.9	.24	.05
	31°	80.6	8.1	2.05	3.90
	40°	80.6	11.5	1.55	.66
	55°	81.1	12.5	.52	.32
Chippewa (Maine)	Harvest	82.7	11.9	.29	.08
	31°/2	82.3	6.7	4.30	2.40
	40°/2	82.2	11.0	.80	.36
	55°	82.7	11.5	.12	.14
Russet Burbank (Washington)	Harvest	74.8	19.6	.10	.05
	31°	74.7	13.2	4.30	2.50
	40°	75.2	18.6	.49	.26
	55°	74.9	18.5	.24	.18

1. Average of duplicate determinations

2. Average of seven replications

of harvest. Varietal differences in response are apparent when the 40°F. storage values are compared. Much less total sugar was accumulated by the varieties Chippewa and Russet Burbank than by Triumph or Green Mountain. Such varietal differences are important in choosing potato tubers for commercial storage, since those varieties accumulating large amounts of sugar during storage are undesirable. However, these varietal differences are no longer apparent in the tubers stored for four months at 31°F. This storage treatment represents an extreme condition of environmental stress. The accumulation of sugars at this temperature represents the maximum amounts attainable for the given lots of potatoes. Continued storage beyond four months at this low temperature leads to a physiological breakdown which has been characterized as internal mahogany browning (51).

A quantitative estimation of fructose in the samples in Tables 1 and 2 was not possible. The routine application of the method of Roe (79) to the sugar solutions gave variable results. The data in Table 3 show the composition of the reducing sugar fraction of the variety Chippewa (Maine) after four months storage at 31° and 40°F. This variety was chosen because of its high proportion of reducing sugars to sucrose. Only with this condition is a reliable estimation of fructose possible. Seven chemical replications of the original sugar extract were made. The data show that the glucose/fructose ratio is close to unity both at 31° and 40°F.

Table 3. Composition of the sugar fraction from Chippewa (Maine) tubers.

Storage	Per cent on a fresh weight basis[1]		
	Glucose	Fructose	Sucrose
4 months at 31°F.	1.95	2.35	2.40
4 months at 40°F.	.35	.45	.36

1. Average of 7 chemical replications

The data in Table 4 give the principal carbohydrate fractions in potato tubers, previously stored at 40°F. for four months, after three weeks at 70°F. The results show that there has been a loss in reducing sugars and sucrose and a gain, although not equivalent, in starch. Considerable variability of response was found in the different varieties as well as lots of the same variety.

As previously stated, the presence of oxygen is considered essential for the formation of sucrose in plant tissue. The results from an experiment which compared sugar accumulation in potato tubers stored at 32°F. both in air and in 3% oxygen are given in Table 5. There is an increase in sugar during the 20 days of the experiment even in the presence of limiting oxygen although it is, in general, lower than the increase in the tubers stored in air. It may be concluded that the process of sucrose accumulation in potato tubers at low temperatures is, within limits, relatively insensitive to the oxygen level.

The results from an experiment which compared the extent

Table 4. Changes in carbohydrates in potato tubers, previously stored four months at 40°F., during three weeks storage at 70°F.

Variety and (source)	Storage	Per cent on a fresh weight basis[1]			
		Moisture	Starch	Reducing Sugars	Sucrose
Triumph (North Dakota)	4 mo.-40°F.	80.4	11.9	.98	0.58
	" -3 wks.-70°F.	81.6	12.2	.77	.35
Green Mountain (Long Island)	4 mo.-40°F.	80.6	11.5	1.55	.66
	" -3 wks.-70°F.	79.7	11.9	.79	.41
Chippewa (Maine)	4 mo.-40°F.	82.2	11.0	.80	.36
	" -3 wks.-70°F.	82.9	10.9	.46	.24
Russet Burbank (Washington)	4 mo.-40°F.	75.2	18.6	.49	.26
	" -3 wks.-70°F.	74.7	18.8	.17	0.21

1. Average of duplicate determinations

Table 5. Sugar accumulation in potato tubers during 20 days at 32°F. as affected by 3% oxygen.

Variety and (source)	Treatment	% on a fresh weight basis[1]	
		Reducing Sugars	Sucrose
Triumph (North Dakota)	air	0.68	1.07
	3% oxygen	.61	.86
Green Mountain (Long Island)	air	.49	1.14
	3% oxygen	.54	1.03
Chippewa (Maine)	air	.44	.88
	3% oxygen	.33	.74
Russet Burbank (Washington)	air	.13	.76
	3% oxygen	0.15	.67

1. Average of duplicate determinations

of sugar loss from tubers, previously stored at 40°F. for four months, after seven and 14 days at 70°F. both in air and in 3% oxygen are given in Table 6. The different varieties are not consistent in their response but, in general, the effect of 3% oxygen has been to retard the extent of sugar loss.

An additional experiment was conducted in which potato tubers were placed in Magness-Diehl (63) respiration chambers at 70°F. under different levels of oxygen. Respiration measurements were taken and after two weeks half of the 20-tuber sample was removed from each chamber for immediate sugar analyses and the other half was left in the respiration chamber under a normal atmosphere for two additional weeks before analyses. The sugar level of the treated samples was compared with that of corresponding 70°F. room controls. The results are given in Table 7. None of the treatments resulted in a significant change in the starch-sugar equilibrium of the tubers. Many of the tubers in these experiments when first removed from the respiration chambers were in the initial stages of blackheart as evidenced by a faint pink spot in the center. Comparable samples, upon being restored to normal atmospheres and cut two weeks later, showed hollowheart.

The respiration data for the above experiment are given in Table 8. The general effect of the low oxygen level was to decrease the rate of respiration, however, the significant feature of the data was the burst in respiration that occurred upon transfer of samples previously exposed to low oxygen

Table 6. Loss in sugar in potato tubers, previously stored at 40°F. for four months, when placed at 70°F. as affected by 3% oxygen.

Variety and (Source)	Treatment	Per cent sugar in tubers at 40°F. minus per cent sugar in tubers at 70°F.	
		Reducing Sugars	Sucrose
Triumph (North Dakota)	7 days at 70°F.-air	0.11	0.08
	" -3% O_2	.07	.08
	14 days at 70°F.-air	.18	.17
	" -3% O_2	.05	.09
Green Mountain (Long Island)	7 days at 70°F.-air	.30	.14
	" -3% O_2	.36	.09
	14 days at 70°F.-air	.57	.22
	" -3% O_2	.27	.07
Chippewa (Maine)	7 days at 70°F.-air	.28	-.03
	" -3% O_2	.11	.04
	14 days at 70°F.-air	.31	.16
	" -3% O_2	.14	.03
Russet Burbank (Washington)	7 days at 70°F.-air	.23	.06
	" -3% O_2	.18	-.04
	14 days at 70°F.-air	.33	-.04
	" -3% O_2	0.16	-0.03

1. Average of duplicate determinations

Table 7. The percentage of reducing sugars and sucrose (fresh wt. basis) in Kennebec (Maine) potatoes after storage at 70°F. under low oxygen levels.

Treatment[1]	After 2 wks. at 70°F.		Condition of tubers	After 2 additional wks. at 70°F. with 21% O_2	
	Reducing Sugars	Sucrose		Reducing Sugars	Sucrose
Initial	.12	.14	-	-	-
Room Control	.06	.11	-	.07	.09
21% O_2	.08	.12	-	.14	.12
8-10% O_2	.09	.07	Normal	.19	.10
4-5% O_2	.08	.09	No sprouts	.18	.16
2-2.5% O_2	.16	.06	Blackheart 0-+	.18	.14
.5-1.0% O_2	.10	.05	Blackheart ++	.16	.16
N_2	.08	.12	Blackheart +++	Decayed	Decayed

1. Average of duplicate determinations

Table 3. Respiration rate in mgs. CO_2/Kg./hr. of potato tubers at 70°F. as affected by low oxygen levels and subsequent restoration to normal atmosphere. [Variety Kennebec (Maine)]

Days at 70°F.	Mgs. CO_2/Kg./hr. of potato tubers in various atmospheres [1]					
	Air	10% O_2	5% O_2	2.5% O_2	1% O_2	N_2
0-2	8.3	7.9	7.0	6.2	6.6	6.3
2-4	7.6	8.0	5.7	4.4	3.8	3.5
4-6	7.7	7.4	5.7	4.3	3.5	3.6
6-8	8.0	7.2	4.0	3.7	3.7	4.5
8-10	7.0	7.5	4.0	3.5	4.4	4.9
10-12	7.7	7.3	3.2	2.7	4.6	5.5
12-14	7.4	7.4	3.7	2.8	4.9	5.9
		Samples restored to normal atmosphere				
14-16	7.4	8.6	25.2	30.0	28.6	30.8
16-18	7.7	8.7	28.0	26.2	29.3	33.2
18-20	7.5	8.0	24.8	21.4	27.4	38.9
20-22	7.1	7.9	22.0	18.4	23.6	Decay
22-24	7.8	8.0	21.3	16.1	18.4	"
24-26	7.2	7.7	18.4	16.7	14.9	"
26-28	7.6	7.9	18.0	14.6	19.6	"

1. Average of duplicate determinations

levels to normal atmosphere. The possible relationship of this response to a similar response that occurs in potato tubers upon transfer from low temperatures to higher temperatures will be discussed later.

The above experiment was repeated and after two weeks the remaining half of each sample, instead of being left at 70°F. under normal atmosphere, was placed in 32°F. under normal atmosphere for seven days. Sugar accumulation in the treated samples was compared with corresponding controls transferred to 32°F. room at the same time. None of the treatments induced a significant change in the starch-sugar equilibrium of the tubers, as compared with the room controls. However, a noteworthy response that was observed in this experiment was that tubers of the varieties Chippewa and Katahdin taken from the low oxygen treatments at 70°F. and transferred to 32°F. for seven days under normal atmosphere developed severe, diffuse blackheart, whereas comparable tubers left at 70°F. for the same period of time even under the conditions of low oxygen showed no injury. The varieties Russet Burbank and Irish Cobbler did not show this response.

Discussion

The similarity in response of potato tubers when placed at 31°F. and 95°F. has been shown. Evidence that the physiological factors leading to this response are similar would represent an important advance. It may be significant that maximum sucrose accumulation in the tubers occurs just prior to the breakdown of cell permeability as evidenced by the development of internal mahogany browning in tubers stored for sufficient time at 32°F. and the development of blackheart in tubers stored at 95°F. or higher. If the above are causally related, it would appear that physiological stress somehow initiates the conditions leading to an accumulation of sugars. This condition of physiological stress could be brought on by an impairment in respiration at both low and high temperatures. However, data from Appleman (7) show that potato tubers at 30°C., while accumulating sugar,, maintain a high level of respiration during an 18 day period and numerous independent workers (9,52) have shown that potato tubers at 32°F. have a higher rate of respiration than comparable tubers stored between 32° and 40°F. These data indicate that respiration is normal at 32°F. and 95°F. However, carbon dioxide production gives no measure of the efficiency of the phosphorylative coupling mechanism that leads to the formation of ATP. Studies by Green, et al. (29) on the cyclophorase system have shown that coincident with any

oxidation process in the intact cyclophorase system inorganic phosphate becomes esterified or incorporated in the enzyme gel in a highly labile form, which they refer to as gel phosphorus, and any reagent which abolishes oxidation also abolishes incorporation of phosphorus and leads to the discharge of gel phosphorus. The point of interest is that, in addition to such agents as dinitrophenol, temperatures of 0° and 38°C. cause a discharge of gel phosphorus. Whether these facts are of any significance in the regulation of the starch-sugar equilibrium in potato tubers is not known. It may be concluded that if there is a causal relationship between physiological stress, sugar accumulation and respiration, the interaction is involved.

The situation is further complicated by the fact that on the one hand, there is the possibility of physiological stress, presumably caused by some defect in metabolism or respiration at high and low temperatures, initiating the conditions leading to sucrose synthesis; and, on the other hand, the process of sucrose synthesis would seem to require a substantial level of active metabolism since sucrose formation or conversion into starch does not take place in the absence of oxygen or upon damage to cell organization. The fact that the results in the present work dealing with the dependency of oxygen are not as clear cut as might be desired is undoubtedly caused by working with intact tubers. Other workers (15) have shown that tubers at 5°C. could be exposed to an atmosphere entirely devoid of oxygen for 42

days before the development of diffuse blackheart.

The study of the effect of the different levels of oxygen on tubers at 70°F. was made to determine if it is possible to have a level of oxygen that is low enough to cause physiological stress and yet sufficiently high to permit the formation of sucrose. As reported, this was not possible. Likewise, the two week period of low oxygen stress had no effect on sugar accumulation in the tubers when allowed to remain for additional periods in normal atmosphere at either 70° or 32°F.

Other results of the study that may or may not be significant should be mentioned. Russet Burbank was found to be the most resistant variety toward the development of blackheart. Similarly, Russet Burbank, over a period of years, exhibits more resistance toward the accumulation of sugars at low temperatures than any of the other leading varieties. However, there are exceptions to this trend. Another result of interest is the burst of respiration obtained when tubers are removed from limiting oxygen levels and restored to normal atmosphere. A similar burst of respiration is obtained when potatoes are moved from low to high temperatures. Kimbrough (56) has shown that, within limits, the initial respiration rate is higher the lower the storage temperature. However, it is not proportional since a difference in storage temperature of 4°F. between 40° and 36°F. more than doubled the initial rate of respiration at 71.6°F. A difference of 4°F. between 40° and 36°F. will

similarly cause sugar accumulation to be more than doubled. Studies by Bennet-Clark and Bexon (14) may be pertinent. These workers found that the application to the outside of the cell of low concentrations of substances, such as organic acids, which were already inside the cell in high concentrations greatly affected respiration. In certain experiments application to the outside of the cells of 1% of the total quantity of some substance inside the cells caused the respiration to increase 40%. They suggested that in the cell the store of active materials is separated from the enzymes with which they react and that when supplied to the outer surface of the cell they make easy contact with the respiratory enzyme systems. It is possible that under anaerobic and low temperature conditions insufficient energy is produced to maintain the strict differentially-permeable nature of the membranes and a consequent leakage of substrate to enzyme occurs. The burst in respiration could be caused by the leakage of organic acids, such as malic and citric which are stored in large excess inside the vacuole, across the vacuolar boundary into the cytoplasm where ready contact with the respiratory centers would be made. That a somewhat similar condition could lead to the accumulation of sugars was postulated by Coville (19) in 1920. However, sucrose synthesis, because of its energy requirement, cannot be so simply explained.

Physiological experiments on intact potato tubers are more or less limited to a study of the effect of storage temperatures and atmospheres. Yet it is only in the intact

tuber that the full effect of organizational control is exerted. Because of this, the experimental difficulties involved in determining a cause and effect relationship between physiological factors and sugar accumulation are increased.

Phosphorylated Intermediates in Potato Tubers in different Storage Temperatures

There is much biochemical evidence to support the view that the synthesis of sucrose involves reactions in which the phosphate esters of either one or both of the hexoses (glucose and fructose) participate (2,44). Table 9 shows the distribution of acid-soluble inorganic, organic, and total phosphorus in potato tubers from 32°, 55° and 95°F. storage. In potato tubers about 35% of the total acid-soluble phosphorus is composed of organic phosphorus. There appears to be no consistent change in organic phosphorus with storage temperature. However, since the combined sample and chemical variation is large, small changes would not be detected.

The organic phosphorus soluble in 7.5% trichloroacetic acid was further studied by determining the extent of hydrolysis under different conditions, according to the methods of Heard (46) and Umbreit, et al. (92). The results from this study are given in Table 10. The predominant portion of the organic phosphorus is composed of phosphorus compounds resistant to hydrolysis. Glucose-6-phosphate would be expected to be present in this fraction, as well as

Table 9. Distribution of phosphorus soluble in 7.5% trichloroacetic acid in potato tubers stored at different temperatures.

Variety and (Source)	Storage	Mg./100 g. of fresh tuber[1]		
		Inorganic P	Organic P[2]	Total P
Triumph (North Dakota)	21 days-32°F.	17 ± 1[3]	9	26 ± 1
	" -55°	19 ± 1	7	26 ± 2
	" -95°	16 ± 1	7	23 ± 2
Green Mountain (Long Island)	" -32°	16 ± 1	11	27 ± 1
	" -55°	14 ± 2	13	27 ± 2
	" -95°	15 ± 1	12	27 ± 3
	4 mos.-32°	20 ± 1	11	31 ± 2
	" -55°	18 ± 1	12	30 ± 3
Chippewa (Maine)	21 days-32°	22 ± 1	10	32 ± 2
	" -55°	21 ± 1	11	32 ± 2
	" -95°	23 ± 2	8	31 ± 3
Russet Burbank (Washington)	" -32°	14 ± 1	10	24 ± 3
	" -55°	16 ± 1	11	27 ± 3
	" -95°	17 ± 1	11	28 ± 3

1. Average of 3 replications

2. Organic P = total P - inorganic P

3. Standard error

Table 10. Distribution of acid-soluble organic phosphorus in potato tubers stored at different temperatures as determined by the rates of hydrolysis.

Variety and (Source)	Storage	Mg./100 g. of fresh tuber[2]				
		P_7-P_i[3]	$P_{alk.}-P_i$[4]	$P_{60}-P_i$[5]	$P_{180}-P_i$[6]	P_T-P_{180}[7]
Triumph (North Dakota)	21 days-32°F.	0[1]	0	0	1	8
	" -55°	0	0	0	1	6
	" -95°	0	0	0	1	6
Green Mountain (Long Island)	" -32°	0	0	0	1	10
	" -55°	0	0	0	1	12
	" -95°	0	0	0	1	11
	4 mos.-32°	0	0	0	1	10
	" -55°	0	0	0	1	11
Chippewa (Maine)	21 days-32°	0	0	0	1	9
	" -55°	0	0	0	1	10
	" -95°	0	0	0	1	7
Russet Burbank (Washington)	" -32°	0	0	0	1	9
	" -55°	0	0	0	1	10
	" -95°	0	0	0	1	10

1. 0 signifies less than .75 mg.

2. Average of duplicate determinations

3. P_7-P_i signifies P after 7 min. hydrolysis in 1 N HCl at 100°C. minus inorganic P

4. $P_{alk.}-P_i$ signifies P after 20 min. hydrolysis in 2 N NaOH at 25°C. minus inorganic P

5. $P_{60}-P_i$ signifies P after 60 min. hydrolysis in 1 N HCl at 100°C. minus inorganic P

6. $P_{180}-P_i$ signifies P after 180 min. hydrolysis in 1 N HCl at 100°C. minus inorganic P

7. P_T-P_{180} signifies total acid soluble P minus P_{180} which is P resistant to hydrolysis

uncharacterized phosphorus compounds. The compounds that are least resistant to hydrolysis are ATP, ADP, glucose-1-phosphate, triosephosphate, and fructose-1,6-diphosphate. These compounds are present in the potato tuber in almost undetectable amounts since any phosphorus liberated by the hydrolysis of these compounds is obscured by the sample and chemical variations of the test. The gain in inorganic phosphorus after hydrolysis of the sample for 130 minutes in 1N HCl at 100°C. could be due to the accumulated hydrolysis of the above compounds as well as the partial hydrolysis of fructose-6-phosphate (74%) and glucose-6-phosphate (10%). These results demonstrate the experimental difficulties involved in detecting a small increase in phosphorus from hydrolysis in the presence of a relatively large inorganic phosphorus fraction when the combined biological and chemical variations of the test are significant.

The acid-soluble phosphorus from potato tubers of the variety Green Mountain (Long Island) were also studied by fractionation of the extracts with barium as described by Umbreit, et al. (92). The results from this study are given in Table 11. Any variations of the phosphorylated intermediates with storage temperature would fall in the range of concentrations listed. It should be pointed out that none of the phosphorylated compounds were identified and the results only establish their range of concentrations, if present. The analytical techniques were inadequate for more accurate evaluation.

Table 11. Composition of acid-soluble organic phosphorus from potato tubers stored at different temperatures as determined by fractionation of the extracts with barium.

Variety and (Source)	Storage	Mg./100 g. fresh tuber[1]			
		HDP[2]	G-1-P[3]	F-6-P[4]	G-6-P[5]
Green Mountain (Long Island)	21 days -32°F.	0-5	0-5	5-10	25-50
	" -55°	0-5	0-5	5-10	25-50
	" -95°	0-5	0-5	5-10	25-50
	4 months-32°	0-5	0-5	5-10	25-50
	" -55°	0-5	0-5	5-10	25-50

1. Three replications
2. Fructose-1,6-diphosphate
3. Glucose-1-phosphate
4. Fructose-6-phosphate
5. Glucose-6-phosphate

The above results are in sharp contrast to those reported by Arreguin-Lozano and Bonner (8). These workers reported striking changes in the concentrations of glucose-6-phosphate and fructose-6-phosphate in potato tubers with storage temperature. They list glucose-6-phosphate as being present in a concentration of 0.7 g./100 g. of dry tuber at 0°C. and 4.50 g./100 g. of dry tuber at 25°C. Similarly, they list fructose-6-phosphate in a concentration of 2.50 g./100 g. of dry tuber at 0°C. and 0.35 g./100 g. of dry tuber at 25°C. It is apparent that their figures are in serious error since, as established by numerous other analyses over a period of years, the phosphorus content of potato tubers will range from 0.15 to 0.55% on a dry weight basis and 4.50% glucose-6-phosphate would more than account for the total percentage phosphorus in an average lot of tubers. Since in the present work it was found that from 40-45% of the total phosphorus is soluble in 7.5% trichloroacetic acid and about 35% of this is in organic combination, it is also probable that the values given by these workers for triose phosphates and glucose-1-phosphate at 0°C. are erroneously high.

Discussion

Other workers (8,41,64) have reported that hexose monophosphates and fructose-1,6-diphosphate accumulate in potato tubers that are in the process of converting starch into sucrose. In the present work this could not be substantiated. However, any accumulation of a phosphorylated intermediate

would be expected to be of small concentration and short duration due to the dynamic position of phosphorus in all phases of cellular metabolism. The detection of an accumulated intermediate would depend on the accuracy of the analytical techniques. The available chemical methods for the determination of the phosphorylated intermediates were found inadequate for the reliable detection of differences of small magnitude. This applies particularly to plant tissue since contamination by traces of sucrose and fructose lead to large errors in the determination of both fructose-6-phosphate and fructose-1,6-diphosphate.

Activity of Some of the Enzymes Involved in Fermentation in Potato Tubers from Different Storage Temperatures

Arreguin-Lozano and Bonner (8) investigated the effect of storage temperature on the activity of phosphorylase, amylase and phosphatase from potato tubers. They reported that the activity of phosphorylase and phosphatase was not affected by the temperature of storage. Amylase activity appeared to increase in activity with storage temperature but no significance was attached to this as far as the regulation of the starch-sugar equilibrium is concerned. These workers were of the opinion that the failure of phosphorylase to attack the starch in potatoes stored at high temperatures was due to the formation of an inhibitor of phosphorylase at high temperatures which disappeared at low storage temperatures. Since this inhibitor was of theoretical interest and could be of possible use in the commercial storage of potatoes where

the accumulation of sugars is undesirable, much of their work was repeated. The results of the study on phosphorylase and amylase are given in Table 12.

Phosphorylase activity was determined after 15 and 30 minutes since it was during these periods of time that the rate of the reaction was most closely proportional to the enzyme concentration. This is an important consideration since, with sufficient time, wide variations in enzyme concentration attain essentially the same final equilibrium. Amylase was similarly determined in order to correct for its activity in the homogenate.

The crude juice from the potato tubers showed strong phosphorylase activity. The high variability was biological in origin since duplicate determinations on the same sample gave satisfactory agreement. In agreement with Arreguin-Lozano and Bonner (8), it appears that the activity of phosphorylase is not affected by the previous storage temperatures of the potato tubers from which the enzyme is extracted. Similarly, variations in phosphorylase activity do not appear to account for varietal differences in response to storage temperature. Due to the high coefficient of variation of the data, it is possible that significant changes in activity were obscured. However, additional replications were not carried through since it was felt that the presence of this biological variability was itself indicative that the level of phosphorylase activity is not critical in determining the response of potato tubers to storage temperature.

Table 12. Phosphorylase and amylase activity in the crude juice from potato tubers stored at different temperatures.

Variety and (Source)	Storage	Activity/g. fresh tuber[1]		
		Phosphorylase		Amylase
		Mg."starch" formed	Mg. inorg.P liberated	Mg. starch hydrolyzed
		30_v-15_v[2]	30_v-15_v[2]	30_v-15_v
Triumph (North Dakota)	21 days-32°F.	2.1	0.32[3]	0.5
	" -55°	2.4	.41	.5
	" -95°	2.4	.35	.5
Green Mountain (Long Island)	" -32°	1.9	.30	.5
	" -55°	2.7	.46	.6
	" -95°	1.6	.34	.5
	4 months-32°	2.1	.35	.5
	" -55°	2.1	.35	.6
Chippewa (Maine)	21 days-32°	2.3	.43	.5
	" -55°	2.6	.36	.5
	" -95°	2.2	.34	.5
Russet Burbank (Washington)	" -32°	2.4	.42	.5
	" -55°	2.1	.31	.6
	" -95°	2.0	0.33	0.5

1. Average of duplicate samples

2. Value obtained after 30 minutes incubation at 25°C. minus the value after 15 minutes incubation at 25°C.

3. Coefficient of variation: 18%

The presence of an inhibitor for phosphorylase in the tubers from 55° or 95°F. storage could not be demonstrated. Since the enzyme preparations used to obtain the activity values in Table 12 were homogenates, this inhibitor presumably should have been present. Arreguin-Lozano and Bonner (8) indicated an inhibition of about 75% in phosphorylase activity in digests treated with the inhibitor. An inhibition of this magnitude, if present, would be readily apparent in the data in Table 12 even with the large coefficient of variation. Similarly, inhibition of phosphorylase could not be demonstrated when the inhibitor was prepared by extracting tubers from 75°F. storage with boiling 80% alcohol, evaporating the alcohol and adding the aqueous solution to the digest (8). Until the presence of an inhibitor for phosphorylase activity in potato tubers from high storage temperatures can be readily and consistently demonstrated, the importance of this factor in regulating the response of potato tubers to storage temperature must be questioned. Theoretically, the effect of an inhibitor on an enzyme such as phosphorylase which catalyzes a freely reversible reaction should be to increase the time required for the reaction to reach equilibrium rather than to change the equilibrium value. Practically, potato tubers, when transferred from low temperatures to high temperatures, convert a large portion of their sugar into starch. This reaction, which presumably involves phosphorylase, takes place at an even more rapid rate than the accumulation of sugars at the lower temperatures.

Hyster (25) found that starch synthesis by squares of etiolated corn leaves floated on glucose or sucrose solutions was inhibited by the juice of onions and related starch-free plants. Dyar (24) reported that the juice of onion markedly but not completely inhibited phosphorylase activity in pea root tip sections. In the present work onion juice was extracted by pressure and heated to destroy the enzymes. The addition of 2 ml. of this juice to a digest containing 5 ml. of enzyme preparation caused no significant inhibition of phosphorylase activity.

Amylase was found to be present in potato tubers but its activity was considerably less than that of phosphorylase. The significance of this enzyme in the potato tuber is not known since, as shown by paper chromatography, maltose is not present in the sugars of the potato tuber. Likewise, dextrins are absent as shown by the failure to form starch of carefully centrifuged homogenates to which glucose-1-phosphate but no primer have been added. This test is sensitive enough to detect the dextrins present in commercial C.P. samples of maltose.

It was not possible to measure the activity of phosphoglucomutase in digests using the diluted enzyme preparation. When glucose-1-phosphate was added to the digests at pH 8.4, there was some indication of a conversion of acid-labile phosphorus to acid-resistant phosphorus during the first 10 minutes of incubation and a partial loss of the acid-resistant phosphorus to acid-labile phosphorus during the next 10 minutes of incubation. Enzyme preparations from tubers stored at

the three storage temperatures showed this indication, but the effect was too variable and inconclusive to present quantitatively. However, it was found that inorganic phosphorus did not change during the 20 minutes of incubation which shows that phosphatases capable of hydrolyzing glucose-1-phosphate are not present in the digest at this dilution and pH.

Similarly, it was not possible to measure the activity of aldolase in digests using the diluted enzyme preparation.

Phosphatase activity was readily demonstrated in the digests. The different enzyme preparations liberated inorganic phosphorus from glucose-6-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate. Under similar conditions glucose-1-phosphate was resistant to the action of the phosphatases present. Acid and alkaline phosphatases were measured colorimetrically (83). The activity of the acid phosphatases (pH 4.8) was from four to five times greater than the activity of the alkaline phosphatases (pH 9.1). In agreement with Arreguin-Lozano and Bonner (8), it was found that the activity of the various phosphatases was not affected substantially by the previous storage temperatures of the potato tubers from which the enzymes were extracted.

Discussion

The mechanisms for the control of carbohydrate metabolism in plant cells and tissues are largely unknown. Many present day workers appear to be of the opinion that these control

mechanisms are somehow related to the enzymes involved in the reactions. When one compares, for example, the high phosphorylase activity found in high starch, high sucrose tissue such as the potato tuber with the, as yet undetected (62), activity of phosphorylase in low starch, low sucrose tissue such as the tomato fruit, this view would appear to have some justification. However, the same reactions undoubtedly occur with equal facility in both organs. The full significance of enzyme concentrations in tissues is not known but there are serious theoretical objections in considering levels of enzyme activity or concentrations as mechanisms for the control of metabolism.

A detailed investigation of the activity of the enzymes involved in the carbohydrate metabolism of potato tubers was not a part of the present work since the labor involved would have prevented other considerations and the validity of applying conclusions drawn from <u>in vitro</u> studies to <u>in vivo</u> processes would be questionable. However, the problem was sufficiently considered, both experimentally and theoretically, to conclude that it is improbable that the level of activity of the involved enzymes controls the regulation of the starch-sugar equilibrium in potato tubers.

Synthesis of Sucrose from Infiltrated Glucose and Fructose

Arreguin-Lozano and Bonner (3) vacuum infiltrated discs from potato tubers stored at 0° and 25°C. with solutions of glucose or fructose, and incubated the discs at both 0° and

25° for 24 hours. They concluded that the amount of sucrose synthesized was greater in discs taken from tubers stored at the low temperatures, and that sucrose synthesis proceeded faster in discs incubated at 0° than in discs incubated at 25°C. They also concluded that in discs from tubers stored at 0° and incubated at 0° the interconversion of glucose and fructose was much greater than similar discs from tubers stored at 25° and incubated at 25°C.

The above results, if shown to occur consistently, would have to be given primary consideration in any studies on the regulation of the starch-sugar equilibrium in potato tubers. However, since the results are not in accord with those of other investigators (38,61) or the general effect of temperature on enzymatic reactions, it was considered worthwhile to repeat the experiments.

Russet Burbank potatoes were stored for 3 weeks at 32° and 75°F. Cylinders 1.5 cm. in diameter were cut out with a cork-borer and sliced into discs one to two mm. thick. The discs were washed in running tap water for 20 minutes and rinsed in distilled water. The discs were then blotted dry and 10 g.-samples consisting of 20-25 discs were vacuum infiltrated for 15 minutes with water, 3% glucose, or 3% fructose. After infiltration the discs were transferred to Petri dishes containing a sheet of filter paper moistened with the same solution used for infiltration. The discs were incubated at 32° and 75°F., and after 24 hours were washed for four minutes with tap water and dropped into hot 80% alcohol. This is the identical procedure used by Arreguin-Lozano and Bonner (8)

with the exception that these authors dried the discs at 70°C. and ground them before analysis. Each treatment was replicated five times in an effort to minimize the effect of sampling, physiological, and chemical variations. The results of this study are given in Table 13.

As is indicated by the results with the water-infiltrated controls, the response of the discs from tubers stored at 32°F. is primarily due to the effect of the different incubation temperatures on the metabolism of the discs. Incubation of these discs at 75°F. for 24 hours resulted in a conversion of sucrose into reducing sugars. There is little evidence that the infiltrated hexoses have been converted into sucrose by the discs from tubers stored at 32°F. whether incubated at 32° or 75°F. The higher resorcinol value/total sugar ratios for the fructose infiltrated samples when compared with the sucrose levels indicate that the fructose has penetrated the discs but that it has not been converted to sucrose.

The discs from the tubers stored at 75°F. are more effective in the conversion of infiltrated hexoses into sucrose than the discs from tubers stored at 32°F. There is little difference in the amount of sucrose formed from the different hexoses but what difference exists is in favor of glucose rather than fructose. What is clearly shown, however, is that with the discs from tubers stored at 75°F. an incubation temperature of 75°F. is more effective in the conversion of the infiltrated hexoses into sucrose than an incubation temperature of 32°F.

No conclusions can be formed on the rate of uptake or

Table 13. The percentage of reducing sugars and sucrose in potato discs after infiltration with glucose or fructose and incubation for 24 hours. (Variety Russet Burbank)

Treatment[1]	Incubation temp.	% of original fresh wt.		Resorcinol value[2]
		Reducing sugars:	Sucrose	: Total sugar
Discs from tubers stored 3 weeks at 32° F.				
original	-	0.06 ± .02[3]	.90 ± .05	7.0 ± 8%[4]
infilt. with: water	32° F.	.08 ± 0	1.04 ± .08	7.0 ± 8%
	75°	.14 ± .02	.86 ± .04	7.5 ± 6%
3% glucose	32°	.14 ± .03	1.07 ± .11	7.5 ± 7%
	75°	.15 ± .02	1.00 ± .05	8.0 ± 6%
3% fructose	32°	.14 ± .02	.94 ± .06	11.0 ± 10%
	75°	.20 ± .02	.99 ± .02	11.0 ± 11%
Discs from tubers stored 3 weeks at 75° F.				
original	-	.03 ± .01	.16 ± .02	6.0 ± 6%
infilt. with: water	32°	.02 ± 0	.17 ± .03	7.0 ± 8%
	75°	.05 ± 0	.23 ± .03	7.0 ± 6%
3% glucose	32°	.07 ± .01	.26 ± .03	7.0 ± 6%
	75°	.10 ± .02	.61 ± .05	8.0 ± 8%
3% fructose	32°	.08 ± 0	.20 ± .01	9.0 ± 8%
	75°	0.07 ± .01	.54 ± .02	9.0 ± 8%

1. Average of 5 replications

2. Values obtained from Roe's test for fructose. The higher the value, the greater the proportion of fructose in the hydrolyzed total sugar.

3. Standard error

4. Coefficient of variation

interconversion of the hexoses since these responses are obscured by the metabolism of the discs as modified by the different incubation temperatures. In addition, experimental attempts to determine the composition of the reducing sugar fraction in the presence of relatively large amounts of sucrose by the method of Roe (79) give only questionable results.

The experiment was repeated using an incubation period of 40 hours. Any contamination of the solutions at 75°F. during this period of time should be of little consequence since the discs were washed for four minutes before being put up for analysis. The results of this study are given in Table14.

Similar to the previous experiment, the response of the discs from the tubers stored at 32°F. reflects the effect of the different incubation temperatures on the metabolism of the discs more than the effect of the infiltrated hexoses. The results indicate that essentially no sucrose is formed from infiltrated hexoses by discs from tubers stored at 32°F. when incubated at 32°F. and only small amounts when incubated at 75°F.

Discs from the tubers stored at 75°F. responded similarly in the two experiments.

The conclusions reached by Arreguin-Lozano and Bonner (3) with respect to the synthesis of sucrose from infiltrated hexoses could not be confirmed as typical responses of potato discs. The present data show that sucrose formation from infiltrated hexoses occurs more readily at 75°F. than at 32°F. Likewise, discs from tubers stored at 75°F. are more effective in sucrose formation from infiltrated hexoses than similar discs from tubers

Table 14. The percentage of reducing sugars and sucrose in potato discs after infiltration with glucose or fructose and incubation for 40 hours. (Variety Russet Burbank)

Treatment[1]	Incubation temp.	% of original fresh wt.		Resorcinol value[2]
		Reducing sugars	Sucrose	Total sugar
Discs from tubers stored 5 weeks at 32°F.				
original	-	0.13	2.16	9.0
untreated	32°F.	.12	2.04	8.0
	75°	.36	1.66	8.0
infilt.with: water	32°	.12	2.02	8.0
	75°	.32	1.68	8.0
3% glucose	32°	.18	2.09	8.0
	75°	.27	1.85	8.0
3% fructose	32°	.17	1.97	8.5
	75°	.44	1.81	9.0
Discs from tubers stored 5 weeks at 75°F.				
original	-	.08	.10	4.5
untreated	32°	.04	.15	5.0
	75°	.09	.24	9.0
infilt.with: water	32°	.03	.11	9.0
	75°	.08	.22	10.0
3% glucose	32°	.06	.33	7.0
	75°	.12	.61	8.0
3% fructose	32°	.09	.14	12.5
	75°	0.08	.53	9.0

1. Duplicate determinations
2. Values obtained from Roe's test for fructose. The higher the value, the greater the proportion of fructose in the hydrolyzed total sugar.

stored at 32°F.

Experiments on Potato Discs

Discs of potato tuber tissue have been employed extensively in physiological investigations (31,32,38). Many investigators wash the discs for periods of 18-24 hours before use to wash out or to remove the damaged cells on the surface and to bring the discs into a more uniform physiological state (32) Schade and Levy (31) have presented data to show that during a period of 9¼ hours of washing the respiration of the discs increased by 130% and that there was a change in the terminal oxidase pattern. The data in Table 15 show the effect of washing with a trickle of tap water on the sugar content of potato discs cut from potato tubers stored at 55° and 32°F. Washing the discs from potatoes stored at 55°F. caused a pronounced increase in reducing sugars and sucrose. The discs from tubers stored at 55°F. could be washed for periods up to 8-10 days without apparent evidence of damage other than a general browning. In contrast, discs from potato tubers stored at 32°F. for three months, which contained a relatively high initial content of sugar, showed a pronounced loss of sugar upon washing, presumably due to leakage. Discs from potato tubers stored at 32°F. for 3 months turned white and were found to be dead after 2 or 3 days of washing.

Another pronounced difference between the discs from the different storage temperatures was the rate at which the oxidation products of the polyphenoloxidase reaction appeared. This difference was measured only in a qualitative manner.

Table 15. Changes in sugars in potato discs during washing.

Variety and (Source)	Storage	Days of washing	% of original fresh weight[1]	
			Reducing sugars	Sucrose
Russet Burbank (Washington)	3 months-55°F.	0	.14	.16
		1	.37	.21
		2	.48	.22
		4	.57	.29
		8	.66	.51
Triumph (North Dakota)	3 months-32°F.	0	1.67	2.32
		1	.93	1.85
		2	.77	1.74
		3	70-80% of discs were dead	

1. Average of duplicate determinations

Discs from tubers stored at 55°F., after cutting and rinsing, remained normal for periods of hours, whereas similar discs from potato tubers stored for 3 months at 32°F. rapidly developed a pink coloration which progressively darkened. Throughout the present work the development of such coloration was taken as an indication of irreversible injury to the cell. It is probable that in the intact cell the enzyme and substrate of this reaction are separated by the vacuolar membrane and that in weakened or injured cells the two are no longer separated. No attempt was made to verify this.

Salt leakage was not measured in the present studies but other investigators (88) have reported that discs from potato tubers stored for several months at 32°F. are no longer capable of retaining salts.

This combined evidence strengthens the view that potato tubers stored at 32°F. have been weakened physiologically. While it is probable that the accumulation of sugars in tubers at low temperatures is a consequence of this weakened condition, such a relationship must be proven, since it is always possible that the two conditions are unrelated. If sugar accumulation is a response to physiological stress then it would appear to be possible selectively to damage healthy discs so as to cause the accumulation of sugars.

As a means of carrying out experiments to test this possibility, potato discs were placed in large test tubes in water which were aerated at a rate sufficient to keep the discs in constant motion. Discs subjected to such treatment in distilled water showed an increase in sugars similar to

the discs that were washed. We might well ask what causes this increase in sugar in healthy, aerated discs. This response, however, is only one of a series of reactions taking place since in addition to accumulating sugars such discs are actively absorbing water (32), synthesizing protein (38), actively absorbing ions (33), if present, forming periderm (17) and increasing in ascorbic acid (35). It hardly can be said that we know the reasons for any of these reactions. What is known is that each of the reactions is aerobic and is either greatly diminished or halted under low oxygen pressures or anaerobic conditions. Caution must be exercised in applying conclusions drawn from the study of these actively metabolising cells to the resting cells in the tuber. Nevertheless, the same physiological conditions could lead to the hydrolysis of starch reserves in both cases. For example, without trying to force a case, both active metabolism and physiological stress caused by low temperature storage could lead to a relatively high inorganic phosphate level which would increase the phosphorylation of starch to glucose-1-phosphate.

In the present work when discs were aerated with 3% oxygen, a decrease rather than increase in sugars was found. Discs allowed to stand in water for 24-48 hours showed a similar response. Since the solutions in which the discs were standing gave a positive test for sugar, it may be assumed that the conditions of limiting oxygen resulted in a leakage of sugar from the discs. Results similar to these were reported by Brauner, et al. (17). These experiments as well as the previous experiments on whole tubers would seem to

preclude the possibility of initiating sugar accumulation by physiological stress (caused by low oxygen pressures) since apparently all synthetic reactions cease.

It is well known that such compounds as toluene, thymol, ethyl ether, and alcohol alter or destroy the permeability of the cell. When potato discs were placed in aerated solutions containing increasing amounts of a saturated solution of thymol the response was either essentially the same as the controls in aerated distilled water or else a complete destruction of the permeability of the cell occurred causing the solutions to become dark brown as a result of the polyphenoloxidase reaction. There was no intermediate behavior either as shown by sugar analyses or by the color of the solutions. Similar results were obtained when ethyl alcohol was added to the solutions in concentrations from 10-20%.

The effect of increasing concentrations of salt solutions, increasing hydrogen-ion concentrations, and growth regulators were investigated according to the procedure just outlined. Thiourea, HCN (33), and ethylene (53) which have been reported to increase sugar accumulation in whole tubers were also investigated. The results from these studies are given in Tables 16 to 20. As seen in Table 16, 0.05 M NaCl and 0.05 M KCl caused a significant increase in total sugar of which sucrose showed the major increase. Concentrations of these salts of 0.1 M and above caused death of the discs during the three days of the experiment. $CaCl_2$ in concentrations up to 0.3 M appeared to produce a slight inhibition

Table 16. Sugar accumulation in aerated potato discs during three days at room temperature. (Variety Irish Cobbler from 55°F. storage)

Treatment[1]	Appearance	Per cent of original fresh wt.	
		Reducing sugars	Sucrose
1) Original discs	-	0.08 ± .01[2]	0.14 ± .01
2) Distilled water	normal	.55 ± .03	.13 ± .01
3) 0.05 M KH_2PO_4-Na_2HPO_4, pH 6.0	"	.48 ± .02	.21 ± .01
4) 0.05 M NaCl	brownish	.60 ± .04	.44 ± .03
5) 0.05 M KCl	"	.62 ± .04	.34 ± .02
6) 0.30 M $CaCl_2$	"	.33 ± .02	.26 ± .02
7) 0.30 M $CaCl_2$ + 15 p.p.m. NAA[3]	"	.31 ± .03	.27 ± .02
8) 15 p.p.m. NAA	normal	.42 ± .02	.26 ± .01
9) 100 p.p.m. NAA	"	.34 ± .02	.19 ± .01
10) 0.1 M 2,4-D, pH 6.0	"	.31 ± .02	.23 ± .02
11) 0.1% thiourea	"	.46 ± .03	.16 ± .01
12) 0.2% thiourea	"	.40 ± .02	.12 ± .01
13) 5 x 10^{-5} M DNP[4], pH 6.0	"	0.51 ± .04	0.26 ± .02

1. Average of 3 replications
2. Standard error
3. Napthalene acetic acid
4. Dinitrophenol

when compared to the controls in distilled water, but caused little damage to the discs during the time of the experiment. Since there was a possibility that the salt solutions could cause an increased accumulation of sugar through an osmotic effect whereby water lost to the salt solutions would cause an accumulation of sugar similar to the accumulation of sugar initiated by water loss in discs upon drying, NAA was added to the 0.3 M $CaCl_2$ solution to increase the uptake of water. The addition of naphthalene acetic acid, however, to 0.3 M $CaCl_2$ or to distilled water at a concentration of 15 p.p.m. had little if any effect on sugar accumulation when compared with the distilled water controls. Recent work has shown that the full effect of NAA on water uptake in potato discs is exerted between the third and sixth days of aeration or incubation (32). The addition of 2,4-D at a concentration of 0.1 M (ca 2000 p.p.m.) had little if any effect on sugar accumulation in the discs when compared with the distilled water controls. Thiourea in concentrations of 0.1 and 0.2% appeared to have a slight inhibitory effect and dinitrophenol at a concentration of 5×10^{-5} M had no pronounced effect when compared with the distilled water controls.

The portion of the above experiment involving the treatment of discs with $CaCl_2$ and NAA was repeated for a six day period. The solutions were changed every two days to check infection, particularly in the $CaCl_2$ treatments which developed cloudiness in the unchanged solutions and gave a positive test for sugars. The results are given in Table 17. The discs in the distilled water and NAA treatments increased

Table 17. Sugar accumulation in aerated potato discs during six days at room temperature. (Variety Irish Cobbler from 55°F. storage)

Treatment[1]	Appearance	Change in fresh wt.	% of original fresh wt.	
			Reducing sugars	Sucrose
1) Original discs	-	-	0.08	0.14
2) Distilled water	normal	11%	.47	.30
3) 20 p.p.m. NAA[2]	"	25%	.33	.42
4) 0.25 M $CaCl_2$	damaged	-9%	.36	.12
5) 0.25 M $CaCl_2$ + 20 p.p.m. NAA	"	-9%	0.28	0.14

1. Average of 3 replications

2. Naphthalene acetic acid

11 and 25%, respectively, in fresh weight during the six days of the experiment. Along with this increase in fresh weight was an increase in sugar. The discs in the $CaCl_2$ treatments were damaged, as indicated by soft spots in the tissue, and no direct comparisons are justified.

Using the aerated tubes previously described, the effect of a 10% mannitol solution on sugar accumulation in the discs was investigated. The experiment was carried out at room temperature and at 32°F. In addition to mannitol, the effect of NAA, DNP, glucose and malic acid were studied at the two temperatures. The results of this study are given in Table 18. None of the treatments produced a change of sufficient magnitude in the sugar levels to make it appear that the starch-sugar equilibrium was being directly affected when compared with the distilled water controls. As expected, the 3% glucose treatment caused a significant increase in both glucose and sucrose and was included chiefly for the sake of comparison. Mannitol at a concentration of 10% had a general, although small, inhibitory effect. This general inhibition by mannitol was not changed by the addition of 10 p.p.m. NAA or 10^{-4} M dinitrophenol. Dinitrophenol at a concentration of 10^{-4} M had no significant effect when compared with the distilled water controls. This was unexpected in view of the high concentration of this inhibitor. Likewise, malic acid was without effect. Malic acid was studied since, as previously mentioned, several investigators have shown a dynamic equilibrium between starch and malic acid in some types of plant tissue. It was introduced into the solution at a

Table 18. Sugar formation in aerated potato discs at room temperature and 32°F. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Per cent of original fresh weight			
	3 days at room temperature		3 days at 32°F.	
	Reducing sugars	Sucrose	Reducing sugars	Sucrose
1) Original discs	0.13	0.19	0.13	0.19
2) Distilled H_2O	.48	.21	.28	.17
3) Distilled H_2O 10 p.p.m. NAA	.43	.24	.31	.18
4) 10% mannitol	.33	.18	.24	.16
5) 10% mannitol + 10 p.p.m. NAA[2]	.31	.21	.25	.17
6) 10% mannitol + 10^{-4} M DNP[3]	.30	.23	.21	.20
7) 10^{-4} M DNP, pH 5.5	.34	.27	.24	.22
8) 3% glucose	.56	.32	.35	.24
9) .05 M maleate, pH 5.0	.41	.23	.27	.20
10) 100 p.p.m. NAA	0.42	0.15	0.22	0.16

1. Average of 2 replications
2. Naphthalene acetic acid
3. Dinitrophenol

relatively low pH (5.0) in an attempt to increase its penetration of the cell membrane. The general effect of the low temperature was to decrease the amount of sugar accumulated in every treatment.

The effect of ethylene on sugar accumulation in aerated potato discs was studied. The discs were supported in Petri dishes so as to break the surface of the solutions and placed in five gallon jars to which ethylene was added at a concentration of 1 part in 5000 of air. The experiment was allowed to run for six days at 70°F. and the solutions were changed once to minimize contamination. The results of this study are given in Table 19. None of the treatments produced a pronounced effect on the starch-sugar equilibrium in the potato discs when compared with the distilled water controls. The ethylene treatment caused an increase in sucrose that apparently is of some significance, but this increase is small when compared to the increase in sucrose found in potato discs after desiccation.

The above experiments indicate that the sugar accumulation occurring in potato discs when exposed to aerobic conditions is, within limits, independent of the gain or loss of water, although under aerobic conditions water uptake takes place to a greater or lesser extent. Osmotic agents such as 10% mannitol or 0.3 M $CaCl_2$ inhibit sugar accumulation in the discs. This makes it likely that the pronounced increase in sugar taking place in discs during desiccation is due to some secondary effect rather than to the actual loss of water. Likewise, the increase in sugar accumulation in the 0.05 NaCl

Table 19. Sugar accumulation and increase in fresh weight of potato discs during incubation at 70°F. for six days as affected by ethylene and naphthalene acetic acid. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Per cent increase in fresh wt.	Per cent of original fresh weight	
		Reducing sugars	Sucrose
1)Original discs	-	0.17 ± .01	0.14 ± .01
2)Distilled water	7.2 ± 1.1[2]	.33 ± .03	.18 ± .01
3)1:5000 C_2H_4	5.8 ± .7	.25 ± .03	.33 ± .05
4)1:5000 C_2H_4 + 10 p.p.m. NAA	6.7 ± 1.2	.23 ± .02	.22 ± .02
5)10 p.p.m. NAA	9.0 ± .8	0.33 ± .06	0.20 ± .02

1. Average of triplicate determinations

2. Standard error

and 0.05 M KCl treatments above the distilled water controls is due to a physiological effect of the salts rather than to an osmotic effect.

An additional experiment which studied the effect of buffer solutions at different pH values upon sugar accumulation in aerated discs was made. McIlvaine's buffer of citric acid and Na_2HPO_4 was used since these compounds at pH 3 caused no obvious injury to the discs. In contrast, 0.1 M acetate buffer at pH 5 caused the death of the discs. The results of this study are given in Table 20. None of the treatments produced a pronounced change in the starch-sugar equilibrium of the discs. Buffer solutions at pH 8 caused a disorganization of the tissue. Maximum sugar accumulation occurred at pH 5, which indicates that sugar accumulation responds either directly or indirectly to the hydrogen-ion concentration since the pH of potato tissue ranges between 5.9 and 6.2. As the hydrogen-ion concentration was increased from pH 5 to pH 3 sugar accumulation was progressively inhibited. Since the maximum penetration of citric acid would occur at pH 3 (84), the above responses would seem to be due to the different hydrogen-ion concentrations rather than to any metabolic response of citric acid. The relationship between pH and phosphate uptake is not known.

Table 20. Sugar accumulation in aerated potato discs as affected by pH during two days at room temperature. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Appearance	Per cent of original fresh wt.	
		Reducing sugars	Sucrose
1)Original discs	-	0.16	0.14
2)Distilled water	normal	.54	.35
3)0.05 M citric acid-0.1 M Na_2HPO_4			
pH 8	damaged	.21	.11
pH 7	normal	.46	.28
pH 6	"	.61	.39
pH 5	"	.68	.47
pH 4	"	.56	.35
pH 3	white	0.39	0.27

1. Average of duplicate determinations

Effects of Metabolic Inhibitors

The effects of metabolic inhibitors on sucrose formation have been studied by a number of investigators in a variety of plant tissues (40,61). Previous work has largely been confined to the study of sucrose formation caused by the addition of hexoses to the external solution. Since the experimental procedure used in the present work made it possible to study the formation of sucrose from starch, a general study of the effect of metabolic inhibitors on sugar accumulation in potato discs exposed to aerobic conditions was made.

In order to enlarge the experiments Petri dishes, in which the potato discs were placed on edge so as to break the surface of the treating liquids, were substituted for the aerated tubes most of the time. The experiments were carried out at 32°F., since preliminary work had shown that potato discs could be treated at this temperature for two weeks or longer with little danger of injury or serious contamination. In addition, the discs incubated at 32°F. accumulated a higher percentage of sugars than similar discs incubated at higher temperatures. This made it possible to evaluate more accurately the effect of the inhibitor. However, it did not appear that the low temperature was causing a response exactly similar to that caused in intact tubers since the sugar levels in the discs incubated at 32°F. were only from 15-25% higher than similar discs stored at 55°F. As previously mentioned, the

metabolically active cells of potato discs are not strictly comparable to the resting cells of whole tubers.

The initial concentrations of inhibitors were the same as those found by Hackett and Thimann to cause an approximately 50% inhibition in the water uptake by potato discs (31). The results of this study are given in Table 21. When compared with the distilled water controls it is evident that the inhibitors in the concentrations used (10^{-4} M DNP, 5×10^{-5} M arsenite, 5×10^{-3} M NaF, and 10^{-4} M iodoacetate) had no pronounced effect on the accumulation of sugars by the potato discs. The question of whether the inhibitors had been taken up by the discs was partially answered in the DNP treatments where the color of the alcoholic solutions used for the sugar analyses showed that DNP had entered the discs.

The use of metabolic inhibitors in concentrations higher than the above should be questioned since at high concentrations the inhibitors cause a disorganization of the cells and their probable specificity of action is lost. Nevertheless, an additional experiment was set up in which the concentrations of the inhibitors, with the exception of NaF, were increased ten-fold. The results from this study are given in Table 22. Marked inhibition is present in the azide and KCN treatments. The discs from these treatments were not examined for turgor since no discoloration was present when they were removed from the Petri dishes and washed. However, the sugar levels would indicate that the discs were severely damaged and that the treatments had prevented the development of the discoloration. The DNP treatment caused about a 50% inhibition in

Table 21. Sugar accumulation in potato discs incubated at 32°F. for 14 days. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Appearance	Per cent of original fresh wt.	
		Reducing sugar	Sucrose
1)Original discs	-	0.18 ± .01[2]	0.12 ± .01
2)Distilled water	normal	.60 ± .02	.41 ± .02
3)10^{-4} M DNP[3], pH 5.5	"	.66 ± .03	.51 ± .03
4)5 x 10^{-3} M NaF, pH 6.0	"	.59 ± .03	.32 ± .02
5)10^{-4} M iodoacetate, pH 5.5	"	.52 ± .02	.52 ± .03
6)5 x 10^{-5} M arsenite, pH 6.0	"	0.53 ± .03	0.45 ± .03

1. Average of 3 replications
2. Standard error
3. Dinitrophenol

Table 22. Sugar accumulation in potato discs incubated at 32°F. for nine days. [Variety Cobbler (Maine) from 55°F. storage]

Treatment[1]	Appearance	% of original fresh wt. Reducing sugars	% of original fresh wt. Sucrose	Leakage of sugars from discs
1) Original discs	-	0.08 ± .01[2]	0.14 ± .01	-
2) Distilled water	normal	.70 ± .04	.28 ± .02	Trace
3) 10^{-3} M DNP,[3] pH 5.5	"	.44 ± .03	.13 ± .01	+++
4) 10^{-2} M NaF, pH 6.0	"	.61 ± .04	.26 ± .02	Trace
5) 10^{-3} M iodoacetate, pH 5.5	damaged	.59 ± .03	.45 ± .03	++++
6) 5 x 10^{-4} M arsenite, pH 6.0	"	.53 ± .03	.23 ± .02	+++++
7) 10^{-2} M KCN, pH 6.0	normal?	.31 ± .03	.11 ± .01	++
8) 10^{-2} M Azide, pH 6.0	normal?	.17 ± .03	-	++
9) 10 p.p.m. NAA[4]	normal	0.68 ± .04	0.29 ± .02	Trace

1. Average of 3 replications
2. Standard error
3. Dinitrophenol
4. Naphthalene acetic acid

the level of sugar accumulation. Some indication of inhibition is present in the NaF and arsenite treatments. The arsenite treatment caused extensive damage to the discs as indicated by the discoloration, but the samll amount of inhibition in sugar accumulation was not what one would expect from the extent of the damage. The iodoacetate treatment was without effect on sugar accumulation but appeared to prevent the conversion of sucrose to reducing sugars. The NAA treatment was without effect.

No conclusions are justified in the treatments causing inhibition since this effect could be due to structural disorganization. In the iodoacetate treatment, where sugar accumulation occurred in the presence of a high concentration of inhibitor, it would appear to be safe to conclude that triosephosphate dehydrogenase is not involved in the process of sucrose synthesis from starch.

The effects of the metabolic inhibitors on sugar accumulation in potato discs under aerobic conditions were studied at room temperature to determine if the same high concentrations of inhibitors were necessary to cause inhibition at this temperature as at 32°F. The results from this study are given in Table 23. It can be concluded from the data that the temperature of incubation has little effect on the response of the potato discs to the metabolic inhibitors. It is probably significant, as shown in this and the previous inhibition studies, that the accumulation of reducing sugars is inhibited to a greater degree than the accumulation of sucrose. Since, in many cases, the sucrose level actually increases in the presence

Table 23. Sugar accumulation in potato discs during incubation at room temperature for 48 hours as influenced by treatment with different inhibitors. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Appearance	Per cent of original fresh wt.	
		Reducing sugars	Sucrose
1)Original discs	-	0.16	0.18
2)Distilled water	normal	.46	.12
Infiltrated with:			
3)distilled water	normal	.40	.19
4)10^{-3} M DNP[2], pH 5.5	"	.24	.30
5)10^{-3} M iodoacetate, pH 5.5	damaged	.14	.33
6)5 x 10^{-4} M arsenite, pH 6.0	normal	.35	.22
7)10 p.p.m. NAA[3]	"	0.37	0.17

1. Average of duplicate determinations
2. Dinitrophenol
3. Naphthalene acetic acid

of the inhibitors, it would appear that it is the conversion of sucrose to reducing sugars that is inhibited.

Potato discs placed at 95°F. were dead and badly infected after 24 hours both in the control and inhibitor treatments.

The effect of DNP on sugar accumulation at 32°F. was repeated using potato discs of the variety Russet Burbank since, as reported later, DNP was found to increase sugar accumulation in discs of this variety during desiccation. The results are given in Table 24. The evidence for inhibition of sugar accumulation in aerated potato discs by DNP is clear cut. Phosphate buffer at a concentration of M/15 showed no pronounced effect. Leakage of sugars from the discs exposed to the different treatments was determined and, while it is small in amount, the leakage increased as the degree of inhibition increased.

The significance of this leakage of sugars from the discs is not known. In general, leakage paralleled inhibition and structural damage of the discs. Similarly, when discs were placed at 70°, 60°, 55°, 40° and 32°F., leakage of sugars from the discs increased as the temperature was decreased. However, the study of this effect is complicated by the fact that contamination by microorganisms occurred more readily at the higher temperatures and, in general, the discs contained less sugar as the temperature was increased.

It is generally agreed that the phosphorylated sugars and intermediates are impermeable to cell membranes. However, Nickerson and Chung (70) reported the uptake of unhydrolyzed glucose-1-phosphate by yeast cells in the presence of 10^{-2} M

Table 24. Sugar accumulation in potato discs incubated seven days at 32°F. as affected by different concentrations of dinitrophenol. (Variety Russet Burbank from 55°F. storage)

Treatment[1]	Discs: Per cent of original fresh weight		Leakage into solution: Per cent of original fresh weight	
	Reducing sugars	Sucrose	Reducing sugars	Sucrose
1) Original discs	0.12	0.16	-	-
2) Distilled water	.43	.54	less than 0.01	-
3) M/15 phosphate buffer, pH 6.0	.39	.47	"	-
4) 10^{-4} M DNP[2]	.38	.46	.01	-
5) 5 x 10^{-4} M DNP	.35	.42	.02	-
6) 10^{-3} M DNP	0.31	0.22	0.02	-

1. Average of duplicate determinations

2. Dinitrophenol

NaF and Dyar (24) has reported data that indicate that it is possible for small amounts of glucose-1-phosphate to penetrate the cell membranes over long periods of time. Krotkov and Bennet (58) reported that glucose-1-phosphate, fructose-6-phosphate and fructose-1,6-diphosphate when infiltrated with a hexose sugar strongly inhibit sucrose synthesis. In the present studies 0.3% glucose-1-phosphate and 0.4% fructose-1,6-diphosphate either alone or in the presence of 10^{-2} M NaF had essentially no effect on sugar accumulation in aerated potato discs during seven days incubation at 32°F. Similarly, ATP at a concentration of 10^{-2} M was without effect.

The addition of 3% glucose or 0.05 M malic acid to the DNP and NaF treatments failed to prevent the inhibition in sugar accumulation caused by these compounds (42).

Sugar Accumulation in Potato Discs during Partial Desiccation

The data in Table 25 show the changes which occurred in the sugar fraction of potato discs during partial desiccation. Both reducing sugars and sucrose accumulated but the major increase took place in sucrose. Additional moisture loss above 20% or additional drying time beyond 48 hours appeared to have little effect , but the various possible combinations of these two factors were not studied. There were no pronounced varietal trends in response although discs from Russet Burbank tubers consistently accumulated the highest amount of sugars during partial desiccation. This response of the Russet Burbank discs could be related to their low moisture and high starch content, but it is more probably related to the stronger

Table 25. Sugar accumulation in potato discs during partial desiccation at room temperature.

Variety and (Source)	Weight loss(%)	Duration of drying(hrs.)	Per cent of original fresh wt.[1]	
			Reducing sugars	Sucrose
Triumph (North Dakota)	original	-	0.31	.10
	20.2	48	.41	.92
	21.4	48	.43	.86
	61.6	96	.46	.79
	64.3	96	.40	.82
Green Mountain (Long Island)	original	-	.33	.17
	22.6	48	.41	1.02
	21.1	48	.46	1.08
	59.6	96	.51	.96
	61.0	96	.43	.92
Chippewa (Maine)	original	-	.19	.16
	19.7	48	.72	.77
	20.8	48	.54	.87
	62.4	96	.58	.91
	64.2	96	.50	.76
Russet Burbank (Washington)	original	-	.06	.03
	22.6	48	.46	1.32
	23.5	48	.64	1.39
	62.7	96	.76	1.09
	64.6	96	0.88	1.21

1. Single determinations

resistance of these discs to the stress of desiccation which is similar to the resistance shown by the tubers (Table 2) to the stress of low temperature.

The data in Table 26 show the effect of rapid desiccation on sugar accumulation in potato discs. When the discs are brought to a low moisture level by a stream of air during a period of 8 hours, sugar accumulation occurred but it was not as pronounced as that occurring under conditions of slow desiccation. These results indicate that during desiccation a critical water level is soon reached that prevents sucrose synthesis. In addition, they indicate that reactions requiring a finite time are either involved in sucrose synthesis or the development of the physiological conditions leading to sucrose synthesis. There was a loss of reducing sugars during rapid desiccation which may be interpreted by the assumption that the reducing sugars were used to form sucrose or that they entered into the metabolism of the discs. Leonard (61) reported that cabbage leaves free from starch formed sucrose from reducing sugars upon desiccation.

The data in Table 27 show that the original moisture content of the discs had little effect on sugar accumulation during partial desiccation. Discs from tubers of the variety Chippewa with a moisture level of 83.3% behaved similarly upon partial desiccation to discs from tubers of the variety Russet Burbank with a moisture level of 71.8%. This occurred notwithstanding the fact that discs from Maine Chippewa tubers would have to lose approximately 50% of their moisture before they

Table 26. Changes in sugars in potato discs during rapid desiccation at room temperature.

Variety and (Source)	Weight loss(%)	Duration of drying(hrs.)	Per cent of original fresh wt.[1]	
			Reducing sugars	Sucrose
Irlumph (North Dakota)	original	-	0.31	0.10
	76.4	8 hrs.	.16	.67
	75.1	8	.21	.77
Green Mountain (Long Island)	original	-	.31	.17
	76.7	8	.06	.38
	76.7	8	.05	.58
Chippewa (Maine)	original	-	.19	.16
	81.2	8	.09	.54
	81.2	8	.08	.52
Russet Burbank (Washington)	original	-	.06	.03
	69.7	8	.05	.46
	71.0	8	0.04	0.33

1. Single determinations

Table 27. Sugar accumulation in potato discs of different moisture levels during partial desiccation at room temperature.

Variety and (Source)	Moisture %	Weight loss(%)	Duration of drying	Per cent of original fresh weight[1]	
				Reducing sugars	Sucrose
Chippewa (Maine)	83.3	original	-	.15	.10
		13.0	68 hrs.	.70	.32
		23.1	83	.82	.29
Russet Burbank (Washington)	71.8	original	-	.15	.22
		10.3	68	1.32	.62
		28.5	83	.78	.76

1. Single determinations

would have the same ratio of moisture to solids as the discs from Russet Burbank tubers. These data show that the moisture level of the cells is under close physiological control and that sugar accumulation during partial desiccation is unrelated to the relative proportions of moisture to solids. When the data in this table are compared with the data in Tables 25 and 26, it can be seen that slow desiccation over a relatively long period increased the proportion of reducing sugars to sucrose. This does not invalidate the view that sucrose is formed first, but indicates that under conditions approaching normal metabolism sucrose is converted into reducing sugars.

Wolf (99) reported that the process of sugar accumulation in potato discs during partial desiccation has a Q_{10} of 1.5. Data obtained in the present work confirmed the observation that the rate of sugar accumulation increases with an increase in temperature. Discs subjected to partial desiccation at 105°F. (40°C.) accumulated more sugar in eight hours than similar discs subjected to partial desiccation for eight days at 32°F. However, since the rates of desiccation were not equivalent, an exact quantitative comparison is not justified. The reaction was stopped by a temperature of 158°F. (70°C.).

Several investigators (61,69) have established the necessity of aerobic conditions for sugar accumulation in plant material subjected to partial desiccation. Results obtained in the present work, as given in Table 28, confirmed

Table 26. Sugar accumulation in potato discs during partial desiccation at room temperature for 48 hours as affected by anaerobic conditions. (Variety Russet Burbank from 55° F. storage)

Treatment[1]	Weight loss %	Per cent of original fresh weight	
		Reducing Sugars	Sucrose
Original discs	-	0.14	0.16
Dried in air	3.0	.76	.93
Dried in air	31.5	.62	.81
Dried in O_2	4.0	.88	.97
Dried in 1% O_2	3.5	.65	.73
Dried in N_2	7.0	.56	.69
Dried in N_2	42.5	.21	.60
Dried in vacuum	7.5	.12	.12
Dried in vacuum	48.0	0.16	0.18

1. Average of duplicate determinations

this. However, the dependency of sucrose formation upon the presence of oxygen is clearly shown only when the potato discs are subjected to partial desiccation in a vacuum. Potato discs when partially desiccated under an atmosphere of nitrogen accumulated sugar but not to the extent of the controls in air. These results emphasize the relative insensitivity of sucrose formation to the partial pressure of oxygen. According to some workers (3), this would indicate that the reaction is fundamental in cell metabolism.

The effect of the original sugar level of the discs on sugar accumulation during partial desiccation was studied by drying discs from tubers stored at different temperatures. The results of this study are given in Table 29. The data show that potato discs from tubers which have accumulated sugars in response to low temperature storage show little or no additional increase in sugars when subjected to partial desiccation. Similarly, discs that have been induced to accumulate sugar by eight days of washing with a trickle of tap water do not respond to partial desiccation by exhibiting an additional increase in sugar. Aeration of the discs in a buffer solution of pH 6.0 containing phosphate and potassium ions had no significant effect on the subsequent response of the discs to partial desiccation.

The effect of several metabolic inhibitors on sugar accumulation in potato discs when subjected to partial desiccation was studied by vacuum infiltration of the inhibitors into the discs and allowing them to dry slowly under uniform

Table 29. Sugar changes occurring in potato discs with different levels of sugar during partial desiccation at room temperature.

Treatment[1]	Weight loss %	Per cent of original fresh weight[1]	
		Reducing sugar	Sucrose
1) Russet Burbank-21 days at 32°F.			
Original discs		.06	.91
Dried 18 hours	22.7	.35	.86
2) Triumph(N.D.)-3 months at 32°F			
Original discs		2.42	3.14
Dried 8 hours	70.6	1.02	3.34
Dried 60 hours	73.2	2.38	1.24
3) Russet Burbank- 55°F.			
A. Discs washed 8 days		.66	.51
B. Discs washed 8 days and aerated 24 hours in H_2O		.77	.32
C. Discs washed 8 days and aerated 24 hours in .05 M Na_2HPO_4 + KH_2PO_4		.83	.31
No. A dried 24 hours	20.4	.83	.42
No. B dried 24 hours	16.0	.83	.67
No. C dried 24 hours	23.4	.66	.60

1. Average of duplicate determinations

conditions in an incubator oven at 70°F. The results of this study are given in Table 30. No evidence of inhibition is present notwithstanding the fact that the inhibitors were added in concentrations some 5 to 10 times larger than those used in comparable physiological investigations. In the 10^{-3} M DNP and 10^{-2} M NaF treatments sugar accumulation is significantly greater than in the distilled water controls.

That portion of the above experiment involving DNP was repeated in greater detail. The results are given in Table 31. Sugar accumulation increased with increase in DNP up to 10^{-3} M. It is also significant that in the presence of DNP the dominant sugar is sucrose.

In additional experiments it was found that infiltration of potato discs with 10^{-2} M DNP at pH 5.5, 10^{-3} M DNP at pH 4.8, and 10^{-2} M NaF at pH 4.8 caused the death of potato discs during desiccation. Some varieties were killed by 10^{-3} M DNP at pH 5.5. The variety Russet Burbank showed the greatest resistance to the inhibitors. However, as long as the infiltrated DNP did not cause the structural disorganization of the cell, an increase in sugar accumulation over the distilled water controls was observed.

Table 30. Sugar accumulation in potato discs during partial desiccation at room temperature (70°F.) for 68 hours as affected by metabolic inhibitors. [Variety Katahdin (Colo.) from 55°F. storage]

Treatment[1]	Appearance	Weight loss %	Per cent of original fresh weight	
			Reducing sugars	Sucrose
1) Original	-	-	0.19	.17
2) Untreated	normal	25.6	.93	.61
Infiltrated with				
3) distilled water	"	22.1	.88	.79
4) 10^{-3} M DNP[2], pH 5.5	"	23.9	.54	1.72
5) 10^{-3} M iodoacetate, pH 5.5	"	20.7	.98	.63
6) 5×10^{-4} M arsenite, pH 6.0	"	19.9	.92	.82
7) 10^{-2} M NaF, pH 6.0	damaged	24.5	.92	1.30
8) 10 p.p.m. NAA[3]	normal	21.0	0.98	.65
9) .2 M acetate buffer, pH 4.8	dead	-	-	-

1. Average of 3 replications
2. Dinitrophenol
3. Naphthalene acetic acid

Table 31. Sugar accumulation in potato discs during partial desiccation at room temperature (70°F) for 68 hours after infiltration with different concentrations of dinitrophenol. (Variety Russet Burbank from 55°F. storage)

Treatment[2]	Weight loss %	Per cent of original fresh weight[1]	
		Reducing Sugars	Sucrose
1) Original discs	-	.12	.16
2) Untreated	22.0	1.21	.99
Infiltrated with:			
3) distilled water	8.0	1.24	.74
4) 0.05 M phosphate buffer, pH 6.0	10.0	1.19	.79
5) 10^{-4} M DNP[3], pH 5.5	15.0	1.08	1.04
6) 5×10^{-4} M DNP, pH 5.5	12.0	.97	1.17
7) 10^{-3} M DNP, pH 5.5	15.0	.63	2.06

1. Average of duplicate determinations
2. All samples normal
3. Dinitrophenol

DISCUSSION

Levitt (59), in a review article on frost, drought and heat resistance in plants, has stated, "It is now clear that frost, drought (i. e. desiccation), and heat resistance are all basically similar, and that any resistance to one of these factors carries with it a resistance to the others. Consequently, a theory proposed to explain one of them must apply to all".

The fact that potato tissue accumulates sugar when exposed to low and high temperatures and to partial desiccation makes it probable that this response is a part of the above complex problem. Any adequate theory that explains frost, drought and heat resistance should give some indication of the physiological changes that initiate sucrose accumulation. Conversely, an explanation of the conditions leading to sucrose synthesis would partially explain the response of the plant to frost, drought and heat. At present, no adequate theory for either exists.

The results presented make it appear improbable that there is a simple biochemical explanation for the accumulation of sugar in potato tissue exposed to low and high temperatures and to partial desiccation. Evidence obtained in the present work indicates that this response is caused by an alteration of the organization of the cell. The possible nature of this alteration, other than that it is largely reversible, is not

known. The finding that 10^{-3} M DNP increases rather than inhibits sucrose synthesis in potato discs being subjected to partial desiccation should aid in understanding this change. Teply (90) reported that the addition of DNP to a cyclophorase system carrying on active oxidation caused some of the gel phosphorus to be discharged from the particulates and to be made available in the form of inorganic phosphorus. Green, et al. (29) have reported that temperatures of 38°C. and 0°C. favor the release of gel phosphorus. As explained previously, any increase in inorganic phosphorus in the cytoplasm should cause the conversion of starch to glucose-1-phosphate which could result in the formation of sucrose. These statements are presented for consideration only since it would appear likely that the actual process is much more involved.

As previously stated 10^{-4} M to 10^{-3} M DNP partially inhibited the accumulation of sugars in aerated potato discs in aqueous media. It is not clear why an inhibition is present here and a stimulation is found in the partially desiccated discs. It has been emphasized that the potato discs aerated in aqueous media are in a state of active growth and are not strictly comparable either to the tubers or to the discs being subjected to partial desiccation. However, it is reasonable to assume that the same physicochemical control mechanisms are in operation. The increased metabolic activity of the potato discs aerated in aqueous media would require an increased utilization of ATP with a resulting liberation of inorganic phosphorus which, in turn, should favor the conver-

sion of starch to glucose-1-phosphate. The presence of DNP would effectively block all synthetic reactions requiring ATP. Presumably, it should also cause the leakage of phosphorus from the particulates and favor the conversion of starch to glucose-1-phosphate. If this happens in aerated potato discs, it does not lead to the formation of sucrose.

Observations from the different experiments strongly indicate that in the potato tuber the reducing sugars arise from sucrose. Most of the results obtained in these studies as well as the experimental studies on photosynthesis fit into a logical pattern if it is assumed that sucrose is the storage sugar of the cytoplasm for plants in general. Since most synthetic reactions occur in the cytoplasm, sucrose would be the first sugar formed. Sucrose could be accumulated in the cytoplasm in relatively high concentrations without interfering with or entering into the metabolism of the cell. In some respects its position would be analogous to lactose in milk and fructose in sperm cells. To postulate further, the inversion of sucrose would take place in the vacuole of the cell and the reducing sugars, when drawn upon for use in metabolism, would be phosphorylated at the vacuolar membrane and pass into the cytoplasm as a phosphorylated sugar. At present, the exact mechanism for sucrose penetration of the cell membrane is not known and the extent to which the above concept approximates the actual picture will depend largely on an experimental elucidation of this problem.

Additional information on the mechanism of sucrose

synthesis was not obtained. However, the relative insensitivity of this synthesis to the oxygen level of the tubers and to the presence of high concentrations of metabolic inhibitors make it probable that the major portion of the energy in the glycosidic bond of sucrose is derived from preexisting bond energy. The exact mechanism by which the bond energy of the precursors is increased to that of sucrose remains a challenge to biochemistry. The continued synthesis of sucrose in the presence of 10^{-3} M DNP makes it unlikely that ATP is involved.

SUMMARY AND CONCLUSIONS

The starch-sugar equilibrium was studied in potato tubers, in potato discs aerated in aqueous media, and in potato discs subjected to partial desiccation.

Potato tubers stored at 31°, 40° and 95°F. responded by converting starch to sucrose and reducing sugars. The conversion of starch to sugars was only partially inhibited when potato tubers were stored in 3% oxygen at 32°F. for 20 days. Potato tubers, previously allowed to accumulate sugars at 40°F. storage, converted sugars to starch when stored at 70°F.

Potato discs when properly aerated in aqueous media at room temperature or at 32°F. increased in reducing sugars and sucrose. This process, which was sensitive to the partial pressure of oxygen, was unaffected by 10 p.p.m. NAA or 0.1 M 2,4-D and was partially inhibited by osmotic agents such as 0.3 M $CaCl_2$ or 10% mannitol. It was slightly stimulated by 0.05 M NaCl or .05 M KCl. Sugar accumulation in these discs at 32°F. showed a high degree of resistance to metabolic inhibitors, being only partially inhibited by 10^{-3} M DNP at pH 5.5.

Potato discs subjected to partial desiccation increased in reducing sugars and sucrose. A slow rate of desiccation increased the amount of sugar accumulated and the proportion of reducing sugars to sucrose. The original moisture level of the discs was without effect, but discs containing rela-

tively high levels of sugar showed little or no increase in sugars upon drying. The process of sucrose synthesis was remarkably resistant to the effect of metabolic inhibitors. As long as cell structure was not damaged, 10^{-3} M DNP at pH 5.5 caused an actual increase in the amount of sucrose accumulated. Sucrose was the dominant sugar accumulated in the presence of the metabolic inhibitors.

The results from the various experiments indicate that the first sugar formed from the hydrolysis of starch is sucrose. The reducing sugars, in turn, come from sucrose.

Acid-soluble organic phosphorus and the activity of phosphorylase were determined in potato tubers from different storage temperatures, but a relationship between these values and storage temperature was not found.

Discs from tubers stored at 75°F. were more efficient in the conversion of infiltrated hexoses into sucrose than discs from tubers stored at 32°F.

Sugar accumulation in potato tubers appears to be a part of the larger problem of frost, drought, and heat resistance in plants. It is improbable that there is a simple biochemical explanation for this response. The available evidence indicates that sugar accumulation in potato tubers is initiated by physiological stress of a structural nature.

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