EXPERIMENTS ON SUCROSE FORMATION BY POTATO TUBERS AS INFLUENCED BY TEMPERATURE[1]

BARBARIN ARREGUIN-LOZANO AND JAMES BONNER

(WITH SEVEN FIGURES)

Received December 29, 1948

Introduction

It has been known that plants and plant parts maintained at low temperature tend to convert stored starch to sucrose. Thus MÜLLER-THURGAU (**15**) early found an accumulation of sugars and a corresponding loss of starch in potatoes kept at temperatures between 0° and 6° C. This work was later repeated and confirmed by WOLFF (**20**). Higher storage temperatures on the other hand result in an accumulation of reserve carbohydrates in the form of starch. Although the sweetening of potatoes, sweet potatoes and other tissues during low temperature storage has then been known for some time, the mechanisms responsible for the starch-sucrose conversion have remained obscure, due mainly to the fact that enzymes of the carbohydrate metabolism have been but little studied in higher plants. In animal tissues on the other hand a great deal is known concerning these matters. CORI and CORI (**2**) isolated from frog muscle glucose-l-PO_4 or Cori ester as the initial product of phosphorolysis of glycogen. Later they obtained the same ester from rabbit muscle (**3**) and showed that the presence of the enzyme phosphorylase, is responsible for the reaction. HANES (**8**) has shown that a similar but not identical phosphorylase is present in pea seeds and in potato tubers (**9**) and that this enzyme attacks starch with the production of glucose-l-phosphate. This ester may indeed be prepared biologically from starch with the aid of potato phosphorylase (HANES **8**, MCCREADY and HASSID **14**). The enzyme has also been found in yeast (**12**), and many other plant and animal tissues.

Although many plant tissues form sucrose on cold storage, the mechanisms involved are unknown. The enzymes of carbohydrate transformation in the potato tuber have been but little investigated, and no investigation appears to have been made on the relation of amount and activity of enzymes of the carbohydrate metabolism in relation to temperature. This paper will consider the carbohydrate metabolism of the potato both as to enzyme mechanisms and as related to temperature.

[1] Report of work supported in part by the Herman Frasch Foundation for Agricultural Chemistry.

Methods

Storage

Potatoes of the Russet variety were obtained from the market and were then randomly divided into groups. Each group was stored in the dark at controlled temperature (± 1° C).

Sugar analysis

Samples of potato tissue were rapidly dried in a forced draft oven at 70° C. The dry samples were ground in a Wiley mill to 20 mesh and aliquots were then weighed out and extracted with 80 per cent. ethanol according to Hassid (**10**). After the extracts were cleared with lead acetate, aliquots were used for determinations of reducing sugars by the ceric sulphate method. Sucrose was determined as the increase in reducing power after hydrolysis with invertase. Non-sugar reducing substances were determined as the reducing power in aliquots of extracts which had been previously fermented with yeast. Fructose gives a characteristic color reaction with alcoholic resorcinol in strong acid solution (**16**) and was determined colorimetrically in a Klett-Summerson photoelectric colorimeter. Starch was analyzed in the residue of the alcohol extraction by hydrolysis either with amylase or with acid and titrated as glucose. In reaction mixtures, starch synthesis by phosphorylase was followed colorimetrically as follows: 1 ml. aliquots were removed from the digest to 1 ml. of 16 per cent. trichloroacetic acid in order to arrest the enzymatic activity. The samples were filtered and the precipitate washed several times. One-half ml. of iodine-potassium iodide solution was added and the sample was made up to 25 ml. final volume. The color developed was then measured in a Klett-Summerson colorimeter and compared with a calibration curve made for potato starch. This calibration is not an absolute one, and can be used only for the purpose of comparisons within an experiment.

Phosphorylated intermediates

A portion of dried tuber sample was extracted with successive portions of 5 per cent. trichloroacetic acid in the cold by a slight modification of the method described by Umbreit, Burris and Stauffer (**18**) until the acid extract gave a negative test for phosphorus. The phosphorylated intermediates in the combined extracts were determined by characteristic and specific reactions detailed in (**18**).

Phosphorus determinations

Inorganic phosphate and 7-minute-hydrolyzable-phosphate were determined by the colorimetric method of Fiske and Subbarow (**6**). One ml. samples for phosphate determination were removed from the digests to 16 per cent. trichloroacetic acid. After filtration 1 ml. of 2N sulphuric acid was added, followed by 1 ml. of 8.3 per cent. ammonium molybdate

solution and finally by 1 ml. of the reducing agent, 1-amino, 2-naphtol, 4-sulphonic acid. In the case of the samples for total phosphate the Cori ester was first hydrolyzed by placing the sample in boiling water for seven minutes after the addition of the sulphuric acid. After cooling the other reagents were added. In all cases the readings were made at exactly 20 minutes of color development.

The phosphate methods of KING (**13**) and ALLEN (**1**) were also used but showed no advantages over that outlined above.

Chemical changes in potatoes at different temperatures

The temperature at which potato tubers are stored has a profound effect not only on their content of sucrose but also on their content of other sugars and sugar derivatives, as is shown by the following experiment. Tubers of the variety Russet were stored at temperatures of 0°, 9°, 16° and 25° C. An initial sample was taken as the potatoes came from common storage and further samples were taken after two weeks of controlled temperature storage. These samples were analyzed by the procedures described above. The results of this experiment which are given in Table I, lead to the following conclusions:

TABLE I

CARBOHYDRATE COMPOUNDS AND DERIVATIVES IN POTATO TUBERS AS INFLUENCED BY TEMPERATURE OF STORAGE

VALUES GIVEN IN PER CENT. OF DRY WEIGHT

SUBSTANCE ANALYZED	ZERO TIME (MARKET)	AFTER TWO WEEKS OF STORAGE			
		0°	9°	16°	25°
Starch	67.00	61.00	65.00	63.00	64.00
Glucose	.62	.79	.73	.49	.56
Fructose	.17	1.50	.34	.22	.15
Sucrose	1.07	6.65	1.25	.75	.84
Non-sugar red. substances	.00	.60	.09	.19	.09
Glucose-1-phosphate	.00	.17	.04	.00	.00
Glucose-6-phosphate	3.50	.70	.66	4.20	4.50
Fructose-6-phosphate	.17	2.50	1.05	.25	.35
Fructose-1,6-diphosphate	.00	.00	.00	.00	.00
Triose phosphates	.37	.94	.11	.57	.26

1. Starch concentration decreased in all conditions, the reduction being most marked at 0° C.
2. Glucose concentration increased slightly during storage at 0° and 9° but decreased a little at the higher temperatures.
3. A remarkable change occurred with respect to fructose which increased in concentration nearly ninefold at 0° and twofold at 9° C but remained approximately constant in concentration at the two higher temperatures.
4. Striking changes occurred in concentrations of glucose-6-phosphate and

fructose-6-phosphate. The first compound was found in greater quantities at 16° and 25° C than at temperatures of 0° and 9° C. The reverse was true for fructose-6-phosphate.

5. Glucose-1-phosphate was found in detectable concentration only in tubers stored at 0° or at 9°.

These results indicate that investigation of the problem of sugar formation in potato should include study of the metabolism of the phosphorylated sugars.

Phosphorylase

The phosphorylase content of potatoes from various storage temperatures was first studied. The standard procedure developed for obtaining

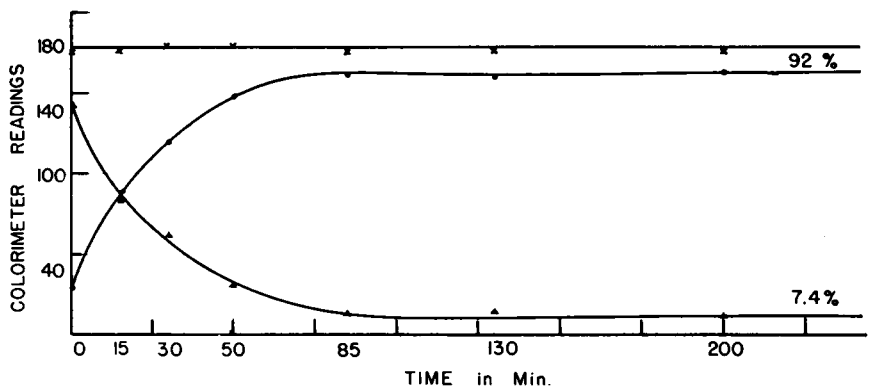

FIG. 1. Activity of potato phosphorylase in synthesis of starch as measured by the changes in phosphates. Digest containing 5 ml. of 5× concentrated enzyme, 3 ml. of maleate buffer pH 5.5, 30 mgs. of Cori ester, and 2 mgs. of starch. Total volume of reaction mixture 20 ml. Incubated at 25° C.

Inorganic phosphate = ●, Cori ester = ▲, total phosphate = ×.

the enzyme from potato consisted of a modification of that used by GREEN and STUMPF (**7**), and by HANES (**9**), both of whom make use of a fractional precipitation of the proteins present in potato juice with ammonium sulphate. A comprehensive study of the method showed that potato proteins should not remain in contact with ammonium sulphate during precipitation any longer than is absolutely essential, since long contact causes losses in enzyme activity. A layer of toluene over the crude juice prevents oxidation. Low temperatures are recommended during extraction, and keeping the enzyme preparation wet or otherwise in solution is also part of the technique.

ACTIVITY

Standard digests consisting of definite quantities of enzyme, substrate and primary substances (starch) were used in the course of the experiments for determination of relative phosphorylase activity. The standard digest consisted of enzyme peparation (semi-purified) 5 ml., Cori ester 30

mgs. (3 mg./ml. solution), starch 2 mgs., and 3 ml. 0.1 M maleate buffer pH 5.5, (total volume 20 cc.) The incubation temperature was 25° C unless otherwise stated. Phosphorylase activity may be followed by determination of the inorganic phosphate liberated during conversion of Cori ester to starch. The results of a typical experiment of this kind are given in figure 1. In a similar manner the amount of starch synthesis can be followed colorimetrically and figure 2 shows the colorimeter readings of the iodine-starch complex plotted against time for the same reaction mixture as shown in figure 1. The color developed rises to a maxi-

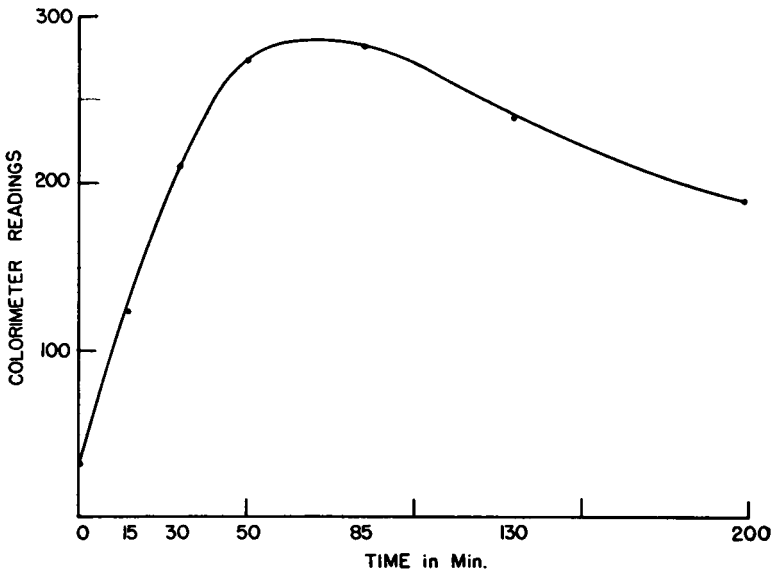

FIG. 2. Determination of phosphorylase activity as measured by synthesis of starch from glucose-1-phosphate. Conditions identical with those of figure 1.
Ordinates represent the colorimeter readings of the iodine-starch complex.

mum and then decreases after equilibrium has been attained as judged by the attainment of constant phosphate concentration in figure 1. During the interval in which these readings were taken the color of the starch-iodine complex solution changed from a deep blue to a violet blue, followed by a violet reddish color, suggesting that the starch has undergone some structural change resulting in decreased absorption of light by the starch-iodine complex. It is known that starch consists of two fractions which are designated as amylose and amylopectin. The former is easily soluble in H_2O and forms a slightly viscous solution, retrogrades from solution, and consists of straight unbranched chains of glucose molecules joined in 1–4 linkages. Amylose produces deep blue color with iodine and is the form of starch produced initially by the action of phosphorylase on Cori ester. The amylopectin fraction is less soluble, gives a highly viscous, opalescent solution in H_2O, produces a violet-red or reddish color

with iodine and is formed of chains of glucose residues linked through 1–4 linkages, but with frequent branches at position 6 of the gluco-pyranose residues. In view of these properties of the starch fractions, the change in color of the starch iodine complex after attainment of phosphate equilibrium suggests and gives some support to the theory that the enzyme preparation contains the "Q" factor of HAWORTH and co-workers (**11**) which enables phosphorylase to form 1,6-glycosidic linkages. This must involve conversion of 1–4 linkages since no net phosphate exchange is involved.

ACTIVITY OF VARIOUS CONCENTRATIONS OF PHOSPHORYLASE

A highly concentrated enzyme was prepared and then diluted to give a range of concentrations. These concentrations were referred to the

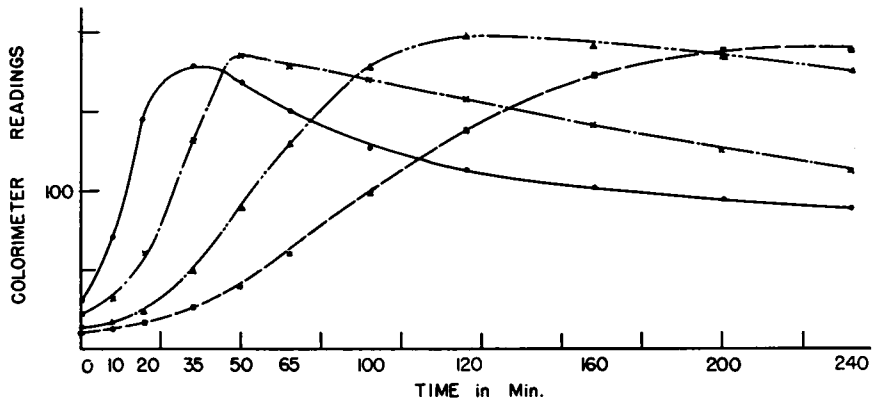

FIG. 3. Rate of synthesis of starch by various concentrations of phosphorylase. The reaction was followed by starch synthesis and the amount of starch measured colorimetrically. Digest consisted of the same amounts and components as for figure 1, with the exception of varying enzyme concentrations.

Enzyme concentrations: 20X ——— ● (20X concentrated over pressed juice)
10X ——— · ——— × 5X ——— - - ——— ▲ 2.5X —— —— —— ■

original volume of potato juice used in the fractionation. Thus a 20x concentrated enzyme is one which, after purification, was taken up in 1/20 of the original juice volume. Various concentrations of enzyme were then incubated with Cori ester as described above and reaction rates followed by determination of the starch formed. Results of such an experiment are represented graphically in figure 3 in which time of incubation is plotted against the colorimeter readings of the starch-iodine complex. From the initial slopes of the curves of figure 3 it can be deduced that the rate of starch synthesis by potato phosphorylase is proportional to the enzyme concentration within the range of concentrations studied. The final equilibria attained and the total amounts of starch synthesized from equal amounts of Cori ester were the same for all enzyme concentrations.

Effect of pH on phosphorylase activity and equilibrium

The pH in the phosphorylase reaction mixtures was adjusted by means of maleate (**17**) and borate buffers, other factors being kept constant. The results of a typical experiment over the range pH 4.5 to 7.5 are summarized graphically in figure 4, where the slopes in the linear portions of

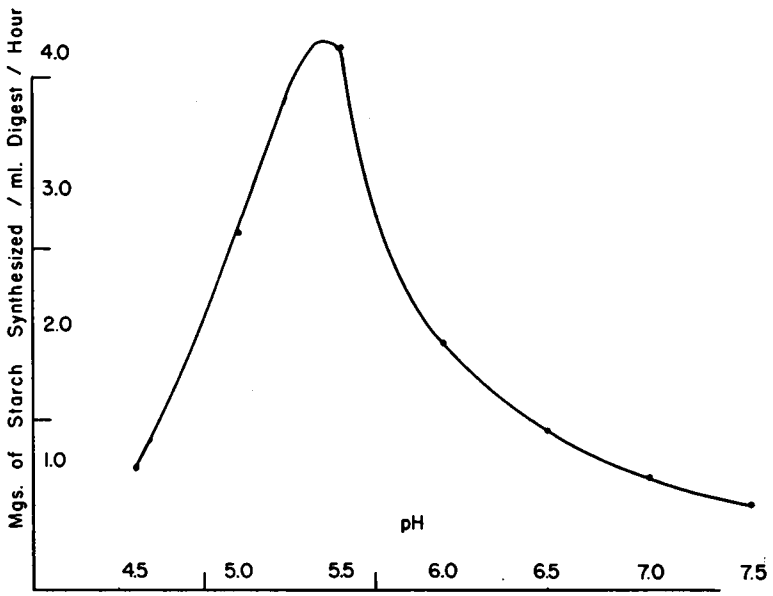

Fig. 4. Effect of pH on the initial rate of starch synthesis reaction by phosphorylase. The digest contained same components and amounts as for experiment of figure 1 except for the pH of the buffer which varied as indicated in the abscissae.

the starch formation-time curves are plotted as a function of pH. As expected, hydrogen ion concentration markedly affected the rate of phosphorylase action, maximum rate occurring at approximately pH 5.5. Above or below this value there is a decrease in rate which is more noticeable towards the less acid side.

TABLE II

Effect of pH on the phosphorylase equilibrium

Digests contained 30 mgs. of Cori ester, 5 ml. of enzyme preparation, 3 ml. of buffer and 2 mgs. of starch. The incubation temperature was 25° C.

pH of digest	4.5	5.0	5.5	6.0	6.5	7.0	7.5
mgs. of starch per ml. of digest at equilibrium	1.04	.99	.98	.95	.90	.85	.78

The pH not only affected the rate of reaction but also the position of the final equilibrium. Under more acid conditions a larger portion of the Cori ester in the digest is converted to starch and inorganic phosphate as can be seen below in table II.

The phosphorylase equilibrium is determined by the concentration of the divalent phosphate and glucose-1-phosphate anions (**4, 9**). Phosphate and glucose-1-phosphate have different dissociation constants. The pH of the medium therefore influences the ratio of total phosphate to total glucose-1-phosphate present in the equilibrium mixture.

Effect of substrate concentration

Several experiments were carried out using 15, 30 and 60 mgs. of Cori ester in digests containing the same enzyme preparation and standard amounts of buffer and priming substance. Figure 5 shows that in such an

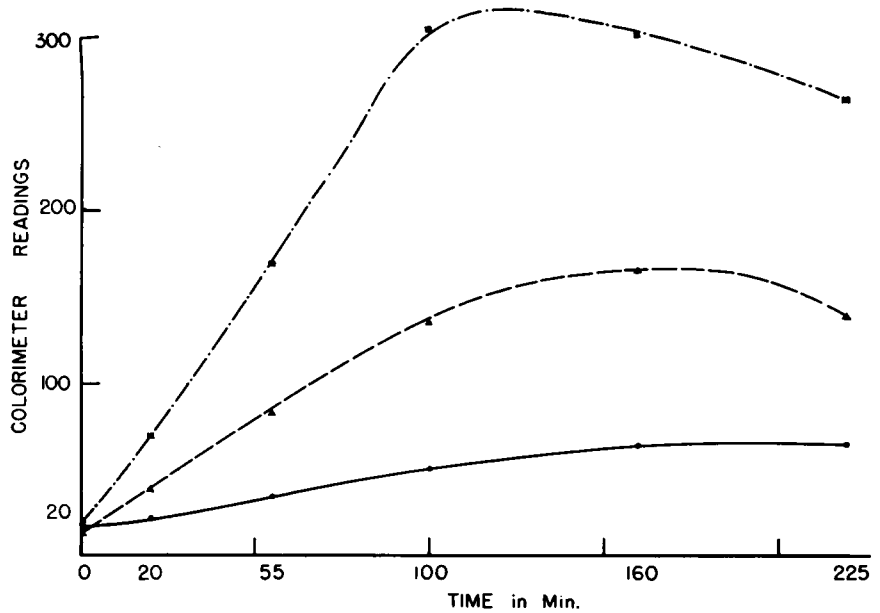

Fig. 5. Effect of substrate concentration on phosphorylase activity as followed by starch synthesis. The digest contained the same components as described for previous experiment 1. Amount of Cori ester was varied in the reaction mixtures as indicated below.

Amount of Cori ester in digest:
▲ —— —— —— 30 mgs.
● ——— 15 mgs.
■ —— . —— . 60 mgs.

experiment, in which all other conditions were held constant, the rate of starch synthesis was proportional to the amount of Cori ester added. Similarly the rate of inorganic phosphate liberation was proportioned to the substrate concentration. The position of the final equilibrium was not however appreciably influenced. Calculating the equilibrium for the three concentrations of substrate, table III was obtained.

Effect of temperature

Experiments were carried out with all factors constant except temperature of the digest. The temperatures used were 0°, 10°, 20°, and 30° ± 0.5° C. Rate of starch synthesis was used as the measure of enzyme activity. The results of figure 6 show that the rate of reaction in-

TABLE III

EQUILIBRIUM ATTAINED IN EXPERIMENT OF FIGURE 5 IN TERMS OF PHOSPHATE

DIGESTS CONTAINED THE SAME CONCENTRATION OF ENZYME BUFFER AND PRIMING SUBSTANCE			
DIGEST NO.	AMOUNT OF CORI ESTER ADDED TO DIGEST IN MGS.	INORGANIC PHOSPHATE % OF TOTAL	ORGANIC PHOSPHATE % OF TOTAL
1	15	11%	85%
2	30	9%	88%
3	60	9%	90%

From these figures we may conclude that the effect of substrate concentration on the position of equilibrium is negligible.

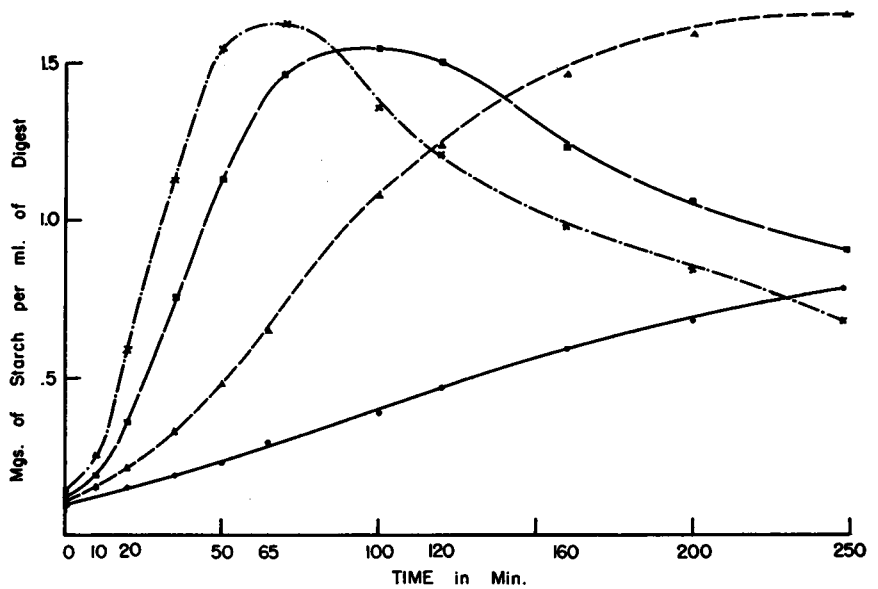

FIG. 6. Effect of temperature of incubation on phosphorylase activity reaction mixtures prepared in the same way as for experiments of figure 1.

The temperatures of incubation were in °C

——— ● 0° — — — — ▲ 10° ——— ——— ■ 20° x —— . —— × 30°

creases with increasing temperature up to 30° C. The final equilibrium position was not affected by temperature since the final amount of starch synthesized when equilibrium was attained was the same at all experimental temperatures used, as must be expected for a process associated with an insignificant production of heat. The rate of reaction was, however, very much influenced by temperature. Calculating the temperature coefficient Q_{10} for the rates plotted in figure 6 at three different points we obtain the following, where

$$Q_{10} = \frac{\text{velocity at } T° + 10°}{\text{velocity at } T°}$$

TABLE IV

EFFECT OF TEMPERATURE ON RATE OF REACTION OF PHOSPHORYLASE

TIME IN MINUTES	Q_{10} BETWEEN			
	0–10°	10–20°	20–30°	30–40°
20	1.41	1.77	1.53	.94
35	2.00	2.17	1.50	.84
50	2.30	2.13	1.32	.74

The values for Q_{10} lie in the range 1.3–2.3 except at the highest temperatures where the Q_{10} is smaller than 1 and decreases consistently with time, suggesting a rapid destruction of the enzyme. It has been shown by others (7) that 61 per cent. of the activity of phosphorylase is lost by heating at 58° for three minutes while heating for the same time at 68° C results in a 97 per cent. loss of activity.

EFFECT OF VARIOUS TEMPERATURES OF STORAGE ON PHOSPHORYLASE ACTIVITY

Experiments were carried out to determine the effect of storage temperature on the phosphorylase activity of potato tubers. The purpose of these experiments was to determine quantitatively the changes in enzyme content and enzyme activity as a response to temperature and time of storage. This is particularly pertinent; it is the starch which is the source of the sugars, formed in response to low temperature storage.

Potatoes from the market were randomized into four lots of approximately 10 lbs. each. The lots were stored at 0°, 10°, 20° and 30° C. Initial determinations of phosphorylase content and activity were made, and after varying periods of storage further samples were taken for study of enzyme content. Comparisons of phosphorylase activity were based on the rate of starch formation at 25° C in reaction mixtures prepared with standard enzyme preparations. The results of a typical storage experiment are given in table V, in which each value represents the average of three enzyme extracts for each batch of potatoes.

TABLE V

TEMPERATURE OF PREVIOUS STORAGE HAS NO INFLUENCE ON THE AMOUNT OF PHOSPHORYLASE CONTAINED IN POTATO TUBERS. FOR DETAILS OF REACTION MIXTURE SEE TEXT

TEMPERATURE OF STORAGE IN °C	TIME OF STORAGE IN WEEKS	TEMPERATURE OF REACTION IN °C	RATE OF STARCH FORMATION IN MGS./ML. DIGEST/HR.	MGS. OF STARCH/ML. OF DIGEST AT EQUILIBRIUM
Market	0	25°	2.0	1.5
0	3	"	2.1	1.3
10	3	"	1.6	1.2
20	3	"	2.0	1.4
30	3	"	2.0	1.5

The results show that the activity of phosphorylase as judged from the partially purified preparation remained approximately constant under all storage conditions. The equilibria were also reached at the same time and the final amount of starch formed was roughly the same in all cases. When the digests were incubated at the same temperature as that of storage the rates of reaction, as is to be expected, were a function of temperature of incubation as discussed above.

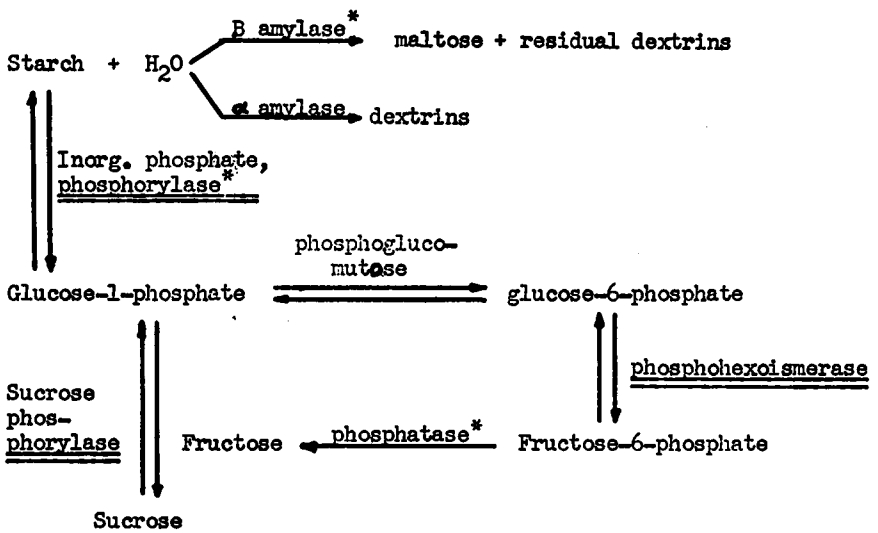

FIG. 7. Pathways of starch metabolism in plant tissues. All of the enzymatic mechanisms noted have been found in one or more species of plants and all but sucrose phosphorylase in higher plants. Those reactions marked * have been studied with isolated enzyme systems in present work. The others have been studied in tissue slices. Those reactions which are doubly underlined are those which appear to be influenced by temperature of storage of the potato tuber.

It can be concluded then that the activity of phosphorylase found in the potato tuber is not affected by temperature of storage. The starch-Cori ester equilibrium is likewise little influenced by temperature.

REGULATION OF PHOSPHORYLASE ACTIVITY

Table 1 shows that there is no appreciable amount of Cori ester in potatoes stored at high temperatures. Since inorganic phosphate and starch are present, a non-equilibrium condition obtains with regard to this system. Why then is starch not attacked by phosphorylase in potatoes stored in the warm as it is in potatoes stored in the cold? It appears that there may be a substance or substances in potatoes stored at 25° C which inhibit phosphorylase activity and that this factor is absent or present in lower concentration in potatoes stored at 0° C. Evidence to substantiate this idea is found in the following experiments.

Potatoes from 0° or from 25° storage were ground in a Waring blendor and extracted with boiling 80 per cent. ethanol. The alcohol was then dis-

tilled off, and the aqueous solution, which will be referred to as inhibitor, was filtered and made to volume, so that each ml. of inhibitor represented 1 gm. of fresh potato. The inhibitor preparations from tubers stored at 0° or at 25° C were added to reaction mixtures containing 5 ml. of 2x enriched phosphorylase, 2 mgs. starch, 20 mgs. of Cori ester, and maleate buffer of pH 6.5, the total volume amounting to 10 ml. One ml. samples were removed periodically and the amount of starch synthesized followed. The results of such an experiment are found in table VI.

TABLE VI

EFFECT OF 80 PER CENT. ALCOHOL EXTRACTS OF POTATO ON THE ACTIVITY OF POTATO PHOSPHORYLASE IN STARCH SYNTHESIS EXPERIMENT

DIGEST NO.	CONCENTRATION OF INHIBITOR	SOURCE OF INHIBITOR	INITIAL REACTION VELOCITY STARCH FORMED MG./HR./ML.
1	none	.....	1.12
2	Represents 0.5 gm./potato/ml.	Tubers Stored at 0°	1.36
3	Represents 0.5 gm./potato/ml.	Tubers Stored at 25°	0.26

The data indicate that the preparation from potatoes stored at 25° C inhibits the synthesis of starch from Cori ester by phosphorylase. The inhibitor seems to be absent from potatoes stored at 0° C, where polysaccharide synthesis took place at a rate approximating that in the controls. Similar results were obtained when the 80 per cent. ethanol extracts were studied with respect to their effects on the degradation of starch by phosphorylase. In the preparation of the enzyme from potato tubers, phosphorylase is apparently separated from inhibitor by the ammonium sulphate precipitation procedure. Thus even though as much phosphorylase is found in potatoes stored at high temperatures as in potatoes stored at low temperatures, still the activity of the enzyme *in vivo* may be decreased at high storage temperatures by the inhibitor.

Amylase

The role of amylase in starch hydrolysis in potatoes stored at various temperatures was investigated. Whole juice pressed from potatoes was used. The enzymes were then precipitated with half saturated ammonium sulphate, the precipitate taken up in water and dialized. Dialysis against distilled water inactivated phosphorylase, permitting a separation of amylase from phosphorylase. The enzyme found in potato juice is a beta-amylase which converts starch into maltose and dextrins. No attempts were made to study the bound amylase in tubers.

ACTIVITY

Activity was determined by a modification of WOHLGEMUTH'S (**19**) method, in which the disappearance of color of the iodine-starch com-

plex is followed as a function of time. Acetate buffer pH 5.0 was used in all digests and the reaction mixtures were incubated at 25°C. In the following experiment the reaction mixtures contained 5 ml. of 2x concentrated enzyme preparation, 3 ml. of acetate buffer, and 2.5 mgs. of starch/ml. digest. The total volume amounted to 20 ml. One ml. aliquots were removed periodically, I_2-KI reagent added and the residual starch determined colorimetrically. The results of this experiment are presented in table VII. Potatoes from 30° storage showed a higher amylase activity

TABLE VII

EFFECT OF TEMPERATURE OF STORAGE ON AMYLASE CONTENT OF POTATO TUBERS

THE FIGURES REPRESENT AVERAGE VALUES OF FOUR EXPERIMENTS

TIME OF STORAGE FIVE WEEKS	TEMPERATURE OF STORAGE			
	0°	10°	20°	30°
Mgs. of starch hydrolyzed in 200 200 min./ml. of digest	0.37	0.29	0.27	0.43
% of starch hydrolyzed in 200 min.	14.8	11.6	10.8	17.2

than potatoes from any other condition. Digests containing amylase and incubated at 0° showed that this temperature essentially suppressed amylase activity. These results taken together suggest that the amylase present in the potato cannot account for the starch hydrolysis in potatoes stored at 0°, and hence cannot be related to the normal sweetening of tubers stored at lower temperatures.

Phosphatase

Among the phosphatases known in plant tissues are those which attack hexose phosphates to yield the corresponding hexoses. Since it is conceivable that phosphatase might in some way be involved in the starch-sucrose transformation in the tuber, the presence and activity of this enzyme in potatoes from various storage temperatures was studied.

ACTIVITY

Whole juice and ammonium sulphate fractionated juice were used as the enzyme source. The precipitate from 50 per cent. saturation of whole juice yielded the most active fraction. The enzyme preparation liberated inorganic phosphate from fructose-6-phosphate and was also able to attack fructose-1,6-diphosphate. The pH optimum of this potato phosphatase is in the neighborhood of pH 8.0 and the enzyme is inhibited by potassium fluoride (50 per cent. inhibition with 0.02 mg. KF per ml.) For the following experiment, 5.0 ml. aliquots of 1× concentrated enzyme were taken from potatoes which had been stored at 0° or at 25°C for three weeks. The reaction mixtures also contained 10 ml. of a saturated solution of the

calcium salt of fructose-6-phosphate, 2 ml. of 0.1M $MgCl_2$ and 3 ml. of borate buffer pH 8.0. The results are given in table VIII. The data show

TABLE VIII

EFFECT OF TEMPERATURE OF STORAGE ON POTATO PHOSPHATASE

INCUBATION TEMPERATURE OF REACTION MIXTURES 25° C.		
TEMPERATURE OF STORAGE	TIME OF STORAGE	MGS. OF INORGANIC PHOSPHATE LIBERATED IN 250 MIN./ML. OF DIGEST
Market	0	0.11
0	3 weeks	0.14
25	"	0.14

that the differences in activity of potato phosphatase from potatoes stored at 0° and 25° were negligible. Phosphatase activity was, further, completely suppressed at 0° C.

Experiments *in vivo*

The experiments reported above have given a clue as to the nature of the temperature-controlled reaction which regulates starch breakdown, e.g. the production of a phosphorylase inhibitor at high temperature. None of the results obtained with isolated enzyme systems have however given any information as to how sucrose is formed in the potato tuber. *In vivo* experiments were therefore carried out, using discs of living tissue cut from potato tubers which had previously been stored at various temperatures.

METHODS

Potatoes were stored for varying periods of time at 0°, 9°, 16°and 25°C. Cylinders then were cut out with a cork-borer and these sliced into discs 1 to 2 mm. thick. The discs were washed in running tap water for 20 minutes and rinsed in distilled water. They were then vacuum infiltrated with the desired substrates. Controls were run in each experiment by infiltrating the discs with distilled water under exactly the same conditions. After infiltration the discs were transferred to Petri dishes containing a sheet of filter paper moistened with the same infiltration solution to keep the atmosphere around the discs moist. The discs were incubated at the desired temperature and after 24 hours were washed and dried at 70° C, ground and subjected to analysis as described earlier.

SYNTHESIS OF SUCROSE BY POTATO DISCS

Sucrose is synthesized by potato discs which are infiltrated with aqueous solutions of glucose and/or fructose, a result which strongly suggests that the two simple sugars are used as intermediates in sucrose synthesis.

Table IX summarizes the results from a typical experiment in which discs were taken from potatoes stored either at 0° or at 25°C and which were then infiltrated with either glucose or fructose. The following conclusions can be drawn from this and similar experiments:

1. In discs from tubers which had been stored at 0°, sucrose synthesis in general occurred at nearly the same rate when glucose was supplied as when the same concentration of fructose was infiltrated.

2. Discs from tubers stored at 25°C formed more sucrose when given fructose than when given glucose, which is in accord with the data of table I, which shows very low fructose concentrations in such tissue.

TABLE IX

SYNTHESIS OF SUCROSE BY POTATO DISCS AFTER INFILTRATION WITH GLUCOSE OR FRUCTOSE. ALL TISSUES INCUBATED 24 HOURS

SUBSTRATE INFILTRATED	INCUBATION TEMPERATURE °C	INCREASE IN SUCROSE CONTENT DURING INCUBATION: % OF DRY WT.	
		STORAGE TEMPERATURE	
		0°	25°
Water	0	1.80	0.52
	25	−0.50	0.46
1% glucose	0	3.00	0.48
	25	0.65	0.60
1% fructose	0	2.85	0.96
	.....	.....	1.90
3% glucose	0	4.00	0.76
	25	2.15	1.85
3% fructose	0	3.65	1.26
	25	2.15	1.97

3. In spite of the fact that discs from tubers stored at 0° contained more sucrose than discs from tubers stored at 25°, the former were more effective in sucrose formation irrespective of temperature of incubation.

4. In discs from tubers stored at 0°, incubation at 0° induced a greater increase of sucrose than incubation at 25°C, indicating that low temperature favors the process of sucrose synthesis itself as well as the process of starch breakdown.

In summary, potato sections synthesize sucrose rapidly when they are provided with hexose. The amount of sucrose synthesized is greater in sections taken from potatoes stored at low temperatures, suggesting that low temperature storage may increase the amount of the sucrose synthesizing enzyme system, whatever it may be. Sucrose synthesis, unlike the other reactions studied above, proceeds even faster in sections incubated at 0° than in sections incubated at 25°C. The mechanism which brings about this unusual reaction to temperature is obscure.

Interconversion of glucose and fructose

It was not possible to demonstrate phosphohexoisomerase *in vitro* in potato preparations. Glucose and fructose were, however, interconverted in potato discs, as was shown by infiltrating discs with glucose or fructose and then analysing for these two hexoses after an incubation period.

The results of an experiment designed to study this interconversion are given in table X, which gives the increases of glucose and fructose con-

TABLE X

Interconversion of glucose and fructose in potato discs after infiltration. Incubated 24 hours

Infiltrated with	Incubation temperature °C	Increases in hexose above level in H_2O infiltrated controls % of dry weight			
		Tubers stored at 0°		Tubers stored at 25°	
		Glucose	Fructose	Glucose	Fructose
10% glucose	0	2.04	1.15	0.40	0.64
	25	3.20	0.38	0.78	0.29
10% fructose	0	1.63	0.69	0.52	0.69
	25	2.80	0.34	0.00	0.18
3% glucose	0	2.65	0.80	0.52	0.75
	25	1.36	0.24	0.30	0.06
3% fructose	0	1.30	0.25	0.63	0.80
	25	1.79	0.25	−0.28	0.07

centrations during an incubation period of 24 hours. The data of this and similar experiments may be summarized as follows:

1. Discs from tubers stored at 0° appear to take up more glucose and more fructose than discs from tubers stored at 25°C.

2. Storage at 0° and incubation at 0° result in large interconversions of glucose and fructose.

3. Storage at 25° and incubation at 25° result in small interconversion of the two sugars.

4. Storage at 25° and incubation at 0° or *vice versa* give intermediate behavior.

In conclusion it can be said that the interconversion of glucose and fructose is promoted by low temperatures. This effect is in the same direction as that found for sucrose synthesis of which the interconversion reaction is undoubtedly a part.

Discussion

The known reactions relating starch, hexoses, and sucrose in the plant and in microorganisms are summarized in figure 7. Two methods for the breakdown of starch are well-established; namely, the hydrolytic breakdown under the influence of amylase and the phosphorolytic breakdown

under the influence of phosphorylase. The potato tuber possesses only weak amylase activity. It does on the other hand possess a powerful phosphorylase. The action of phosphorylase on starch results in the production of glucose-1-phosphate. The breakdown of starch in potatoes stored at low temperature evidently proceeds through the phosphorolytic system since (a) low temperatures conducive to *in vivo* starch breakdown in the potato essentially completely suppress amylase activity and (b) during active starch breakdown glucose-1-phosphate appears in the potato in detectable quantities, whereas it is absent from potatoes which are not undergoing starch breakdown. Potatoes stored at high temperature possess as much phosphorylase as potatoes stored at low temperature. They also possess inorganic phosphate and a pH favorable to phosphorylase activity. Why then does starch remain unattacked by phosphorylase in potatoes stored at high temperature?

The answer appears to lie in an inhibitor of phosphorylase activity which is found in potatoes stored at high temperatures and which is absent or present in lower concentrations in potatoes stored at low temperatures. Since, however, starch is presumably formed in the tuber even at temperatures in which starch breakdown does not occur during subsequent storage, it would appear that this inhibitor must be formed relatively late in the development of the tuber. In general much remains to be explained concerning the formation, degradation, and nature of the phosphorylase inhibitor.

The formation of sucrose from glucose-1-phosphate involves the production of fructose. The sole known method for the biological interconversion of glucose and fructose is through the enzyme phosphohexoisomerase, an enzyme which establishes an equilibrium between glucose-6- and fructose-6-phosphate. This enzyme could not be demonstrated in potato tubers during the present investigation, owing perhaps to inadequate preparative techniques. *In vivo* experiments of many workers have shown however that glucose and fructose are interconvertible and this has been shown again for the potato tissue. Lastly glucose and fructose once formed are combined in some way to sucrose. In the microorganism *Pseudomonas saccharophila* DOUDOROFF, KAPLAN, and HASSID (**5**) have shown that sucrose synthesis is achieved from glucose-1-phosphate and fructose in the presence of an enzyme, sucrose phosphorylase. The existence of this reaction and of this enzyme in potato tuber tissue could not be established during the course of the present work and the exact mode of sucrose formation is hence unknown. Sucrose synthesis was however readily achieved *in vivo* after infiltration with either glucose or fructose and in particular with tissue taken from tubers which had previously been stored at the low temperatures conducive to the sweetening reaction.

The regulations of starch degradation and sucrose synthesis by temperature in the potato tuber would appear to reside in at least three re-

actions, (a) regulation of the amount of phosphorylase inhibitor which is produced in high temperatures, (b) regulation of the activity of the glucose-fructose interconverting system which is increased in amount in low temperature storage, and (c) regulation of the activity of the sucrose synthesizing enzyme which is increased in amount in low temperature storage.

Summary

1. Analysis of potato tubers during storage at low and high temperatures shows that changes take place in the composition of the tubers, not only with regard to starch (which decreases in low temperatures), hexoses and sucrose (which increase at low temperatures), but also with regard to hexose phosphates. The balance between glucose-6 and fructose-6-phosphate in particular is greatly affected, the latter being favored at the lower storage temperatures.
2. Potatoes contain only low amylase activity and this enzyme is essentially inactive *in vitro* at 0°. This enzyme appears not to be a factor in starch degradation and sugar formation at low temperatures.
3. Phosphorylase is equally active in potatoes from all storage temperatures. At no temperature was equilibrium between Cori ester and starch attained *in vivo*. The fact that phosphorylase does not attack starch in potatoes stored at high temperatures seems to have its explanation in the fact that an inhibitor of phosphorylase is formed at high storage temperatures, but disappears at low storage temperatures.
4. Sucrose formation from hexose infiltrated *in vivo* is promoted at low temperatures. Whether this is due to an increase in amount of the enzyme system responsible for sucrose synthesis or to a decrease in a possible inhibitor cannot be stated.
5. Hexose interconverting enzymes are more active at low temperatures and in tubers stored at low temperatures than at high temperatues. This observation is correlated with the fact that tubers stored at low temperatures are high in fructose and fructose-6-phosphate whereas those stored at high temperatures are high in glucose and glucose-6-phosphate.

WILLIAIM G. KERCKHOFF LABORATORIES OF BIOLOGICAL SCIENCES
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA 4, CALIFORNIA

LITERATURE CITED

1. ALLEN, R. J. L. The estimation of Phosphorus. Biochem. Jour. 733–756. 1940.
2. CORI, C. F., and CORI, G. T. Mechanism of formation of hexose-monophosphate in muscle and isolation of a new phosphate ester. Proc. Soc. Exp. Biol. and Med. **34**: 702–705. 1936.
3. CORI, C. F., COLOWICK, S. P., and CORI, G. T. The isolation and syn-

thesis of glucose-1-phosphoric acid. Jour. Biol. Chem. **121**: 465–477. 1937.

4. Cori, G. T., and Cori, C. F. The kinetics of the enzymatic synthesis of glycogen from glucose-1-phosphate. Jour. Biol. Chem. **135**: 733–756. 1940.
5. Doudoroff, M., Kaplan, N., and Hassid, W. Z. Phosphorolysis and synthesis of sucrose with a bacterial preparation. Jour. Biol. Chem. **148**: 67–75. 1943.
6. Fiske, C. H., and Subbarow, Y. The colorimetric determination of phosphorus. Jour. Biol. Chem. **66**: 375–400. 1925.
7. Green, D. E., and Stumpf, P. K. Starch phosphorylase of potato. Jour. Biol. Chem. **142**: 355–366. 1942.
8. Hanes, C. H. The breakdown and synthesis of starch by an enzyme system from pea seeds. Proc. Roy. Soc. B. **128**: 421–450. 1940.
9. Hanes, C. H. The reversible formation of starch from glucose-1-phosphate catalyzed by potato phosphorylase. Proc. Roy. Soc. B. **129**: 174–208. 1940.
10. Hassid, W. Z. Determination of sugars in plants. Ind. Eng. Chem. Anal. **9**: 228–229. 1937.
11. Haworth, W. N., Peat, S., and Bourne, E. J. Synthesis of amylopectin. Nature. **154**: 236. 1944.
12. Kiessling, W. Über die Reindarstellung von glucose-1-phosphorsäure (Cori ester). Biochem. Zeits. **298**: 421–430. 1938.
13. King, E. J. The colorimetric determination of phosphorus. Biochem. Jour. **26**: 292–297. 1932.
14. McCready, R. M., and Hassid, W. Z. The preparation and purification of glucose-1-phosphate by the aid of ion exchange adsorbents. Jour. Amer. Chem. Soc. **66**: 560–563. 1944.
15. Müller-Thurgau, H. Über Zuckeranhäufung in Pflanzentheilen in Folge niederer Temperatur. Landw. Jahrb. **11**: 751–828. 1882.
16. Roe, J. H. A colorimetric method for the determination of fructose in blood and urine. Jour. Biol. Chem. **107**: 15–22. 1934.
17. Temple, J. W. Sodium maleate a buffer for the pH region of 5.2 to 6.8. Jour. Amer. Chem. Soc. **51**: 1754–1755 1929.
18. Umbreit, W. W., Burris, R. H., and Stauffer, J. E. Manometric techniques and related methods for the study of tissue metabolism. Minneapolis. 1945.
19. Wohlgemuth, J. Über Eine Neüe Methode zur Quantitativen Bestimmung des Diastatischen Ferments. Biochem. Zeits. **9**: 1–9. 1908.
20. Wolff, C. J. de. Die Saccharose-bildung in Kartoffeln während des Trocknens. Biochem. Zeits. **176**: 225–245. 1926.