RELATIONSHIP BETWEEN LEVELS OF SOLUBLE CARBOHYDRATE AND STARCH SYNTHESIS IN DETACHED EARS OF WHEAT

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[*Manuscript received April* 20, 1970]

Summary

Detached ears of wheat were cultured on solutions of sugars. A highly significant correlation was observed between the concentration of sucrose in the endosperm and the rate of starch synthesis. Fluctuations in the level of sucrose in the endosperm were accompanied by parallel changes in the rate of starch synthesis. It is suggested that the concentration of sucrose in the endosperm regulates the rate of starch synthesis.

A linear relationship held over a wide range in concentration between the external level of sucrose and the amounts of sucrose in the floral organs and rachis. However, in the endosperm the relationship was curvilinear and the maximum level of sucrose recorded at high external concentrations was very much lower than the concentration in the rachis.

These findings are discussed in relation to the regulation of starch synthesis in cereal grain.

I. INTRODUCTION

It is generally accepted that sucrose is the principal form in which carbohydrate is transported from photosynthetic tissues to organs where starch is deposited. In recent years much progress has been made towards elucidating the biochemical aspects of starch synthesis. However, comparatively little is known about the way in which the rate of starch synthesis is normally controlled. Ghosh and Preiss (1965, 1966) have proposed that in leaves of spinach the level of adenosine-5′-diphosphate glucose (ADPG) in the chloroplasts regulates the rate of deposition of starch. In turn, the production of ADPG is controlled by the activity of ADPG pyrophosphorylase, which enzyme is stimulated by 3-phosphoglycerate, the primary product incorporating carbon dioxide of the carbon reduction cycle of photosynthesis.

On the other hand, Stoy (1965) considers that in wheat the production of photosynthate is the factor limiting the accumulation of starch in the grain. This view would appear to be supported by numerous observations (Welbank, French, and Witts 1966; Bingham 1967; Lupton 1968; Simpson 1968) that the yield of grain is related to the area of photosynthetic tissues supplying carbohydrate to the grain, and the length of time during which these tissues are capable of fixing carbon dioxide. These findings imply that the production of sucrose by photosynthesis controls in turn the level of sucrose in the endosperm, and the rate of deposition of starch. However, there are no published data comparing the amounts of sucrose in the grain, and, concomitantly, the quantities of starch laid down.

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Using detached ears of wheat cultured on solutions of sucrose (Jenner 1968), the inference that the concentration of sucrose in the endosperm governs the rate of starch synthesis has been examined.

II. Materials and Methods

Grain of wheat, cv. Gabo, and barley, cv. Prior, was sown in sandy loam in clay pots, and the plants were raised in the glasshouse with the temperature maintained within the range 18–27°C. In winter the day length was extended to 14 hr with incandescent lamps. After emergence the seedlings were thinned to eight per pot, and all tillers were removed. A few days prior to anthesis the pots were transferred to a cabinet maintained at 25°C and illuminated continuously with fluorescent and incandescent tubes. At the level of the ear the light intensity was 2000 f.c.

In the cabinet starch began to accumulate in the grains on the fourth or fifth day after flowering, and the grains grew and developed more rapidly than in the field.

The method of measuring short-term changes in the amounts of starch in the grain by using one-half of an ear for the initial sample and the other half of the same ear for the final sample, the procedure for culturing detached half ears, and the techniques used for the extraction and measurement of soluble sugars and for the extraction and determination of starch are all described fully by Jenner (1968).

(*a*) *Dissection of Grain*

After removal from the ear, the outer pericarp was peeled from the grain and discarded. The endosperm, still bounded by the adherent testa and the chlorophyllous layer of pericarp, was weighed on a torsion balance and extracted in boiling 80% (v/v) aqueous ethanol.

(*b*) *Experiments with* [^{14}C]*Sucrose*

Uniformly radioactive [^{14}C]sucrose (specific activity 463 mCi/mmole) was purchased from The Radiochemical Centre, Amersham, England. Ears were cultured on radioactive sucrose in essentially the same manner as described by Jenner (1968) for non-radioactive sucrose.

(*c*) *Measurement of Radioactivity*

Copper planchets (32 mm diam. by 3 mm deep) with four annular rings were boiled in detergent, rinsed with water, and dried. A thin smear of silicone grease was applied to the wall and the outermost ring, and the weights of the planchets were recorded.

Samples of solutions of starch (1 ml), containing 4–5 mg of starch in 0·05N NaOH, were pipetted on to the central portion of the planchets. Samples of solutions of sugars (1 ml) eluted from the paper chromatograms were mixed with 0·5 ml of a solution of starch to make the counted weight comparable with the radioactive starch samples and to act as an adhesive. This was prepared by dissolving 0·8 g of wheat starch in 10 ml of hot 0·5N NaOH and diluting to 100 ml with water. 1 ml of the sugar–starch mixture was plated as before, and the planchets were dried down under infrared lamps. The planchets were removed from the lamps as soon as they had dried.

The planchets were reweighed and counted with an automatic low-background (4 counts/min) gas-flow counter (Nuclear-Chicago, Des Plains, Illinois, model C 110 B) with a thin window (Micromil).

To correct for self-absorption the observed count was corrected to a value predicted for a counted weight of 5 mg using a self-absorption curve obtained by counting equal amounts of radioactive sucrose plated with different weights of starch. The relationship between the observed count and weight per planchet was linear from 3 to 8 mg.

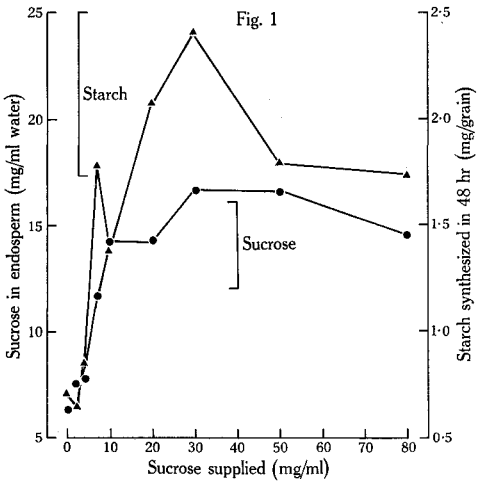
(*d*) *Expression of Data*

For each ear the amount of starch in the initial sample was subtracted from the amount in the final sample, and the results are expressed as a change in starch content (mg/grain).

The amounts of sucrose are expressed as mg/grain or as mg/ml of endosperm water where the amount of water in the endosperm is taken as the difference between the fresh weight and the starch content. As it is likely that the amount of non-starch dry matter as a proportion of the fresh weight of the grain varies with age (Jennings and Morton 1963), sucrose concentrations in grains of widely differing ages are not compared.

III. Results

In a preliminary experiment detached ears were cultured on solutions of different sugars and the amounts of starch produced in the grain were measured. On the sixth day after flowering, ears were detached from plants growing in the lighted cabinet, and from one side of each ear an initial sample of grain was taken. The culms were placed in water or in solutions (50 mg/ml) of four different sugars in the dark for 48 hr. On water the amount of starch in the grain increased by 0·75 mg per grain, compared with increments of 1·26 for sucrose, 1·16 for glucose, 1·05 for fructose, and 0·99 for maltose. Sucrose was used for most of the work in this report.



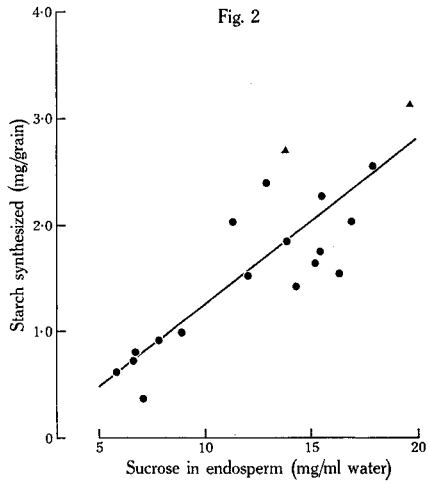


Fig. 1.—Final concentration of sucrose (●) and the amounts of starch (▲) produced in 48 hr in grain from detached ears of wheat cultured on solutions of sucrose. In this and subsequent figures (except Fig. 9) vertical bars indicate least significant differences ($P = 0·05$).

Fig. 2.—Linear regression of the increases in starch during 48 hr in grain from detached ears of wheat cultured on solutions of sucrose (●), and in ears growing on illuminated plants (▲), on the concentration of sucrose in the endosperm. The equation to the regression line is $y = -0·282 + 0·153x$.

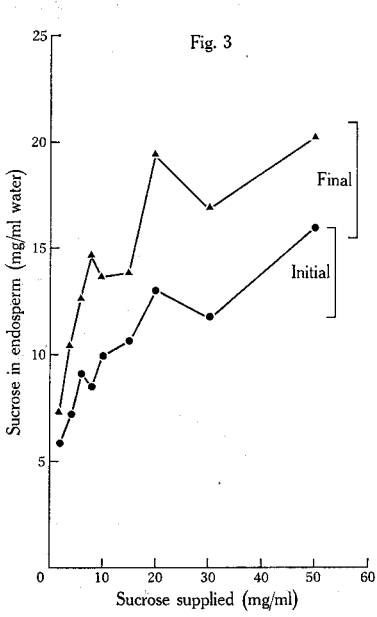
(a) *Variation in Starch Synthesis and Sucrose Content of the Endosperm*

Ears were detached on the sixth day after anthesis and the culms placed in water or solutions of sucrose varying in concentration from 2 to 80 mg/ml. The ears were held in the dark at 27°C for 24 hr and from one side of each ear an initial sample was taken. The remaining portions of the ears were transferred to fresh solutions and returned to the darkened incubator for a further 48 hr.

As the concentration of the sucrose supplied was increased from 0 to 30 or 50 mg/ml (Fig. 1), the level of sucrose in the endosperm increased progressively

towards a maximum value of 16·7 mg/ml of water in the endosperm. From 50 to 80 mg/ml there was a slight, though not significant, reduction in the internal level of sucrose. In grain of the same age taken from ears growing on continuously illuminated plants the concentration of sucrose was 16·8 mg/ml of endosperm water.

The amounts of starch produced followed the changes in the internal levels of sucrose, save for a proportionately greater reduction in starch as the external concentration of sucrose was raised from 30 to 80 mg/ml. Linear regression analysis (Fig. 2) showed that the increments of starch were highly significantly ($P < 0{\cdot}01$) related to the internal levels of sucrose; the correlation coefficient was 0·847. A threefold increase in sucrose was related to a fourfold increase in starch. Values for two samples taken from ears growing on continuously illuminated plants are recorded in Figure 2 for comparison with detached ears.



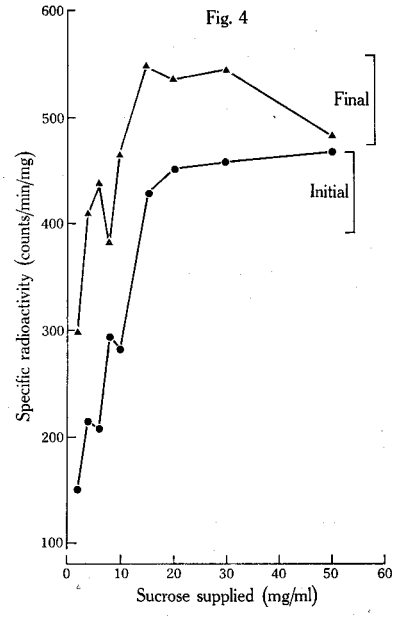


Fig. 3.—Concentration of sucrose in the endosperm of initial (●) and final (▲) samples of grain from detached ears of wheat cultured on solutions of radioactive sucrose.

Fig. 4.—Specific radioactivity of sucrose in the endosperm of initial (●) and final (▲) samples of grain from detached ears of wheat cultured on solutions of radioactive sucrose.

The next experiment was conducted with grain at a more advanced stage of development, beginning on the fifteenth day after anthesis when the grain was almost fully grown and the pericarp had begun to turn yellow. The endosperm was firm and dough-like in consistency and each grain contained between 15 and 20 mg of starch. Since the anticipated increment in starch during 2 days would only have been of the order of 10% of the starch content at this stage, an alternative method of measuring the increase in starch was used.

Ears were placed in solutions of [^{14}C]sucrose (specific activity 998 counts/min/mg) ranging in concentration from 2 to 50 mg/ml. After 24 hr the initial samples were taken from one side of each ear, and the half ears returned to the dark in fresh solutions

of radioactive sucrose for a further 48 hr. In both the initial and final samples of endosperm the radioactivity in the starch and the amounts of sucrose and carbon-14 in the sucrose were determined.

From Figures 3 and 4 it is evident that the concentration of sucrose, and the specific activity of the sucrose in the endosperm increased progressively with each increment in the external concentration up to 15 to 20 mg/ml, but from 20 to 50 mg/ml there was little further change. And during the experimental interval the level and specific activity of the sucrose in the endosperm rose, the rise in amount being greater at the highest external concentrations, while the increase in specific activity was greater at the lowest concentrations.

The net amount of radioactivity incorporated into the starch during the interval of 48 hr was taken as the difference between the initial and final counts in the starch at each level of sucrose supplied (Table 1). To convert radioactivity to quantities of

TABLE 1

INCORPORATION OF RADIOACTIVE SUCROSE INTO STARCH IN WHEAT GRAIN

Detached ears of wheat cultured for 24 hr on solutions of radioactive sucrose. Initial samples were taken and the remaining half-ears cultured on solutions of radioactive sucrose for a further 48 hr

Concn. of Sucrose Supplied (mg/ml)	Radioactivity in the Starch (counts/min/grain)			Specific Activity of the Sucrose in the Endosperm (counts/min/mg)*	Calculated Increase in Starch (mg/grain)
	Initial	Final	Increase in 48 Hr		
2	19	62	44	225	0·20
4	31	119	88	312	0·28
6	43	198	156	324	0·48
8	50	248	200	339	0·59
10	70	308	238	374	0·64
15	113	387	274	489	0·56
20	132	568	436	495	0·88
30	144	474	330	502	0·66
50	186	507	321	477	0·67
L.S.D. (P=0·05)	36	139	n.d.†	n.d.	0·23

* Mean of initial and final values. † n.d., not determined.

starch produced it was assumed that at each level of sucrose the specific activity of the hexose moieties incorporated into the starch was equal to the average specific activity of the sucrose in the endosperm during the experiment. This assumption has been verified for ears at an earlier stage (6–8 days after flowering) cultured on radioactive sucrose at concentrations of 30 or 50 mg/ml (Jenner, unpublished data). The calculated values showed that starch synthesis was maximal at an external concentration of 20 mg/ml. However, the amount produced at that level during 48 hr (0·88 mg/grain) was less than half of the comparable value for the younger grain (see Fig. 1). Linear regression of starch produced on internal level of sucrose was highly significant ($P < 0·01$), and when the data for both experiments were plotted as a percentage of the amount of starch produced at 50 mg/ml of sucrose, both young and old grain were

seen to respond in a similar way to changes in the external concentration of sucrose (Fig. 5).

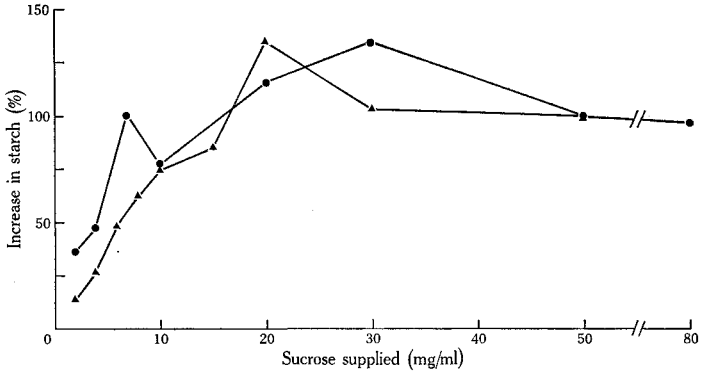
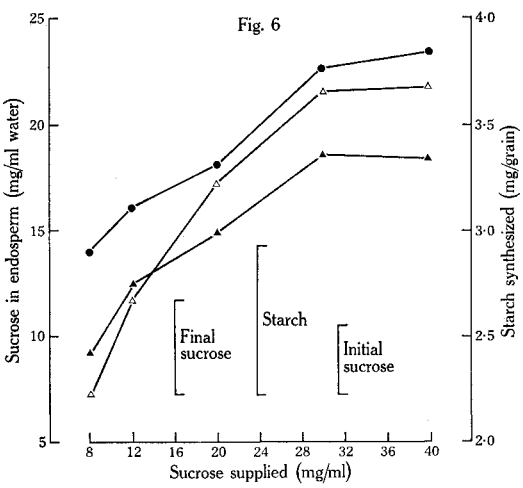


Fig. 5.—Starch synthesis in grain taken from ears of wheat detached 6 (●) or 15 (▲) days after anthesis and cultured for 48 hr on solutions of sucrose. For comparison the increase in starch is expressed as a percentage of the value at 50 mg/ml of sucrose.

Although it seemed clear that over a relatively wide range of concentration, starch synthesis and the internal concentration of sucrose were highly correlated, there remained the possibility that within the critical range of 20 to 30 mg/ml a small



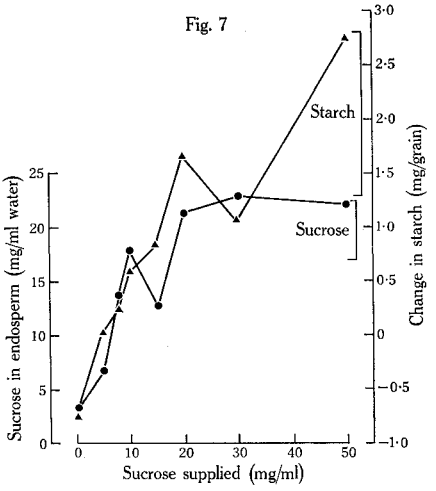


Fig. 6.—Relationship between starch synthesis (△) and the initial (●) and final (▲) concentration of sucrose in the endosperm in grain from detached ears of wheat cultured for 72 hr on solutions of sucrose.

Fig. 7.—Relationship between starch synthesis (▲) and the final concentration of sucrose (●) in the endosperm of grain from detached ears of barley cultured for 48 hr on solutions of sucrose.

change in sugar would not bring about a proportionate change in the rate of starch synthesis. Ears detached on the sixth day after flowering, in an experiment with five replications and lasting 3 days, were used to investigate this range in more detail.

Whereas an increase from 30 to 40 mg/ml (Fig. 6) had no effect on the initial or final internal levels of sucrose, or on the amounts of starch produced, a change from 30 to 20 mg/ml caused parallel changes in all three variables.

Data from an experiment of 48 hr duration with detached ears of barley (Fig. 7) indicated that the relationship between sucrose and starch was similar to that observed for wheat. Moreover, the maximum level of sucrose attained in the endosperm (22·7 mg/ml of endosperm water) and the maximum rate of starch synthesis (1·7–2·7 mg starch/grain/48 hr) were in the same range as corresponding values of 21·6 mg/ml and 1·97 mg/grain measured in ears attached to illuminated plants of barley, and in the same order as the values recorded in Figure 1 for detached ears of wheat. In contrast to the findings for wheat, grain from ears of barley cultured on water had a lower content of starch at the end of the experiment than at the beginning. However, the apparent loss of 0·76 mg of starch from each grain in ears cultured on water was not statistically significant.

TABLE 2

COMPOSITION OF SOLUBLE CARBOHYDRATES AND STARCH SYNTHESIS IN WHEAT GRAIN

Detached ears cultured for 72 hr on solutions of sucrose or mixtures of sucrose and glucose

Concn. of Sugars Supplied (mg/ml)		Final Content of Sugars in the Endosperm (mg/grain)			Starch Synthesized (mg/grain)
Sucrose	Glucose	Sucrose	Glucose	Fructose	
10	0	0·199	0·0082	0·026	1·65
10	1	0·191	0·0093	0·023	1·39
10	2	0·167	0·0090	0·022	1·21
10	5	0·233	0·0094	0·029	1·90
15	0	0·243	0·0099	0·029	1·92
30	0	0·433	0·0207	0·049	2·97
L.S.D. ($P = 0·05$)		0·056	0·0042	0·017	0·59

(*b*) *Dependence of Starch Synthesis on Levels of Sucrose, Glucose, and Fructose in the Endosperm*

Endosperm from a fresh wheat grain sampled on the sixth day after flowering weighed about 15 mg and contained about 0·45 mg of sucrose, 0·05 mg of fructose, and 0·025 mg of glucose. In detached ears provided with solutions of sucrose, alterations in the external concentration of sucrose caused parallel changes, proportionate to their concentration, in the amounts of all three sugars in the endosperm. Thus the question was raised whether the relationship between sucrose and starch synthesis would be affected if the levels of glucose and fructose were altered independently of sucrose. Since of the three sugars, glucose was present in smallest amount, detached ears were supplied with solutions of sucrose or mixtures of sucrose and glucose. After 3 days the endosperm was analysed for sucrose, glucose, and fructose, and the changes in starch measured. The results are set out in Table 2.

The addition of 1 or 2 mg/ml of glucose to sucrose at 10 mg/ml caused slight reductions in the amounts of sucrose and fructose in the endosperm, and in the quantities of starch produced, whereas the level of glucose appeared to increase slightly. However, none of these effects were statistically significant. The composition of soluble sugars and the amounts of starch synthesized were almost identical in ears cultured on a mixture of sucrose and glucose (10 plus 5 mg/ml) and on sucrose alone at 15 mg/ml.

Multiple regression analysis was used to examine in greater detail the dependence of starch synthesis on the levels of the three sugars. The mean square for the regression of starch on sucrose was highly significant, and the mean square for the additional sum of squares due to adding glucose was also significant ($P = 0{\cdot}02$). The corresponding value for fructose was without significance. Set out in the following tabulation are the regression coefficients for the two significant effects:

Independent variates	Sucrose	Glucose
Partial regression coefficients	1·324	−0·473
Standard errors	0·217	0·190
Significant differences	6·10***	2·49*

*** $P = 0{\cdot}001$. * $P = 0{\cdot}02$.

Here it can be seen that the value for glucose, in contrast to that for sucrose, is negative, showing that glucose directly depressed the production of starch independently of its effect on the level of sucrose. This result probably explains the observation in Table 2 that 1 or 2 mg/ml of glucose appeared to cause proportionately greater reductions in starch than in the amounts of sucrose extracted from the grain. Fructose, on the other hand, was not significantly different from sucrose in its effect on starch synthesis.

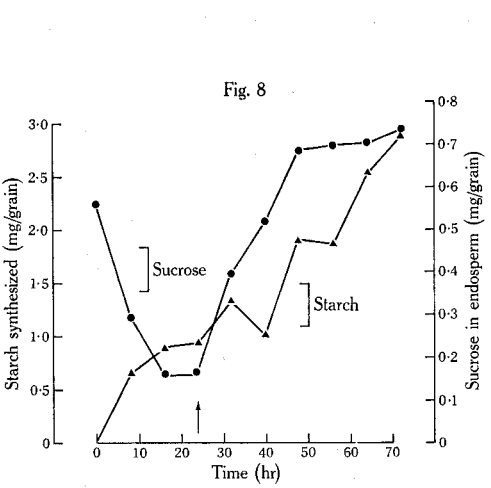
Data from another experiment in which detached ears of wheat were cultured on solutions of sucrose or glucose or fructose, each at 10 or 30 mg/ml, were analysed in a similar way. During 48 hr grain from ears cultured on glucose produced 1·46 (mean of both levels) mg starch per grain compared with values of 1·62 for fructose and 1·82 for sucrose. There was no significant difference between sugars. In the multiple regression analysis, although the mean square for the regression of starch on sucrose was highly significant, the additive effect of glucose was not significant. Although little credence can be attached to the partial regression coefficient for glucose, it is worth noting that is was negative.

(c) *Response of Starch Synthesis to Fluctuating Levels of Carbohydrate*

Under natural conditions of alternating light and darkness it is conceivable that the internal levels of soluble carbohydrate in the grain could rise and fall in a diurnal pattern. It was of interest to establish, therefore, whether temporal variation in the level of sucrose induced experimentally in detached ears would cause parallel fluctuation in the rate of starch synthesis.

Ears of wheat were detached on the seventh day after flowering and the initial samples taken immediately from one side of each ear. After 24 hours of culture on water in the dark, the ears were transferred to solutions of sucrose at 30 mg/ml for a further 2 days.

On water (Fig. 8) the concentration of sucrose in the endosperm fell rapidly to a value which at 16 hr became relatively constant for a further 8 hr. Similarly, the increments of starch became progressively smaller during the intervals on water. After transfer to sucrose, the internal content of sucrose rose significantly during the first interval, and then progressively to a value which remained constant after 48 hr. Starch began to accumulate again after the transfer to sucrose, and the rate of synthesis appeared to become maximal and steady between 48 and 72 hr. However, closer inspection of the values for starch showed that after transfer to sucrose at 24 hr, the first statistically significant increment was synthesized between 40 and 48 hr. Because the data for starch between 24 and 48 hr were rather variable, the period immediately following transfer from water to sucrose was examined in greater detail in an attempt to decide whether there was a real lag in the resumption of starch synthesis.



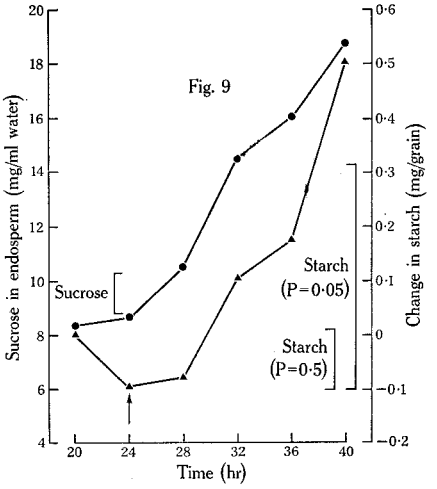


Fig. 8.—Accumulation of starch (▲) and the amounts of sucrose (●) in grain taken from detached ears of wheat cultured for 24 hr on water and then transferred (arrow) to solutions of sucrose (30 mg/ml)

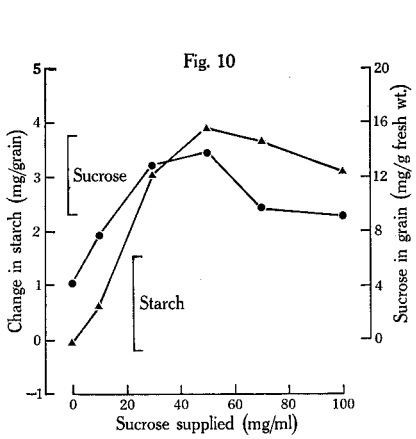
Fig. 9.—Accumulation of starch (▲) and the amounts of sucrose (●) in grain taken from detached ears of wheat cultured for 20 hr on water. Initial samples were taken and the ears were replaced on water and transferred (arrow) after a further 4 hr to sucrose (30 mg/ml). Least significant differences for sucrose ($P = 0{\cdot}05$) and starch ($P = 0{\cdot}5$ and $0{\cdot}05$) shown by vertical bars.

Ears had been cultured on water for 20 hr when initial samples were taken and the remaining portions of the ears replaced in water. After a further 4 hr the ears were transferred to solutions of sucrose (30 mg/ml) and the changes in sucrose and starch in the endosperm followed at 4-hourly intervals. Four hours after transfer to sucrose (Fig. 9) the level of sucrose in the endosperm had risen significantly from 8·6 to 10·5 mg/ml of endosperm water, while the amount of starch had not changed. From 28 to 40 hr both sucrose and starch appeared to increase in parallel. The small, and not significant decrease in starch during the final 4 hr on water was equivalent to a 3% decrease in the starch content of the grain. Moreover, the value for starch after 40 hr was alone significantly greater than the value at 24 hr.

(*d*) *Sucrose Content of the Rachis and Floral Organs of Detached Ears*

On its passage from the external solution to the grain sucrose is transported through the culm and rachis. There is some evidence (Jenner 1968) that at least a fraction of the sucrose entering the grain passes through the glumes and perhaps through the pales. Hence any barrier in these organs to the accumulation or transport of sucrose might be expected to influence the accumulation of sucrose in the grain.

Six days after anthesis ears were detached and cultured on water or solutions of sucrose varying in concentration from 10 to 100 mg/ml. Next day the initial samples of grain were taken and the ears transferred to fresh solutions and held in the dark for a further 72 hr. Five complete spikelets were taken from the centre of the ear and the two basal grains were removed from each spikelet. Without prior dissection the whole grains were killed in ethanol. The five pairs of empty glumes and the five included pairs of basal florets were combined and extracted in ethanol, and the section of rachis subtending the sampled spikelets was cut up and treated in the same way.



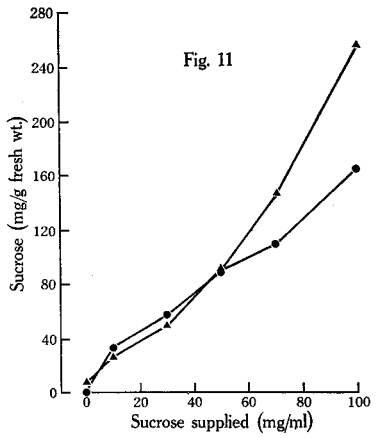


Fig. 10.—Relationship between the increase in starch (▲) and the amount of sucrose (●) in wheat grain from detached ears cultured on solutions of sucrose for 72 hr.

Fig. 11.—Comparison between the amounts of sucrose in the floral organs (▲) and rachis (●). Experimental conditions are the same as in Figure 10.

Within the range 0 to 50 mg/ml (Fig. 10) the relationships between the external concentration of sucrose and the production of starch and levels of sucrose in the endosperm were essentially the same as observed in the earlier experiments. And in agreement with the trend noted in Figure 1, both the sucrose content of the grain and the production of starch appeared to decrease as the external concentration of sucrose was raised, in this experiment, from 50 to 100 mg/ml.

In marked contrast to the response in the grain, the quantities of sucrose in the floral organs and rachis rose roughly in proportion to the concentration of sucrose supplied externally (Fig. 11). At the external level of 100 mg/ml the amounts of sucrose

accumulated in the floral organs and rachis were, respectively, 257 and 166 mg/g fresh weight, compared with a value of 9·1 for the grain. Linear regressions of the content of sucrose in the floral organs and rachis on the external concentration of sucrose were highly significant with correlation coefficients of 0·969 and 0·978 respectively.

IV. Discussion

In support of the contention that in detached ears the synthesis of starch and the metabolism of carbohydrates are operating normally, the following evidence can be cited. Jenner (1968) showed that just as much starch was produced in detached ears cultured in the dark on sucrose at 50 mg/ml, and for periods of up to 4 days, as in ears growing on illuminated plants. Here it is clear [Section III(*a*)] that when the external concentration is in the range 30–50 mg/ml the internal level of sucrose in the endosperm of detached ears is maximal and comparable to values for ears growing on plants continuously illuminated at 2000 f.c. These findings apply equally to barley (Fig. 7). Moreover, in detached ears cultured on water for 24 or 48 hr (Jenner 1968) the accumulation of starch resumes after transfer to sucrose and attains rates similar to those measured in ears cultured continuously on sucrose.

The only apparent difference between wheat and barley seems to be that in detached ears of barley supplied for 48 hr with water alone there is a net loss of starch from the grain (Fig. 7). In wheat no comparable reductions are recorded here (Figs. 1, 9, and 10) or in ears cultured in water for up to 96 hr (Jenner 1968).

The high degree of correlation between the internal level of sucrose and the rate of starch synthesis strongly suggests a causal connection. Since the change in the rate of starch synthesis is temporarily closely linked with the fall in the amount of sucrose in detached ears cultured on water (Fig. 8) it appears unlikely that control is effected by alterations in the quantities of enzymes in the synthetic pathway. The swiftness of the response, and the quantitative nature of the relationship suggest rather that synthesis is regulated by changes in the activity of the enzymes or in the concentrations of intermediates in the pathway to starch. Whether sucrose itself directly influences a rate-determining step in the synthetic pathway, or whether it acts indirectly through other compounds cannot be resolved with the information available. Indeed, the mechanism in wheat may be similar to that proposed for leaves (Ghosh and Preiss 1965, 1966; Sanwal *et al.* 1968) except that in leaves illumination is the ultimate controlling factor, whereas in wheat it appears to be the level of sucrose in the endosperm. These possibilities are under investigation.

The experiments in which ears were transferred from water to sucrose (Figs. 8 and 9) neither rule out nor confirm the existence of a delay in the resumption of starch synthesis as sucrose accumulates in the endosperm. If there is a lag it is only of the order of a few hours, and other techniques would be required to measure it accurately. Nevertheless, if confirmed, the lag phase is the only case reported here where the relationship between sucrose level and starch synthesis does not hold. Whether or not confirmation would invalidate the conclusion reached earlier would depend upon whether the nature of the regulation at abnormally low carbohydrate levels was the same as under normal conditions.

Beyond remarking that the apparent effect of glucose in depressing the synthesis of starch is intriguing, little further can be said until the effect can be examined more reliably. Other methods of investigating this effect are necessary because it has not been possible by culturing detached ears on mixtures of sugars to alter significantly the composition of sugars in the endosperm. Clearly, on their passage through the tissues of the culm and the ear or in the endosperm itself the sugars are rapidly interconverted.

Even though the quantities of sucrose in the floral organs and the rachis rise in step with the concentration of sucrose supplied to detached ears (Fig. 11), the level in the endosperm does not increase at external concentrations above 30–50 mg/ml. To account for these findings, two alternative explanations could be advanced. The rate at which sucrose is transported through the rachilla and the vascular bundle running along the fused margins of the pericarp (Frazier and Appalanaidu 1965) becomes saturated, or the level of sucrose is governed by some metabolic mechanism in the tissues of the endosperm itself. Whatever the explanation, it seems reasonable to propose that the same type of regulation operates *in vivo* since the maximum level observed in detached ears is close to the value for ears of the same age growing on continuously illuminated plants. However, the reduction in the amounts of sucrose in detached ears provided with sucrose at concentrations above 70 mg/ml (Fig. 10) may be an artefact and caused perhaps by disturbances in the water balance of the tissues at high osmotic concentrations of sugar.

The relationship between the carbohydrate status of the grain and the rate of deposition of starch has not so far been investigated in the field. Nor has the possibility been mooted that there may be a restriction imposed, within the ear or the grain itself, on the accumulation of sucrose in the grain. If both kinds of regulation operate *in vivo* as they appear to do in detached ears, it could be concluded that so long as the amount of carbohydrate available for transport to the grain is adequate to maintain at a maximum the level of sucrose in the endosperm, variation in the production of carbohydrate will not alter the rate of starch synthesis. Thus the interpretation of the correlation between rate of photosynthesis and yield or accumulation of starch (see references cited in Introduction) will be valid only if the rate of photosynthesis is inadequate to maintain the level of sucrose in the grain at its maximum. In any case, the correlation cited is susceptible of another interpretation as King, Wardlaw, and Evans (1967) pointed out when they found in wheat that photosynthesis in the flag leaf could be regulated directly by altering the demand for assimilates by the ear. Removing the ears caused reductions in the net rate of photosynthesis, while inhibiting photosynthesis in the ears by spraying them with 3-(3,4-dichlorophenyl)-1,1-dimethylurea led to increased fixation of CO_2. In an attempt to discriminate between these two opposing views, the postulate that the provision of assimilate to the ears in the field is not limiting the level of sucrose in the grain, or the accumulation of starch, is currently under investigation.

V. Acknowledgments

This work was supported by a grant from the Commonwealth Wheat Industries Fund. The author wishes to acknowledge the help of Dr. D. Aspinall in the preparation of the manuscript, and the technical assistance of Miss Christiane Ziolkowski.

VI. References

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