SYNTHESIS OF STARCH IN DETACHED EARS OF WHEAT

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Summary

Detached ears of wheat were provided with water or solutions of sucrose and the effects of light and temperature on the synthesis of starch in the grain were investigated. During 24 hr similar amounts of starch are produced in ears cultured on water or sucrose. Thereafter no further synthesis takes place on water, but with solutions of sucrose synthesis continues at a steady rate for several days. Variation in light intensity has no effect on the synthesis of starch in ears supplied with sucrose. In ears cultured on water or sucrose more starch is made at 30 than at 15·5°C.

Little or no starch is produced in isolated grain or in slices of grain cultured on sucrose solution. Compared with detached, but still intact ears, less starch is made when spikelets are cut from the rachis and cultured separately, and the reduction is accompanied by a decrease in the level of sucrose in the grain. Although the removal of glumes from intact ears causes a reduction in starch synthesis, the amounts of sucrose in the grains are not affected.

I. INTRODUCTION

Early investigations on the environmental control of the formation of starch in leaves have been reviewed by Nurmia (1935). With carbon dioxide as the only external source of carbon, leaves produce starch in the light but not in the dark. However, detached leaves supplied with solutions of sucrose will form starch in the light and in the dark. Moreover, although starch is not normally found in the leaves of many monocotyledonous plants, or in the yellow regions of variegated leaves, starch is formed when such leaves are provided with sucrose.

The balance between synthesis and degradation of starch *in vivo* is markedly influenced by temperature. In the leaves of some species starch is not produced at temperatures below 6–8°C, and in potato tubers held at low temperatures starch breaks down and sucrose accumulates (Müller-Thurgau 1882, cited by Nurmia 1935; Arreguin-Lozano and Bonner 1949).

Buttrose (1960) used the electron microscope to examine the effects of environmental factors on the fine structure of starch granules. Concentric rings were clearly visible in sections of granules from several species of cereal grain grown in the field. The number and form of the rings is consistent with the view that the granule is synthesized by apposition of concentric shells around a nucleus, and that one shell is formed per day. No shells were visible in granules prepared from grain of wheat and barley plants grown in continuous light and at a constant temperature

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(Buttrose 1960), and shells could be induced in granules of wheat starch by growing the plants in alternating periods of light and darkness (Buttrose 1963). Clearly, in cereal grain the fine structure of the granules is controlled by environmental factors, but the extent to which these factors influence the synthesis of starch has not been investigated.

Accordingly, in the present study, detached ears of wheat, or organs derived from ears, have been used to examine the influence of some environmental and physiological factors on the synthesis of starch in wheat grain.

II. Materials and Methods

(*a*) *Growth of Wheat Plants*

Wheat seed, cv. Gabo, was sown in sandy loam in clay pots (8 in. diam.), and the plants were raised in a 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 seedlings were thinned to eight per pot and all tillers were removed.

A few days prior to anthesis the pots were transferred to a growth cabinet maintained at 25°C and illuminated continuously with fluorescent and incandescent tubes. At the level of the ears the light intensity was 1500 f.c. for some experiments and 2000 f.c. for others. Each ear was marked with the date of anthesis.

(*b*) *Sampling Procedure*

Six days after anthesis ears with 28–40 fertile florets (14–20 spikelets) were cut and placed in water. A sample of 10 grains was taken from five spikelets in the central region of one side of each ear. These were designated initial samples, and the ears were ranked in order according to the fresh weight of the grain. The heaviest and lightest ears were rejected if they deviated greatly from the mean. Sets of ears most alike in weight were allotted to replications (3–5 in an experiment) and within each replication the ears were randomized among treatments.

Since comparisons within a single ear are more accurate than comparisons between different ears (see Table 1), half ears were used for all experiments, the initial sample being taken from one side of the ear, and the final sample from the opposite side of the same ear.

(*c*) *Treatment of Detached Half Ears*

All spikelets were cut from the rachis on the side of the ear used for the initial sample with a sharp pair of scissors. The rachis was cut obliquely (60° to the horizontal) under water about 13 cm from the basal spikelet. The ears were quickly transferred to tubes (150 by 15 mm) containing 15 ml distilled water or solutions of sucrose. Each day the rachis was cut back by about 2 mm and the ears transferred to fresh solutions. Unless stated otherwise the ears were placed in an incubator in the dark at 25°C. After treatment 10 grains were removed from spikelets inserted opposite those taken for the initial samples.

(*d*) *Preparation of Spikelets and Isolated Grains*

Glumes and paleas were forced downwards and away from the grain until they broke free at the point of insertion. Lemmas and infertile florets were cut close to the base. The rachis was cut between the spikelets, and the basal 2-mm section of rachis subtending a spikelet was cut again under water. Grains to be cultured in isolation were pulled gently from the florets.

The spikelets and isolated grains were inserted in holes countersunk in sheets of Perspex with their basal ends dipping into water or solutions of sucrose.

(*e*) *Extraction and Chromatographic Separation of Soluble Sugars*

Immediately after removal from the ear the samples of grain were dropped into boiling 80% (v/v) aqueous ethanol and boiled for 10 min. When cool the alcohol was decanted and the

grains homogenized in a glass homogenizer (Kontes Glass Co., Vineland, New Jersey) with two successive portions of ethanol. The suspension was centrifuged and the combined supernatants were dried *in vacuo* at about 35°C. The residue was dissolved in 20% (v/v) aqueous ethanol and portions of the extract were applied to Whatman No. 3MM chromatography paper. After development for 17–20 hr in ethyl acetate–pyridine–water (10 : 4 : 3 v/v/v) the sugars were located with the silver nitrate reagent described by Anet and Reynolds (1954).

Zones corresponding to sucrose were eluted from the paper with water by the technique of Dent (1947) and the quantities of sugar in the eluate were measured with the anthrone reagent described by Yemm and Willis (1954).

(*f*) *Starch Determinations*

Samples of fresh grain, or the residues after extraction with ethanol, were heated with water for 15 min on a boiling water bath. When cool, the samples were dispersed in perchloric acid and the starch precipitated with iodine by the method of Pucher, Leavenworth, and Vickery (1948). After decomposing the iodine complex the precipitate was dissolved by warming in 0·5N NaOH (McCready and Hassid 1943) and the starch determined with anthrone (McCready *et al.* 1950).

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

III. Results

(*a*) *Dependence of Starch Synthesis on the Initial Quantities of Starch in the Grain*

The relationship between the fresh weight and the amount of starch in the grain is shown in Figure 1 for a typical set of 24 ears. The content of starch varied from 1·10 to 2·26 mg/grain and there was a significant linear regression ($r = 0{\cdot}738$) of starch on fresh weight. (Hence the procedure used for allocating ears to replications results in a stratification on the basis of the initial starch content of the grain.)

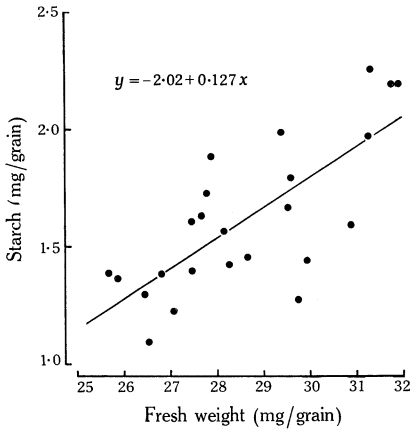


Fig. 1.—Relationship between starch content and the fresh weight of wheat grain sampled at 6 days after anthesis.

The amounts of starch in paired samples of grain taken from the opposite sides of the same ear are recorded in Table 1. The variance of the differences between samples taken from opposite sides of the same ear is 0·025 compared with a value of 0·356 for the variance between ears. Table 1 also shows the wide variation in starch content between ears sampled at a fixed interval after flowering. On the sixth day after flowering 12 detached ears were cultured on 5% sucrose solution

in a growth cabinet at a light intensity of 1500 f.c. During 48 hr the level of starch in the grains rose from a mean value of 1·69 to 3·86 mg/grain. Regression analysis of starch synthesized on initial content of starch gave a correlation coefficient of 0·086. Clearly, the amounts of starch synthesized are independent of the initial quantities.

TABLE 1

VARIATION IN STARCH CONTENT OF WHEAT GRAIN BETWEEN EARS AND BETWEEN OPPOSITE SIDES OF THE SAME EAR

Ears sampled on the sixth day after flowering

Ear	Starch Content (mg/grain)			Ear	Starch Content (mg/grain)		
	Side 1	Side 2	Difference		Side 1	Side 2	Difference
1	2·22	2·11	0·11	9	3·12	3·12	0·00
2	1·56	1·48	0·08	10	2·97	2·65	0·32
3	2·27	1·70	0·57	11	3·12	3·12	0·00
4	1·56	1·53	0·03	12	2·42	2·50	0·08
5	2·97	2·81	0·16	13	2·65	2·34	0·31
6	2·97	3·12	0·15	14	1·33	1·50	0·17
7	2·65	2·65	0·00	15	1·86	1·69	0·17
8	2·31	2·33	0·02				

(*b*) *Starch Synthesis from Solutions of Sucrose at Different Concentrations*

Detached ears were provided with water or solutions of sucrose varying in concentration from 1 to 8%, and placed in an illuminated cabinet (light intensity 1500 f.c.). For comparison, some ears were left attached to plants growing in the same cabinet. During 24 hr a mean of 1·02 mg of starch was produced per grain and there were no significant differences between the treatments. However, during 48 hr and in the dark more starch is produced in ears provided with 5 or 8% sucrose than in ears cultured on water, as the following tabulation shows [L.S.D. = 0·66 ($P = 0·05$)]:

Sucrose concn. (g/100 ml)	0	1	2·5	5	8
Change in starch (mg/grain)	1·08	1·44	1·42	1·79	1·99

The change in amount of starch in grain in ears attached to plants illuminated at a light intensity of 2000 f.c. was 2·52 mg/grain, which is not significantly greater than the value for detached ears provided with 8% sucrose solution.

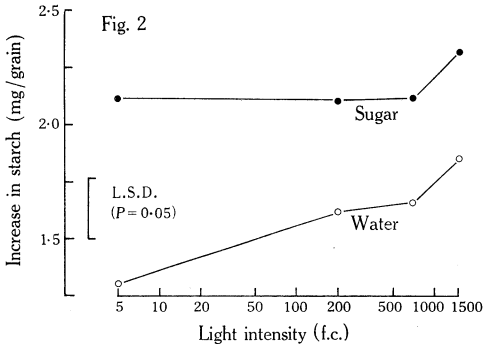
(*c*) *Influence of Light Intensity*

Detached ears, cultured on water or 5% sucrose solution, were placed in a lighted cabinet at 1500 f.c. and with an ambient temperature of 25°C. Large beakers, one uncovered and three covered with layers of white paper or aluminium foil, were inverted over the ears, and each beaker was shielded from the light source with a filter of 5 cm of water. The light intensity under each beaker was measured with

an omnidirectional probe, and the temperature with an unshielded mercury thermometer. In this way light intensities of 1500, 700, 200, and about 5 f.c. were obtained. The temperature at 1500 f.c. was 28°C, and at 5 f.c. 25·5°C.

The amounts of starch produced during 48 hr are plotted against light intensity in Figure 2. There was no significant difference between means for each light intensity, nor was the interaction of light with water and sucrose significant. Moreover, the values at 1500 f.c. may have been influenced by temperature [see Section III(*d*)].

In another experiment detached ears were cultured on 5% sucrose solution for 40 hr at a light intensity of 1000 f.c. or in complete darkness. A flow of air was maintained over both sets of ears and there was no detectable difference in temperature between the treatments. In the dark 1·35 mg starch was produced per grain compared with a value in the light of 1·40 mg/grain.



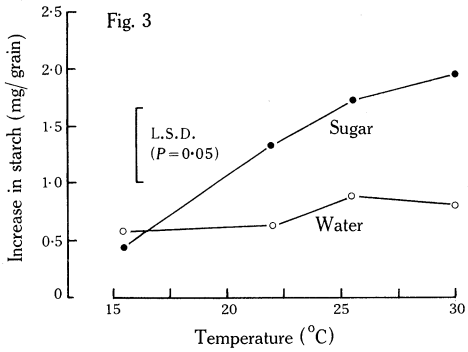


Fig. 2.—Influence of light intensity on starch synthesis in wheat grain. Detached ears were cultured for 48 hr on water or 5% sucrose solution.

Fig. 3.—Influence of temperature on starch synthesis in wheat grain. Detached ears were cultured for 48 hr in the dark on water or 5% sucrose solution.

(*d*) *Effect of Temperature*

Sets of ears were provided with water or 5% sucrose solution and placed in the dark at temperatures ranging from 15·5 to 30°C. During 48 hr similar amounts of starch are produced at 15·5°C in ears cultured on water or sugar (Fig. 3). With sucrose greater quantities of starch are made for each successive increment of temperature. At 30°C the value is 4·4 times greater than at 15·5°C. Compared with 15·5°C slightly more starch is produced at 25·5°C in ears supplied with water, but no greater production takes place when the temperature is raised from 25·5 to 30°C.

Although in ears cultured on sucrose greater quantities of starch are made at 30 than at 15·5°C, it is uncertain whether an increase in temperature accelerates the conversion of sucrose to starch or the transport of sucrose to the grain. To distinguish between these two possibilities ears were cultured at 15 or 25°C on water or 5%

sucrose and after 48 hr the amounts of sucrose and changes in starch were determined. The results are shown in the following tabulation:

Temperature (°C)	Solution	Change in Starch (mg/grain)*	Final Sucrose Content (mg/grain)*
15	Water	0·35	0·46
	Sucrose	0·69	0·77
25	Water	0·57	0·34
	Sucrose	1·49	0·64

* Least significant difference ($P = 0·05$) between any pair of means is 0·35 for starch and 0·07 for sucrose.

These values can be compared with a change in starch content of 2·03 mg/grain and a final sucrose content of 0·62 mg/grain for ears attached to plants in the light (1500 f.c.) at 25°C. Compared with 15°C greater quantities of starch are produced at 25°C in ears supplied with sugar. On the other hand, the level of sucrose is higher at 15 than at 25°C whether the ears are supplied with water or sugar. Thus temperature directly affects the conversion of sucrose to starch. It will be noted also that for ears cultured on sucrose at 25°C the sucrose content is similar to the value for ears attached to illuminated plants.

It has already been established that variation in light intensity does not affect starch synthesis in detached ears provided with sucrose. However, the yield of starch in ears attached to illuminated plants is greater than for detached ears cultured on sucrose in the darkened incubator (see tabulation). It seemed possible that although the ambient temperature was the same in both cases, the temperature within the grain might vary between the two locations. Accordingly, small thermocouples (about 2 mm diam.) were inserted between the lemma and adaxial surface of the subtended grain in ears growing in the lighted growth cabinet and in detached ears cultured in the incubator. Under illumination the temperature recorded at the surface of the grain was 0·5–1 degC higher than the ambient one, while in the incubator it was 1·5–2 degC lower. A difference in temperature of this magnitude could account for a major fraction of the observed difference in starch synthesis. Further evidence in support of this contention is provided later.

(e) *Starch Synthesis in Ears Transferred from Water to Solutions of Sucrose*

Detached ears were cultured in the dark on water or 5% sucrose solution, and after 1 or 2 days some ears were transferred from water to sugar. The amounts of starch synthesized during 4 days are shown in Figure 4. After 24 hr similar amounts of starch are produced with water or sugar. However, for the next 3 days synthesis in ears supplied with sugar continues at a fairly constant rate whereas on water there is little further change. During the first day after transfer from water to sucrose little starch is produced, but thereafter the rate of synthesis increases to values not appreciably different from ears cultured continuously on sucrose.

The ambient temperature in the incubator was 27·5°C and that measured with a small thermocouple at the surface of a grain was 26°C. Conversely, in the illuminated growth cabinet where the ambient temperature was 25°C the temperature within a floret was 0·5–1 degC higher. Hence within the grain the temperature may have been similar at both locations. Indeed after 4 days almost identical amounts of starch were produced in ears growing in the illuminated cabinet and in detached ears cultured on solutions of sucrose in the darkened incubator (Fig. 4).

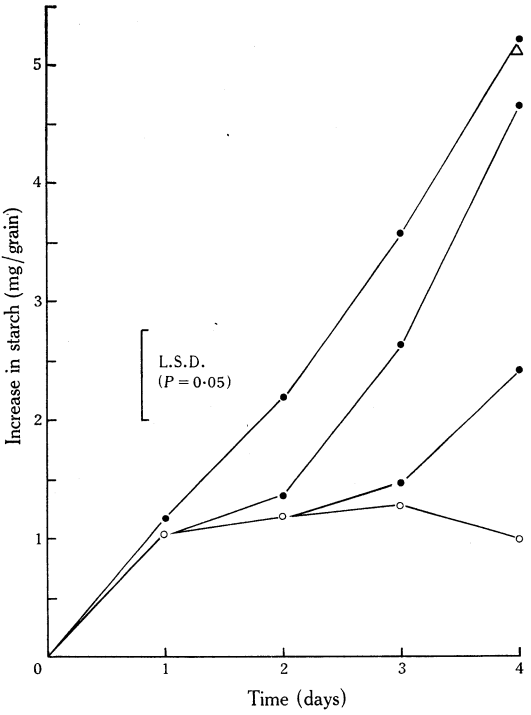


Fig. 4.—Synthesis of starch in detached ears of wheat provided with water (○) or 5% sucrose solution (●) in the dark. Sets of ears were transferred from water to sucrose at 1 and 2 days. One set of ears remained attached to plants growing in the light (△).

(f) Starch Synthesis in Detached Spikelets and Isolated Grains

In all experiments described so far the grains were enclosed within spikelets connected to the rachis through which the substrate was absorbed. The question of whether starch synthesis in the grain in any way depends on the associated floral organs or the rachis was next investigated.

In spikelets cut from the rachis and cultured in the dark with the subtending stump of rachis dipping into sucrose solution (5%) 1·25 mg/grain starch were produced during 48 hr. This value is lower ($P < 0·1$) than the 1·69 mg/grain formed within detached but still intact ears. Only 0·03 mg/grain (value not significantly different from zero) was produced by isolated grains held with the rachilla dipping into solutions of sugar.

The following tabulation shows the quantities of starch formed in isolated spikelets cultured on 5% sucrose solution for 48 hr in the dark, with the glumes, paleas, and lemmas removed [L.S.D. = 0·63 (P = 0·05)]:

	Change in Starch (mg/grain)
Detached ears	2·01
Isolated spikelets	1·07
Isolated spikelets, with glumes removed	0·55
Isolated spikelets, with glumes and paleas removed	0·67
Isolated spikelets, with glumes, paleas, and lemmas removed	0·62
Isolated grains	0·04

Here significantly less starch is made in isolated spikelets than in whole ears. Moreover, removing the glumes causes further reductions but the removal in addition of paleas and lemmas has no greater effect. Again, no starch is produced in isolated grain.

TABLE 2

AMOUNTS OF STARCH AND SUCROSE IN THE ENDOSPERM OF WHEAT GRAIN CULTURED IN ISOLATION OR WITHIN SPIKELETS OR EARS

Organs provided with water or 5% sucrose solution for 48 hr in the dark. L.S.D. for starch (between treatment means) is 0·35, and for sucrose (between any pair of means) 0·08 (P = 0·05)

Source of Grain	Culture	Change in Starch in Endosperm (mg/grain)	Final Sucrose Content in Endosperm (mg/grain)
Detached ears	Water	1·25	0·19
	Sucrose	2·24	0·49
	Mean	1·75	
Isolated spikelets	Water	1·17	0·12
	Sucrose	1·55	0·15
	Mean	1·36	
Isolated grains	Water	0·52	0·05
	Sucrose	0·98	0·09
	Mean	0·75	

To account for these findings it might be advanced that the movement of sucrose to the endosperm becomes limiting when the ear is cut up into spikelets or when the floral organs are removed. Similarly, sucrose may not be absorbed by grain cultured in isolation. To test this hypothesis detached ears, isolated spikelets, or isolated grains were cultured on water or 5% sucrose solution and, in addition to starch, the amounts of sucrose in the grain were determined. In earlier experiments with isolated grain the space between the outer transparent pericarp and the inner green layer occasionally became injected with culture solution so starch and sucrose in this experiment were measured in the endosperm alone. After 48 hr the outer pericarp was peeled from the grain and discarded. The endosperm, still bounded by the adherent testa and chlorophyllous layer of the pericarp, was rinsed with water and killed in ethanol. The results are shown in Table 2. Whether cultured on water

or sugar, less starch is produced in isolated spikelets than in detached ears. In contrast to earlier experiments significant quantities of starch are produced in isolated grains, and the difference of 0·46 mg/grain between sugar and water is significant ($P < 0·1$). Moreover, it is manifest that for all treatments the trends in the values for the amounts of sucrose in the endosperm closely parallel those for starch.

(*g*) *Experiments with Isolated Grain*

Grains isolated from the ear and cultured on water or sucrose increase in fresh weight but produce little or no starch and the internal level of sucrose is low. This suggests that the pathway of transport of sucrose through the severed rachilla is blocked, and that the tissues enveloping the endosperm act as a barrier to the uptake of sucrose. Therefore in a series of experiments the basal portion of the grain was cut off, or the grain sliced longitudinally or transversely. In some cases the slices were floated, with the cut face of endosperm downwards, on droplets of 5% sucrose solution, or laid on polyurethane foam soaked in solution. One experiment was conducted under sterile conditions. In summary, the results were variable and no clear pattern emerged. In some samples there were small increases in starch while in others the amounts appeared to decrease.

TABLE 3

EFFECTS ON STARCH SYNTHESIS AND SUCROSE CONTENT OF REMOVING FLORAL ORGANS FROM SPIKELETS CONNECTED TO THE RACHIS

Detached ears provided with water or 5% sucrose solution for 72 hr in the dark. L.S.D. for starch is 0·29 between means and 0·41 between six treatments, and for sucrose 0·051 between means and 0·089 between six treatments ($P = 0·05$)

Parts Removed	Culture	Change in Starch (mg/grain)	Final Sucrose Content (mg/grain)
None	Water	0·96	0·106
	Sucrose	3·97	0·641
	Mean	2·47	0·373
Glumes	Water	0·79	0·087
	Sucrose	3·49	0·662
	Mean	2·14	0·374
Glumes and paleas	Water	0·66	0·081
	Sucrose	2·87	0·446
	Mean	1·77	0·264

(*h*) *Effects of Removing Floral Organs from Spikelets Connected to the Rachis*

Initial samples were taken from one side of a set of detached ears in the usual way. The spikelets on the opposite side were left attached to the rachis and from some ears the glumes and paleas were removed. After culture for 72 hr on water or 5% sucrose solution the amounts of starch and the levels of sucrose in the grain were measured (Table 3). Whether cultured on water or sugar smaller amounts of starch are formed in ears stripped of floral organs. For ears provided with sucrose the difference of 0·48 mg/grain between untouched spikelets and spikelets with

glumes removed is significant. However, the removal of glumes alone from ears provided with sucrose does not cause a reduction in the amounts of sucrose extracted from the grain, while removal of both glumes and paleas significantly reduces the level of sucrose.

TABLE 4

INCREASES IN FRESH WEIGHT AND FINAL CONCENTRATION OF SUCROSE IN THE GRAIN

Data are for ears cultured on 5% sucrose solution and experimental details are given in Table 3

Parts Removed	Increase in Fresh Weight (mg/grain)	Final Fresh Weight (mg/grain)	Final Starch Content (mg/grain)	Water Content (by difference) (mg/grain)	Concentration of Sucrose (mg/ml of water in grain)
None	10·7	42·7	5·1	37·6	17·0
Glumes	11·7	43·2	5·1	38·1	17·4
Glumes and paleas	3·7	35·2	4·2	31·0	14·4

Removal of both organs results in less rapid growth (Table 4) and the smaller grains contain less water than those of the other two treatments. Consequently, with both organs removed the concentration of sucrose within the grain is only 15% lower than the value for untouched spikelets while the difference in starch between the same two treatments is about 30% (Table 3).

IV. DISCUSSION

The procedure used here to reduce random variability between ears by comparing opposite sides of the same ear affords a reasonably reproducible system for short term investigations on the influence of environment on starch synthesis in wheat grain. The chief disadvantage is that precise measurements can only be made during the early stages of development. Later, as the dry weight of the grain increases, the increments of starch for a given interval become progressively smaller relative to the total amount of starch.

Given an adequate supply of sucrose, variation in light intensity from complete darkness to 1500 f.c. has no effect on the rate of starch synthesis. In Figure 2 the small increase in starch from 700 to 1500 f.c. might be attributed to a difference in temperature. Hence, just as in leaves where starch is produced from sucrose supplied in darkness (see the references cited by Nurmia 1935), so in the wheat grain starch synthesis is not directly associated with the photosynthetic assimilation of carbon dioxide. However, in a few instances an apparent requirement for light has been demonstrated. Phillis and Mason (1937) floated disks cut from cotton leaves on solutions of sucrose and observed that starch was produced in weak light but not in the dark. Using tobacco leaves Porter (1953) found, inexplicably, that starch was produced in the dark in some experiments but not in others. Porter points out that the formation of starch in disks floating on sucrose solution is very slow compared with synthesis from carbon dioxide in the light, and suggests that movement of sugar across the disk might be limiting. In fact Phillis and Mason (*loc. cit.*) conclude that

their results are consistent with the view that the passage of sucrose across the disks is accelerated by light. Thus even where light has been shown to affect starch synthesis it may operate indirectly.

Compared with the magnitude of the differences in the external concentration of sucrose [see tabulation in Section III(*b*)], variation in the rate of starch synthesis is small. However, since the levels of sucrose within the grain were not determined no conclusions can be drawn on the relationship between the internal level of sucrose and the rate of starch synthesis.

For periods up to 4 days (Fig. 4) just as much starch is synthesized in detached ears provided with sucrose in the dark as in ears attached to plants illuminated continuously at 1500 f.c. No less important is the fact that the rate of synthesis in detached ears was never found to exceed that for ears growing in the light. From Figure 4 the average rate of synthesis for ears cultured continuously on sucrose is about 1·25 mg/grain/day. For cv. Gabo growing in the field, Jennings and Morton (1963) recorded a maximum value of 1–1·2 mg/grain/day. Taken together these observations suggest that, given an adequate supply of carbohydrate, the upper limit to the rate of synthesis is determined by factors operating within the ear itself. Moreover, the mechanism of synthesis does not appear to be impaired by temporarily withholding sucrose (Fig. 4), nor is there any appreciable breakdown of starch up to the fourth day on water.

In a discussion on the origin of the concentric shells in starch granules, Buttrose (1960) points out that the pattern reflects sudden discontinuous changes in crystalline structure rather than gradual variations, and considers that any variation in the supply of carbohydrate to the ear in the field is likely to be gradual rather than sudden, and such smooth fluctuations alone could not determine the observed shell structure. An observation from the present investigation supports the argument that carbohydrate supply is not the chief determinant of shell structure. Detached ears cultured on water in the dark produce in 24 hr almost as much starch as ears provided with sucrose (Fig. 4). Since the ears were cut from plants growing under a much lower light intensity than normal daylight, this finding implies that in the field sufficient reserves of carboyhdrate are accumulated in the ear to maintain synthesis at a maximum rate throughout the normal period of darkness.

Variation in temperature within the range normally encountered in the field markedly affects the production of starch [see tabulation, Section III(*d*); Fig. 3]. In the field diurnal fluctuations in temperature could therefore be expected to cause regular variations in the rate of starch synthesis. However, unless the temperature within the grain changes sharply it is difficult to understand how fluctuation in temperature could induce the formation of shell structure in the granules.

The only information that can be adduced to explain why little or no starch is produced in grain cultured in isolation is the fact that the grains did not take up sucrose (Table 2). This explanation, although less plausible, may hold for sliced grain, but here other factors may operate directly to inhibit synthesis. Of greater interest is the fact that the removal of floral organs restricts the uptake of sucrose (Table 3) and reduces the synthesis of starch (Tables 2 and 3).

To account for the effect of removing floral organs on the uptake of sucrose by the grain the following mechanism is assumed to operate. Most of the sucrose entering the cut rachis is transported to the ear in the transpiration stream. (Cutting the rachis may, for example, impair the ability of the phloem to translocate the sugar.) Most of the water taken up is lost through the floral organs and little from the enclosed grain. The sucrose accumulating within the floral organs is then transported to the grain in the normal way. Thus, removing the floral organs would give the observed result. However, in detached ears (Table 3) removing the glumes reduces the synthesis of starch without affecting the level of sucrose in the grain. And it is by no means certain that a reduction of 15% in the concentration of sucrose when both glumes and paleas are removed can wholly account for a 30% reduction in starch synthesis (Tables 3 and 4). Further investigations on the relationship between the concentration of sucrose in the grain and the rate of starch synthesis are in progress. Without this information it is not possible to assess reliably the inference that, in addition to carbohydrate, starch synthesis in the grain requires other factors produced by the floral organs.

V. Acknowledgments

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