THE EFFECT OF THE sh_2 FACTOR ON CARBOHYDRATE RESERVES IN THE MATURE ENDOSPERM OF MAIZE

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THERE are many mutants in maize which produce striking differences from normal in the texture, form and amount of the endosperm. Since an overwhelming proportion of the normal endosperm of maize is composed of reserve carbohydrate, principally starch, it might be anticipated that studies on the action of such mutant forms would have significance for the problem of the biosynthesis of carbohydrates. This is a report of some rather striking effects of one such mutant gene, shrunken-2 (sh_2), whose occurrence and linkage relations were reported by MAINS (1948). Mature endosperms having the constitution *sh sh sh* are highly collapsed, opaque and brittle and have a weight of 75 percent that of normal endosperms. Judging by appearance, the normal allele is completely dominant to sh_2, *Sh Sh sh* and *Sh sh sh* endosperms being indistinguishable from normal (*Sh Sh Sh*). Because of its close linkage with the a_1 factor, sh_2 has been employed as a technical aid in studies of certain of the alleles at the former locus. In the course of these investigations it was noted that the shrunken kernels are unusually sweet and have a pleasant, malty flavor. Biochemical studies have confirmed the former observation and indicate that the high concentration of sugars in these endosperms is due to sucrose primarily and is attained at the expense of polysaccharides.

MATERIALS AND METHODS

The gene su_1 is the basis for the " sugary " endosperm which is characteristic of our present commercial varieties and hybrids of sweet corn and of many strains of maize indigenous to Mexico, South America and our Southwest (STURTEVANT 1899). Previous studies (PEARL and BARTLETT 1911; LINDSTROM and GERHARDT 1926, 1927; ANDREW, BRINK and NEAL 1944) have established that mature sugary endosperms (*su su su*) have from two to three times as much total sugar as those of starchy (*Su Su Su*). In view of the higher sugar level associated with *su* and of an anticipated effect of shrunken-2 (hereafter designated shrunken or *sh*) on the distribution of carbohydrate reserves, the present study was carried out with progenies permitting a valid comparison of the effects of both factors. Accordingly, two different plants of commercial Golden Cross Bantam (*su su Sh Sh*) were crossed with a single plant of the constitution *Su Su sh sh*. The F_1 plants from these two ears were grown in two families in the summer of 1952 and self-pollinated. Since both normal alleles carried by the F_1 plants are dominant, four phenotypic classes

are expected in the F_2: starchy (*Su Sh*), sugary (*su Sh*), shrunken (*Su sh*) and sugary-shrunken (*su sh*); these are described in detail in a later section. Ears bearing these F_2 progenies were harvested and dried when the non-starchy endosperms had begun to show signs of collapse but were not yet hard.

Analyses of the various carbohydrate fractions were carried out on kernels from individual ears in groups of four samples representing the four phenotypic classes in the F_2. From one to five grams of entire kernels, the weight depending on the relative sugar content, were ground in a Wiley mill to pass a 50-mesh screen, weighed and dried to determine the percent of moisture. The dried sample was extracted for six hours in a Soxhlet apparatus with 80 percent ethanol to remove sugars. Reducing sugars and sucrose were determined by the method of HASSID (1936, 1937) according to which sucrose is estimated from the difference between reducing sugars preceding and following enzymatic hydrolysis with invertase. An analytical grade of the latter obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio, was employed. The error in duplicate titrations for reducing sugars and sucrose ranged from zero to two percent and a series of duplicate determinations involving original dried samples gave an average error of 3.9 percent.

The method of SUMNER and SOMERS (1944) was followed in the determination of water-soluble polysaccharides. The residue from the sugar extraction was dried and extracted repeatedly with aqueous, ten percent trichloracetic acid. The residue was recovered and the filtrate treated with two volumes of 95 percent ethanol after which the precipitated polysaccharides were collected, washed and dried prior to weighing.

Starch was determined by the procedure of BRIMHALL and HIXON (1945). The amount of starch is estimated as the difference between the weight of water extractables and the loss in weight of the original sample following the specified treatment with ammonium persulfate.

EXPERIMENTAL RESULTS

Mature endosperms of the four phenotypic classes among the progeny of self-pollinated *Su su Sh sh* plants are illustrated in figure 1. Shrunken endosperms (*Su sh*), shown in figure 1 C, are more collapsed than sugary (*su Sh*) endosperms (fig. 1 B), the axial and abaxial surfaces are invariably concave, and the sides and crown region generally have only one to several regions of deep depression. The prominent ridges which border the shrunken areas of these kernels are either straight or gently curved and their surfaces are smoothly rounded. They resemble starchy (*Su Sh*) kernels (fig. 1 A) in being relatively opaque. In contrast, sugary endosperms are translucent and may have only slight or no depressions of the axial and abaxial surfaces. The areas of collapse on the crown are more numerous than in the case of shrunken endosperms and are bounded by ridges which are angular in outline and not so smoothly rounded in cross-section. Sugary-shrunken kernels (*su sh*) representing the double recessive class (fig. 1 D) have some of the characteristics of both sugary and shrunken endosperms. Similar to that of the shrunken type, the sugary-

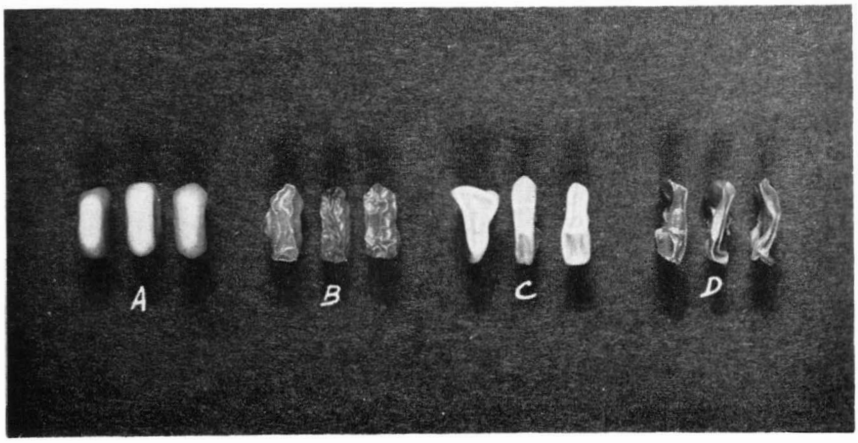

FIGURE 1.—Mature kernels of the four phenotypes among the progeny of selfed *Su su Sh sh* individuals. A, normal (*Su Sh*); B, sugary (*su Sh*); C, shrunken (*Su sh*); D, sugary-shrunken (*su sh*).

shrunken kernel has highly collapsed axial and abaxial surfaces and relatively few but prominent shrunken areas on the sides and crown. The translucent nature of endosperms of this class and the rather angular character of ridges which border the depressions are characteristic of the sugary type. The sugary-shrunken kernel is the most collapsed of the four types as is evident from the data on the relative dry weights of kernels in table 1.

Since *su* and *sh* are in different linkage groups the four phenotypic classes described above are expected to appear in the progenies of selfed heterozygotes in the familiar 9 : 3 : 3 : 1 ratio. Classification of grains representing the entire population of 22 ears bearing F_2 progenies gave 5324 starchy, 1705 sugary, 1551 shrunken and 559 sugary-shrunken individuals. Though these data are in only fair agreement with expected values, the frequency of the sugary-shrunken class is close to the theoretical value indicating that these endosperms are readily distinguishable from the other classes.

Of the four phenotypic classes employed in the chemical analyses reported below, only the sugary-shrunken endosperms are of identical genotype (*su su su sh sh sh*). Since *Su* and *Sh* are dominant, endosperms in the three remaining

TABLE 1

The quantities of various carbohydrates in entire kernels of the four phenotypes among the progeny of selfed Su su Sh sh individuals.

Phenotype	Mean dry weight per kernel	Reducing sugars	Sucrose	Total sugars	Water-soluble polysaccharides	Starch	Total carbohydrates
	grams	%	%	%	%	%	%
Su Sh	0.185	0.33 ± .04	1.44 ± .14	1.77	1.3	65.0 ± 1.5	68.0
su Sh	0.166	1.87 ± .13	2.66 ± .22	4.53	35.8	30.0 ± 2.2	70.3
Su sh	0.139	2.69 ± .21	16.13 ± 1.34	18.82	1.6	24.8 ± 0.9	45.2
su sh	0.113	4.00 ± .34	28.00 ± 1.38	32.00	1.8	7.7 ± 0.7	41.5

classes may have from one to three doses of the particular dominant factor involved. If there are differences in carbohydrate fractions, attributable to the accumulation of additional doses of *Su* and *Sh* beyond the first, they apparently are small in magnitude compared with the effect of the first dose of either of these alleles since phenotypic classes corresponding to these genotypes are absent in the F_2. Moreover, the coefficients of variability of mean values for reducing sugars, sucrose, water-soluble polysaccharides and starch are generally not less for the sugary-shrunken class in which the endosperms have identical

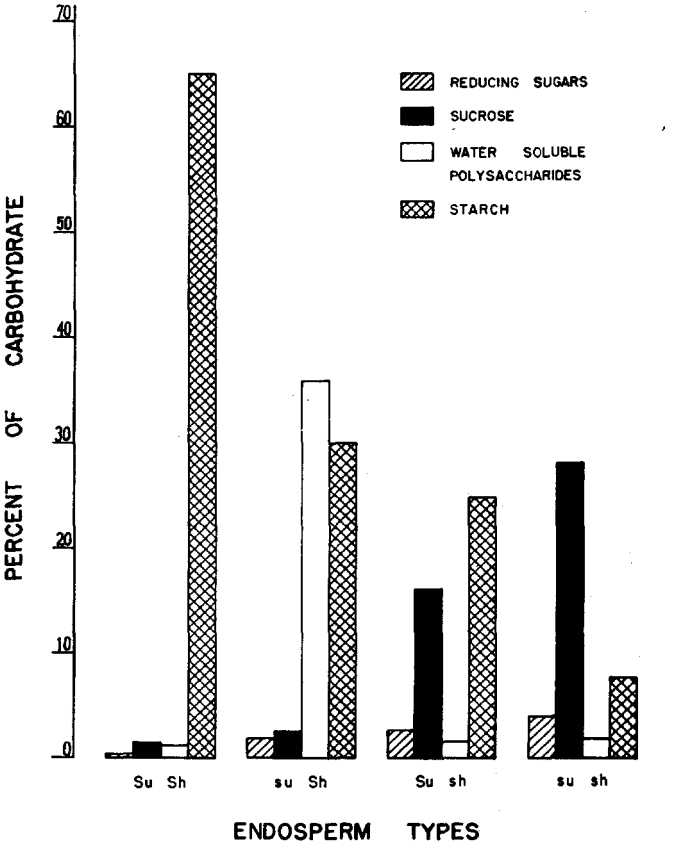

FIGURE 2.—The quantities of various carbohydrates in kernels of the four indicated phenotypes expressed as percentages of the dry weights.

genotypes, than for the other classes each of which is composed of endosperms representing several genotypes. Finally, the percentages of various carbohydrates reported here for sugary and starchy endosperms agree reasonably well with previously published values in spite of the fact that in this study these endosperms vary in the doses of *Sh* and of *Su* and *Sh* respectively.

In table 1 are presented the data on the quantities of the various carbohydrates in entire kernels of the four types, expressed as percentages of the dry weight. They are reproduced in the form of histograms in figure 2. It is apparent that the substitution of *su* for the dominant member, *Su,* is not associated

with a reduction in the amount of total carbohydrate if the latter is expressed in terms of percent of dry weight of the kernel. Even on the basis of total carbohydrate stored per kernel, which may represent a more valid criterion for the comparison of the effects of gene substitution, it is apparent, from a consideration of kernel weights presented in table 1, column 2, that the values for *su Sh* kernels are only slightly below those for the *Su Sh*, or normal class. However, the *su* gene is associated with striking changes in the amounts of the various classes of carbohydrates, an observation confirming that of previous investigators (PEARL and BARTLETT 1911; CULPEPPER and MAGOON 1924; LINDSTROM and GERHARDT 1926, 1927; BERNSTEIN 1943; ANDREW, BRINK and NEAL 1944). Compared with normal kernels, those of sugary show an increase of more than two-fold in total sugars. Most of this difference is accounted for by reducing sugars for which there is a five-fold increase in sugary kernels. The most striking characteristic of sugary kernels is their high content of water-soluble polysaccharides in which they exhibit a thirty-fold increase over *Su Sh* kernels. From figure 2 it is noted that the excessive amount of this carbohydrate in kernels of the *su Sh* class is produced at the expense of starch, which is the only measured carbohydrate in which normal kernels exceed those of the sugary class.

The high levels of sugars and soluble polysaccharides are important factors determining the quality of commercial sweet corn (CULPEPPER and MAGOON 1924, 1927). Following the initial report by MORRIS and MORRIS (1939) indicating that sugary endosperms contain a water-soluble carbohydrate which stains red with iodine and resembles animal glycogen, SUMNER and SOMERS (1944) confirmed this finding and established that the water-soluble polysaccharide fraction of sugary endosperms contains not only this glycogen-like polymer, which they named phytoglycogen, but another fraction which stains blue with iodine and which they called glycoamylose. They found that together these fractions constitute about thirty percent of the dry weight of the kernel, approximately two-thirds of this being phytoglycogen.

The names which were applied to the water-soluble polysaccharides by SUMNER and SOMERS suggest that these authors contemplated a similarity in structure between glycoamylose and amylose on the one hand, and between phytoglycogen and amylopectin on the other. Earlier, HASSID and MCCREADY (1941) had studied the corn glycogen of MORRIS and MORRIS, finding, by the method of end-group assay, that it has an average chain length or repeating unit of 12 glucose molecules. They concluded that it is similar to animal glycogen. In view of the staining properties of glycoamylose and phytoglycogen, and of the established similarity of the latter to animal glycogen, it is most reasonable to consider that phytoglycogen and glycoamylose represent, respectively, the branched and unbranched, water-soluble counterparts of amylopectin and amylose, an interpretation which is consistent with the terminology employed by SUMNER and SOMERS.

No attempt was made in the course of the present investigation to determine the relative amounts of phytoglycogen and glycoamylose in the soluble-polysaccharide fraction but it may be noted that CAMERON (1947), in a study of

the effects of the genes *su*^{am} (an allele of *su*$_1$) and *Du* (the normal allele of dull), made the interesting finding that changes in the relative amounts of amylose and amylopectin in starch, occasioned by varying doses of these factors, are accompanied by similar, but not quantitatively identical, changes in the amounts of glycoamylose and phytoglycogen in the water-soluble polysaccharide fraction. This observation places certain limitations on hypotheses relating to the mechanism of starch synthesis in maize and will be discussed later.

In considering the effects of the shrunken factor (table 1, row 3; fig. 2) it may be noted that on a percentile basis endosperms of the *Su sh* type have only two-thirds the normal amount of total carbohydrate. However, since these seeds also have a mean dry weight which is considerably below normal, the amount of carbohydrate stored per kernel is calculated as only half of that present in normal seeds. Moreover, the substitution of *sh* for its normal allele is accompanied by profound changes in the proportions of the various carbohydrate fractions. Almost 20 percent of the dry weight of shrunken kernels is represented by sugars, an increase of more than ten-fold over normal grains. Most of this increase is due to sucrose which alone accounts for approximately 16 percent of the dry weight of the shrunken kernel. This increase in sugar content is more than balanced by a decrease in starch. Calculations of the absolute amounts of starch stored by normal and shrunken kernels indicate that, in spite of the higher sugar content of the latter, the associated reduction in starch is of such magnitude as to lead to the expectation of a discrepancy, greater than that observed, in the dry weights of the kernels of these two types. Preliminary investigations, not reported in detail here, indicate that most of this discrepancy is due to an enhanced production of lipids in kernels of the shrunken type. The possible significance of this observation is discussed in a later section.

In contrast to sugary kernels, shrunken kernels store only a small amount (1.6 percent) of the water-soluble polysaccharides. There is no reason to believe that this is a significant increase over normal especially since measurements of this fraction were carried out on only two series of samples. Moreover, when allowance is made for the discrepancy between normal and shrunken kernels in respect to their dry weights, the amounts of this fraction stored per kernel are found to be nearly identical for these two types.

The trend favoring higher sugar concentrations and lower starch levels which accompanies the substitution of either *su* or *sh* for their corresponding normal alleles is accentuated in the double recessive class. Thus the amount of starch in *su sh* kernels (7.7 percent), when calculated on the basis of individual kernels, is only seven percent of that stored by kernels of the normal (*Su Sh*) class and one-fourth of that present in seeds of the shrunken (*Su sh*) class. Conversely, reducing sugars and sucrose are correspondingly increased in the sugary-shrunken class, having a mean value of almost one-third (32 percent) of the dry weight of these kernels. Among the seven ears for which sugars were determined in all four phenotypic classes, one yielded the highest values for both reducing sugars and sucrose in the *su sh* class, 5.5 and 33.4 percent,

respectively, suggesting that the selection of a strain having 40 percent of total sugars would not be difficult. As in the comparison of normal and shrunken classes, calculations of the absolute amounts of sugar and starch in normal (*Su Sh*) and sugary-shrunken (*su sh*) kernels lead, on the assumption that the amounts of other stored materials remain unchanged, to the expectancy of a greater-than-observed discrepancy between the weights of kernels of these two classes. The quantitative studies on lipids, to which reference has already been made, suggest that, in this case also, the greater-than-expected weight of *su sh* kernels may be explained largely in terms of their enhanced production of lipids.

Since sugary kernels accumulate a large bulk of water-soluble polysaccharide whereas shrunken seeds have only small amounts of this class of carbohydrate it is of particular interest to learn whether the double recessive type, in which both recessive factors block the synthesis of starch, exhibits an intermediate effect or resembles sugary or shrunken kernels in regard to this component. Again, if a correction for differences in dry weights is applied, the absolute amount of soluble polysaccharide in *su sh* kernels approaches the values for both normal (*Su Sh*) and shrunken (*Su sh*) individuals. Thus with respect to the content of this carbohydrate fraction, in which there resides the greatest difference between individuals of the sugary and shrunken classes, the doubly blocked recessive is similar to the latter. This evidence is discussed later in connection with sequence of steps controlled by the *Su* and *Sh* factors.

The total sugar contents of kernels of the shrunken and sugary-shrunken classes, figured on a percentage basis, exceed those of the sugary type by 4-fold and 7-fold increases, respectively. A number of instances have been reported in which the combination of the *su* gene with a second recessive factor produces an increase in total sugars. Thus, the presence of the factor for waxy (*wx*) in sugary endosperms causes an increase of about ten percent in total sugars (ANDREW, BRINK and NEAL 1944) though in this case it was considered that the higher values may have been due to greater amounts of dextrin extracted from the endosperms of the double recessive type. There is some evidence that kernels of the type designated supersugary, which are homozygous for *su* and for the gene dull (*du*), exceed sugary seeds (*su Du*) in sugar content (MANGELSDORF 1947) but there are conflicting data on this point (CAMERON 1947). Sugar concentrations approaching 14 percent have been reported for kernels which are homozygous recessive for the genes su_1 and su_x (HOROVITZ, MARCHIONI and FISHER 1941). By comparison, the shrunken (sh_2) gene is four times as effective in sugar production as any other single recessive factor and exceeds in this regard all of the known multiple recessive combinations involving *su*. The value of 32 percent sugar found for sugary-shrunken individuals is the highest ever reported for mature seeds of maize. In the discussion which follows, the high levels of sugar associated with the *sh* factor are attributed to the nearly complete blocking of a reaction which occurs relatively early in the synthesis of starch.

DISCUSSION

Although the data reported here are insufficient to indicate the exact nature of the steps controlled by the *Su* and *Sh* factors, they do permit reasonable inferences concerning the sequence of these steps. In accordance with the prevailing viewpoint concerning the relation between the mutated gene and the changed phenotype, it will be assumed that *Su* and *Sh* control specific biochemical steps involved in the biosynthesis of starch in maize endosperms, and that their known recessive forms are associated with complete or partial blocks of these reactions. Unfortunately, little is known concerning the nature and sequence of steps involved in the synthesis of starch in maize, and no case is known for maize in which a particular gene is identified with a known step in starch synthesis. Thus for the present it must suffice to arrive at some general idea concerning the types and times of reactions which are controlled by the *Su* and *Sh* genes. In the absence of more direct evidence some information may be gained by a consideration of the metabolites which accumulate as a result of the genetically blocked reaction in the mutant individual. It may be assumed that sugars represent the ultimate precursors of starch and that the soluble polysaccharides are intermediate substrates in the synthesis. It will be remembered that *su* kernels are higher than normal in all measured carbohydrate fractions except starch. Moreover, as has long been recognized (SALISBURY 1849, table 57; PEARL and BARTLETT 1912), sugary seeds have a higher than normal content of lipids. Calculations based on the data of PEARL and BARTLETT and those of LINDSTROM and GERHARDT (1926, 1927) suggest that this increase is of the order of 50 percent. Significantly, this increase in lipids occurs primarily in the endosperms rather than in the embryos of sugary seeds (LINDSTROM and GERHARDT 1926, 1927; JUMP 1951; LAUGHNAN, unpublished) from which it appears that the effect may be attributed to the higher level in sugary endosperms of those carbohydrates which are precursors of lipid and which accumulate as a result of the genetic block. The most revealing clue concerning the nature of the block interposed by *su* is provided by the water-soluble polysaccharides for, among the carbohydrates which are present in sugary endosperms in greater than normal concentration, these show the greatest increase and are the most complex and the most closely related to starch itself. Their presence in low concentration in normal endosperms, suggests that they are normal precursors of starch, and their occurrence in sugary endosperms in excessive amounts indicates that the normal *Su* gene acts after the step or steps which fashion these lower polymers. On the simplest scheme, one which allows several alternative interpretations, the normal *Su* gene controls a step or steps in the formation of starch from these short-chain, glucose polymers. This might be accomplished by direct condensations between these lower polysaccharides, through their attachment to higher polymers or via the phosphorylase system which would involve the lengthening of these chains by the successive additions of individual glucose residues. Further discussion of these alternatives is postponed to take account of additional evidence which has some significance for *Su* action.

In a study of the distribution of carbohydrates in endosperms carrying all possible combinations of the factors su^{am} (an intermediate allele of su_1), *Du* and their recessive alleles, CAMERON (1947) found that sugars and water-soluble polysaccharides are decreased and starch is increased with increasing doses of su^{am} and *Du*. There is a tendency for the percent of amylopectin in the starch fraction to rise with doses of either of these factors, in which regard *Du* is more effective than su^{am}, although doses of the latter occasion greater increases in percent of starch. As noted previously, CAMERON found that among the various genotypes the ratio of glycoamylose to phytoglycogen (components of the water-soluble fraction) varies as do the proportions of amylose and amylopectin in the starch fraction. He has proposed the interesting hypothesis that *Du* increases the amount or activity of an enzyme responsible for branching of glucose polymers, thus accounting for the increase in total starch and in the amylopectin : amylose ratio with which it is associated. According to CAMERON the effect of su^{am} is to originate short glucose chains thus accounting for the increased starch observed in endosperms carrying su^{am}. The higher ratio of amylopectin : amylose in such endosperms is explained as due to the preference of the branching enzyme for relatively short chains in the starch fraction, a higher proportion of which are expected in the presence of su^{am}. Thus in the *su du* type fewer short chains are available than in su^{am} *du* and greater numbers of these are lengthened beyond the point where they will become branched. While this would account for the higher proportion of amylopectin in the starch of su^{am} *du* endosperms as compared with the *su du* type, it would not explain the corresponding increase in the phytoglycogen (branched) fraction of the soluble polysaccharides since chains of glycoamylose in this fraction would not be expected to exceed a length beyond the threshold for selective action by the branching enzyme. Furthermore, if su^{am} and the dominant member of the series, *Su*, are concerned with the origination of short glucose chains the accumulation of lower glucose polymers would not be expected in the absence of these factors; the data indicate that the accumulation of large reserves of water-soluble polysaccharides is the dominant feature of *su* endosperms, suggesting that the biochemical block in starch synthesis occasioned by *su* occurs after, rather than prior to, the formation of short glucose chains.

On the argument that the *Su* gene controls a step in the synthesis of starch from the lower, water-soluble polymers, it is still necessary to account for the similarity in the ratio of straight-chain to branched polymers in the water-soluble polysaccharides and in the starch fraction of sugary kernels. It is possible that *Su* is in control of a transglucosidase similar to that reported by HAWORTH, PEAT and BOURNE (1944) and by PEAT, BOURNE and BARKER (1948). This enzyme, which does not require the presence of inorganic phosphate, was isolated from potatoes and is reported to break α-1,4-glucose bonds of polymers and attach the reducing end to other polymers through an α-1,6 bond, thus accounting for the branching of such chains. If such an enzyme were responsible for rupturing amylose and amylopectin starch chains and the *su*

gene were associated with its failure to accomplish the α-1,6 attachments, the excessive accumulation of water-soluble polysaccharides in sugary endosperms would be explained. If moreover, this enzyme were responsible for the rupture of amylose and amylopectin chains indiscriminately, the proportions of straight- and branched-chain soluble polysaccharides would be expected to approximate those of amylose and amylopectin in the starch fraction of sugary kernels. The evidence might also be satisfied if, as suggested by BERNFELD (1951), there is a non-enzymatic breaking of glucosan chains when they have reached a certain length, thus providing the source of excessive amounts of water-soluble polysaccharides in sugary endosperms. On this scheme the *Su* gene might be considered in control of phosphorylase (CORI and CORI 1936; CORI, SCHMIDT and CORI 1939; HANES 1940) which is responsible for the lengthening of glucose chains through the successive addition of single glucose residues. An impairment of this function in the absence of *Su* would then account for the accumulation of excessive amounts of the lower polymers in sugary endosperms. Obviously, tests of these and other schemes must await more detailed evidence on the nature of reactions involved in starch synthesis in the maize endosperms.

Concerning the role of *Sh* in starch synthesis, the evidence favors the view that this gene acts prior to *Su*. Thus in shrunken endosperms, in which the *sh* factor may be presumed to represent a partial block of the reaction controlled by *Sh*, not only is there less starch stored than in sugary endosperms but there is no striking accumulation of water-soluble glucose polymers, a situation which is expected if *sh* blocks a reaction occurring prior to the formation of these lower polysaccharides. Additional support for this argument comes from a consideration of the carbohydrate reserves of sugary-shrunken individuals. Starch synthesis in such endosperms is doubly blocked since they are homozygous recessive for both *su* and *sh*. If the normal *Su* gene were in control of a reaction which is prior to that governed by *Sh*, the doubly blocked sugary-shrunken endosperms would be expected to accumulate large amounts of soluble polysaccharides just as do sugary endosperms. However, since *su sh* individuals are similar to normal, and differ strikingly from sugary kernels, in regard to the amount of this component it may be concluded that the *Sh* gene acts prior to *Su* in synthesis.

According to LOOMIS (1945) there is in maize a strongly polarized movement of sugars into the ear shortly after pollination; the great bulk of these sugars is moved in the form of sucrose. Sucrose then must be considered the primary source of carbohydrate available for starch synthesis in maize endosperm. This fact suggests that abnormally high levels of sucrose in mature endosperms of genetically blocked individuals are to be interpreted, not in terms of their enhanced synthesis of sucrose, but rather as the failure of this initial metabolite to be utilized in synthesis at the normal rate. Thus the block conditioned by *su* permits the accumulation in greater than normal amounts not only of the soluble polymers but of reducing sugars and sucrose also. The earlier block conditioned by *sh* is not associated with the accumulation of large

amounts of soluble polysaccharide but instead is accompanied by a sharp increase in sugar content, principally sucrose. The increase in fat content of sugary endosperms is readily explained on this basis as due to the higher level in this tissue of sugars which are the presumed ultimate precursors of lipid. With certain reservations, it would be predicted that shrunken endosperms, because of the advantage they have in sugar content, exceed both normal and sugary types in lipid content and, by the same argument, that *su sh* endosperms have the highest lipid content of all four types studied here. Preliminary studies bear out these predictions, indicating that while the percentages of lipid in the embryos of the four types of kernels are nearly identical, the endosperms of shrunken (*Su sh*) and of sugary-shrunken (*su sh*) kernels show 9-fold and 14-fold increases in lipid compared with those of normal (*Su Sh*), and 4-fold and 6.5-fold increases compared with those of sugary (*su Sh*) endosperms, respectively. These results may be taken as confirmatory evidence that the reaction controlled by *Sh* precedes the step controlled by *Su* and suggest that the precursors of lipid are drawn from the pool of relatively simple carbohydrates which, in these genetically blocked tissues, are present in excess. They suggest also that the shrunken gene, though it blocks a reaction in starch synthesis at a relatively early stage, does not block at a point involving substrates which are common precursors of lipid and starch but impairs the synthesis of the latter at some later point. It might be expected that qualitative studies of the carbohydrates in such genetically blocked tissues would aid in establishing the identity of carbohydrates which are immediate precursors of lipid; they might at least eliminate certain carbohydrates as having such a role.

The data presented here do not permit arguments concerning the specific action of *Sh*. It may be concerned with any one of a number of steps between sucrose and the soluble glucose polymers. Furthermore, it may not be assumed, a priori, that starch synthesis in maize proceeds solely or even predominantly through the phosphorylase mechanism which involves the successive attachment of individual glucose residues to preexisting polymers. Several cases are known in bacteria (HEHRE and SUGG 1942; HESTRIN and AVINERI-SHAPIRO 1944; HEHRE 1949; TORRIANI and MONOD 1949) in which nonphosphorolytic transglucosidases bring about a direct, in vitro synthesis of polysaccharides from disaccharides. In contrast to the action of the phosphorylases, which utilize hexose phosphate ester as their substrate, these enzyme systems split the glycosidic bond of disaccharides which contain glucose, synthesizing polysaccharide directly from the latter and freeing the other hexose residue of the disaccharide. Since in several of these cases sucrose is the known substrate for such syntheses the possibility cannot be excluded that such a pathway for starch synthesis exists in maize, even though its counterpart has so far not been discovered in higher plants. If a mechanism of this type is responsible for a significant amount of starch synthesis in maize, it might be anticipated that the genetic block which the *sh* gene interposes resides in this pathway and thus accounts for the low starch level and high concentrations of sugars and lipids in shrunken endosperms; on this scheme corresponding reductions in

starch would be expected in leaf tissue of *sh sh* plants. However, if synthesis of starch from sucrose by this direct pathway is absent or, in comparison with the phosphorylase system, relatively unimportant in maize, the ease with which sucrose is synthesized or degraded in leaf tissue may have little significance so far as starch synthesis is concerned. Thus, though the shrunken gene may cause a serious reduction in starch in shrunken endosperms by blocking in some way the degradation of sucrose, it may be found that the green tissues of the corresponding " shrunken " plants are quite normal in starch production.

Although our present understanding of the steps in carbohydrate synthesis in plants and of the relation between such steps and known genetic blocks does not yet permit the kind of precise analyses and inferences which, in Neurospora, have been so rewarding for the physiologist, there is reason to believe that maize may be amenable to such studies. Thus, the finding of LA RUE (1949) that maize endosperm may be grown in culture should facilitate studies on the effects of various added substrates with the view to determining the nature and sequence of steps involved in the synthesis of carbohydrates and lipids, and identifying known mutants with these steps. There are those who feel, with some justification, that the extension of this sort of evidence to increasing numbers of steps has the significance for the geneticist only of confirming what has long been suspected, namely, that genes control reactions, without adding a great deal to an understanding of the fundamental problem of how the variety of mutant forms of a particular gene are related to the various degrees of potency of the reaction, or to the nature of the enzymes, which they control. However, the assignment of steps to specific genes is a requirement which must be satisfied before this latter type of analysis may be undertaken. Considered from this standpoint maize offers many advantages. The fact that gene-conditioned blocks in carbohydrate synthesis often are manifested as gross changes in the morphology of the maize endosperm permits easy isolation of new mutant forms with a high probability that they effect this synthesis. From the numbers of these already available it is suggested that carbohydrate metabolism in maize endosperm is relatively complex, thus offering a variety of steps for detailed genetic analysis. Moreover, it appears that sugars, especially sucrose, are sensitive indicators of blocked starch synthesis. This is true for the mutants dealt with here, and preliminary studies indicate that this relation holds also for the shrunken-1 and brittle-1 factors. Finally the feasibility of growing maize endosperms in artificial culture makes possible a convenient and continuous source of enzymes which should prove to be an advantage in the analysis of mutant forms at specific loci.

Because of the high concentration of sugars with which it is associated, the sh_2 factor may have an application in the sweet corn industry, especially if it is found that shrunken endosperms, at the stage when corn is harvested for table use or processing have, in comparison with *su* strains, an advantage in sugar content which is of the same order as that observed for mature endosperms. The decline in quality which occurs when our present sugary strains are held too long after picking is usually attributed to loss of sugars due to their con-

version into glucose polymers. If shrunken strains are not undesirable from other standpoints, it would be anticipated that their use in the sweet-corn program would have an advantage in extending the period during which the product could be held after picking since shrunken endosperms carry a genetic block which reduces greatly this conversion of sugars. However, if it is legitimate to argue from the data on mature kernels, the shrunken strains are expected to have a serious disadvantage. They have at maturity only a trace of water-soluble polysaccharides, a component which is considered an important factor contributing to the texture of our present sugary strains. While this effect may be mitigated through the use of other genetic factors in combination with sh_2, the relatively early block in starch synthesis effected by the shrunken gene suggests that a search for such factors would be an unrewarding one.

SUMMARY

1. Quantitative determinations were made of reducing sugars, sucrose, water-soluble polysaccharides and starch in mature endosperms of normal (*Su Sh*), sugary (su_1 *Sh*), shrunken (*Su* sh_2) and sugary-shrunken (su_1 sh_2) maize. Kernels having these phenotypes were obtained from progenies of selfed *Su su Sh sh* individuals.

2. While normal kernels store carbohydrate predominantly in the form of starch, the substitution of *su* for its normal allele results in a decrease in the starch fraction and a two- to three-fold increase in total sugars. Water-soluble polysaccharides constitute over one-third of the dry weight of sugary (*su Sh*) kernels whereas normal grains have only one percent of this component. These observations are in agreement with those of earlier studies.

3. Shrunken (*Su sh*) endosperms store less starch than normal and sugary endosperms but, in regard to total sugars, exhibit approximately ten-fold and four-fold increases over normal and sugary types, respectively; most of this increase is due to sucrose. The amounts of water-soluble polysaccharides in shrunken kernels are low and do not differ significantly from those in normal grains.

4. The trend favoring lower starch levels and higher sugar concentrations, which accompanies substitution of either *su* or *sh* for their normal alleles, is accentuated in the case of *su sh* kernels, which contain less than ten percent of the normal amount of starch and have a sugar content of almost one-third of their dry weight. Again, most of this sugar is sucrose. The sugary-shrunken kernels store small amounts of water-soluble polysaccharides and are similar in this respect to normal and shrunken grains.

5. The higher than normal fat content of sugary, shrunken and sugary-shrunken endosperms is attributed to the accumulation, in greater than normal amounts, of carbohydrates which are precursors of lipid.

6. Since sugary endosperms accumulate large reserves of water-soluble polysaccharides it is considered that the *Su* gene acts after the steps which fashion these lower polymers and probably controls a step in the formation of starch from them.

7. It is suggested that the *Sh* factor acts prior to *Su* in starch synthesis since *Su sh* and *su sh* endosperms, in contrast to the sugary type, accumulate only normal amounts of soluble polysaccharides; their higher content of reducing sugars, sucrose and lipids also favors this interpretation. The *Sh* gene may control a step in the degradation of sucrose but, on the basis of an alternative pathway for starch synthesis known in certain bacteria, it could be involved in the direct synthesis of glucose polymers from sucrose.

8. The high level of lipids in shrunken and sugary-shrunken endosperms suggests that the *sh* gene blocks starch synthesis at some point not involving substrates which are common precursors of lipid and starch.

9. The possibility that sh_2 may have some application in the sweet corn industry is discussed.

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