The Effects of Fertilization and Growth-regulating Substances (Hormones) on Carbohydrate and Hexose-phosphate Metabolism During the Early Stages of Growth of Corn Kernels (Fruit)[1]

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It has been shown by Murneek (*1*) and Marrè and Murneek (*2*) that the hormones produced in the seed after fertilization have an important role in regulating the movement of carbohydrates and nitrogen toward the developing fruit. Similarly, externally applied growth regulators (hormones) stimulate the accumulation of sugars and the formation of starch reserves in the young tissues of the flower and of the fruit (*2, 3*).

The purpose of these investigations was to establish a relationship between fertilization and hormone treatment with regard to their effects on carbohydrates and phosphate esters in young kernels (fruits) of corn, a plant in which parthenocarpy can be induced by the treatment with growth-regulating substances (*4*).

Ears of an inbred line of sweet corn, grown at 25° C in a well-lighted, thermoregulated greenhouse, were treated, after partial removal of the husks, as follows: (*a*) the silks were stripped and the kernels sprayed with an emulsion of water and lanolin without hormone—not pollinated controls; (*b*) the silks were pollinated with fresh pollen; (*c*) the silks were stripped and the ears sprayed with a hormone-lanolin emulsion.

The hormones used were the ethyl ester of indoleacetic acid (EtIA) and naphthaleneacetic acid (NA). Both substances were used at a concentration of 1000 ppm, which appeared effective in promoting the parthenocarpic development of the kernels. The ears were protected by a small bag from undesired pollen and from excessive evaporation. Treatments were performed on ears bearing silks ripe for pollination on different days in order to permit a simultaneous collection of material 0, 1, 3, 5, and 7 days after the treatment.

The chemical determinations were made on plant material from at least 6 ears; kernels from the lower third of the ear were used. Starch, sucrose, and reducing sugars were assayed as in a previous work (*2*). The hexose phosphates were determined according to procedure B of Umbreit *et al.* (*5*). Three precipitations with barium and alcohol removed practically all reducing substances other than the hexose phosphates. Significant losses in this procedure were observed only for glucose-1-phosphate, data on which therefore are not reported. The other esters, after purification and fractionation, were determined from their reducing values by the Somogyi reagent as used by Cori (*6*), and with the Roe method (*6*).

A. *Carbohydrates in the kernels.* No significant changes occurred in the carbohydrate content in the not pollinated, lanolin-treated kernels (Fig. 1). On the contrary, in both the hormone treated and the pollinated-fertilized ones marked changes were induced in sucrose, starch, and reducing sugar concentrations. This reaction was qualitatively similar in cases of pollination, EtIA, and NA treatments and consisted of: (*a*) a distinct increase in starch content from a very low initial amount; (*b*) in a decrease,

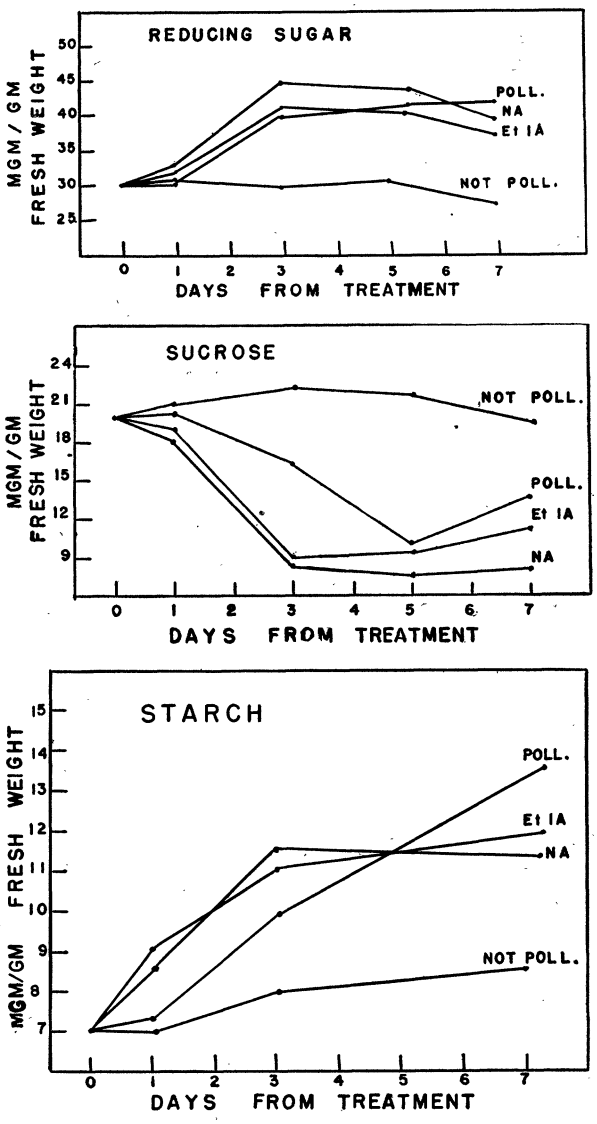

FIG. 1. Effect of fertilization and of hormone treatment on the carbohydrate content of corn kernels. Poll., pollinated; EtIA, treated with ethyl ester of indoleacetic acid; NA, treated with naphthaleneacetic acid; Not Poll., not pollinated, treated with lanolin.

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chiefly during the first 3 days after treatment, of sucrose; and (*c*) in an increase of reducing sugar concentration.

This reaction is very similar to the one previously observed in tomato ovaries (*2*). It appears closely linked to changes in the enzymatic equilibria concomitant with an increase in growth of the fruit.

B. *Carbohydrates in the cob.* The carbohydrate changes in the cob (Fig. 2) followed the same trend as those in the kernels. Here again an increase of starch and reducing sugars and a decrease of sucrose were observed. This similarity of behavior indicates that the action of the hormones (natural or artificially applied) on the carbohydrate metabolism is not confined to the seed- and fruit-forming organs but may have analogous effects on neighboring tissues.

C. *Hexose phosphates in the kernels.* The changes in the hexose phosphates in kernels after the different

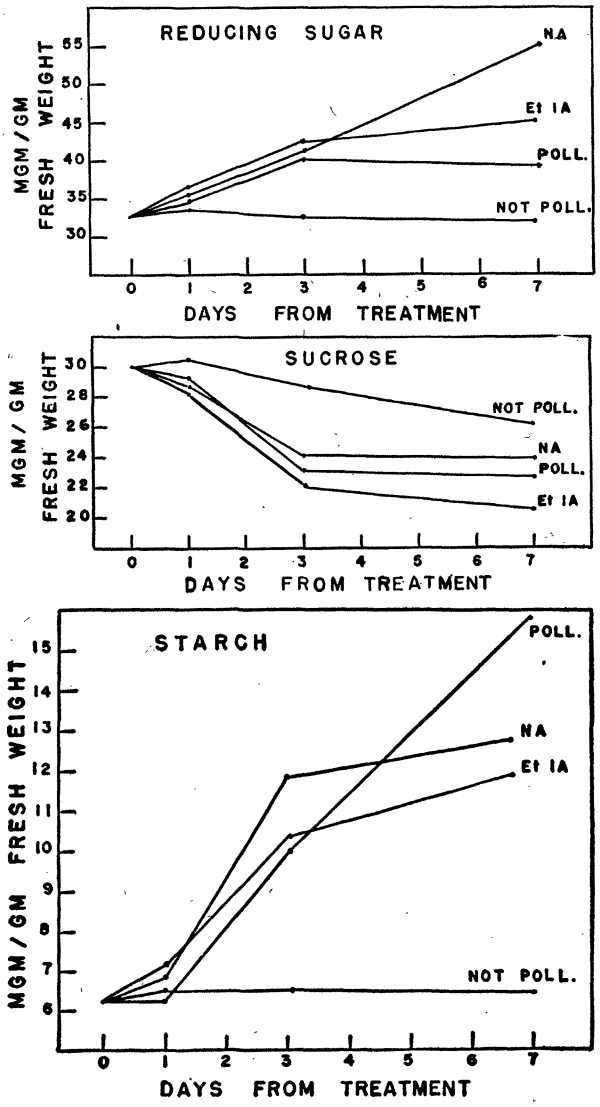

FIG. 2. Effect of fertilization and of hormone treatment on the carbohydrate content in corn cobs.

TABLE 1

CONCENTRATION OF THE HEXOSE PHOSPHATES IN FERTILIZED, HORMONE-TREATED, AND NOT POLLINATED CORN KERNELS, 3 AND 7 DAYS AFTER TREATMENT

(In γ/g fresh weight)

	3 days after treatment	
	Fructose-6-P and Glucose-6-P (determined together)	Fructose-1-6-P
Pollinated	475	48
EtIA	503	51
NA	515	54
Not pollinated	425	41

	7 days after treatment		
	Fructose-6-P	Glucose-6-P	Fructose-1-6-P
Pollinated	158	375	48
EtIA	187	405	52
NA	193	395	54
Not pollinated	117	305	35

treatments seem worthy of some attention, though the behavior of these compounds in relation to growth is, as yet, very little known. The data in Table 1 show that fertilization, as well as the treatment with growth regulators, induced a moderate but significant increase of all considered hexose phosphates. The interest in these data lies in the fact that they may indicate a relationship between growth and the rate of phosphorylation of the carbohydrates. It seems reasonable to assume that in the actively growing ovary, the growth resulting from either hormone treatment or fertilization, the hexose phosphates should be utilized at a higher rate than in a resting one. Notwithstanding this most probable situation, the hexose phosphate concentration actually increased in the growing ovary, thus showing that the production of these compounds was, under these circumstances, larger than their utilization. This fact suggests a significant increase in the rate of phosphorylation of the hexoses. Such a condition could result from two different mechanisms: (*a*) from a hormone-induced activation of respiratory enzymes (*7*) with a consequent increase in the production of high-energy P-bonds; (*b*) from the activation of the P-transferring mechanisms, as of the enzymes of the hexokinase type. It should be considered that in the second consideration (*b*) the rate of respiration should increase, as a result of the faster turnover of ATP (or similar compounds) and therefore of the greater availability of acceptors of high-energy phosphorus from the respiratory reactions (*8*). It is known that both fertilization and auxin treatment are usually followed by an increase in respiration (*9*).

In an attempt to correlate the behavior of the hexose phosphate with that of the soluble carbohy-

drates, a relation seems probable between the stimulation in synthesis of starch and the higher level of the phosphorylated sugars in the growing kernels. There is every reason to believe that in corn kernels, as in other plant structures, the enzyme primarily concerned with starch synthesis is phosphorylase, acting on glucose-1-phosphate. As there is some evidence of the common occurrence in plants of the enzymes necessary for the interconversion of the different hexose phosphates, a greater activity of phosphorylase should naturally be considered favorable for starch synthesis.

The close analogy between the effects on carbohydrate metabolism of the hormones naturally released after fertilization and those of the growth regulators artificially applied should be emphasized. This analogy suggests a corresponding similarity in regard to metabolism. The fact that the response following pollination is always somewhat delayed, compared to the response following hormone treatment, seems to indicate that fertilization, which in the corn follows pollination in about 24 hr, rather than pollination, is the process responsible for the maximum production of metabolically active hormones (*10*).

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Rate of Turnover of Epinephrine in the Adrenal Medulla

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Experiments designed to determine the precursors and intermediates involved in the biosynthesis of epinephrine have yielded the observation that the rate of formation and the normal rate of secretion of adrenal epinephrine are extremely slow. C^{14}-labeled phenylalanine or tryosine administered orally or intraperitoneally to rats or rabbits was incorporated into adrenal epinephrine much more slowly than into the tyrosine of plasma protein (Table 1). The resulting radioactive adrenal epinephrine disappeared slowly after the administration of the labeled amino acid was discontinued; the half-life in rats was about 9 days. Estimates of half-life were based upon measurements of the specific activity of adrenal epinephrine[3] in individual rats which were sacrificed under Evipal anesthesia at various time intervals after discontinuing administration of C^{14}-phenylalanine (Fig. 1). In three additional experiments adrenal glands were compared in the same rats, the glands being removed one at a time several days apart (Table 2).

TABLE 1

INCORPORATION OF C^{14} FROM PHENYLALANINE AND TYROSINE INTO PLASMA PROTEIN TYROSINE AND ADRENAL EPINEPHRINE OF RATS

Expt.	C^{14}-amino acid administered*	Days	Time after last dose	Specific activity (cpm/μmole)		
				Free plasma tyrosine	Plasma protein tyrosine	Adrenal epinephrine
1	Phenylalanine	1	7 hr	80	34	<5
2	Tyrosine	1	7 "	110	46	<5
3	Tyrosine	6	24 "	700	—	280
4	Phenylalanine	6	24 "	930	340	180
5	Tyrosine	6	24 "	600	450	310
6	Phenylalanine	12	20 "	—	—	980
7	Phenylalanine	12	12 days	150	610	420

* 3-C^{14}-D-L-phenylalanine (2.6×10^5 cpm/μmole) or 2-C^{14}-D-L-tyrosine (2.0×10^5 cpm/μmole) were administered in doses of 1 mg/day for the number of days indicated.

In an attempt to stimulate the synthesis of epinephrine, the adrenal glands of rabbits were depleted of epinephrine by the subcutaneous administration of insulin.[4] Epinephrine was determined in the adrenals of individual rabbits sacrificed at various time intervals following insulin administration. The chemical method used was a modification of the fluorimetric procedure of Lund (*1*), which can measure epinephrine in the presence of nor-epinephrine and other catecholamines. It was found that about half of the epinephrine which had disappeared from the adrenal glands after insulin administration was restored in 72 hr (Table 3). No measurable quantities of nor-

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[3] A portion of an adrenal extract was assayed for epinephrine by the procedure of Lund (*1*). To the rest of the extract a measured quantity of nonisotopic l-epinephrine (about 10 mg) was added as a carrier. The carrier was recrystallized repeatedly until isotopic homogeneity was achieved. In a number of cases the recrystallized carrier was converted to a derivative, iodoadrenochrome (*2*), with no change in isotopic composition. The quantity of epinephrine in the adrenal extract (m), the quantity of added carrier (M), and the specific activity of the isolated carrier (C_c) permit calculation of the specific activity of the adrenal epinephrine (C_a) according to the equation $C_a = C_c M/m$, derived from isotope dilution principles.

[4] After insulin administration the blood sugar levels fell to negligible values in 2–3 hr and returned to normal by 8 hr.