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Movement of ^{14}C-Labeled Assimilates into Kernels of *Zea mays* L.

I. PATTERN AND RATE OF SUGAR MOVEMENT[1]

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ABSTRACT

Carbon-14, photosynthetically fixed in leaves of *Zea mays* L. and translocated to developing kernels, passed through specialized basal endosperm cells prior to movement into the starchy endosperm and embryo. Radioactivity migrated in the endosperm at a maximum rate of 2.7 millimeters per hour, and there was no difference in the rate of movement in kernels treated 14 to 30 days after pollination.

Sucrose contained over three-fourths of the radioactivity in the kernal base (fruit stalk) 1 to 6 hours after $^{14}CO_2$ treatment of the plant. Conversely, in the basal endosperm three-fourths of the radioactivity was in glucose and fructose. A high proportion of the radioactivity was retained in the monosaccharides of the starchy endosperm the first 3 hours after the $^{14}CO_2$ treatment. With additional time after treatment there was a decline in the percentage of radioactivity in the monosaccharides and an increase in sucrose-^{14}C. From these data we suggest that translocated sucrose is cleaved to glucose and fructose during entry into the endosperm and that the monosaccharides diffuse throughout the endosperm. Once the sugars arrive in the cells active in starch synthesis, they are rapidly converted to sucrose which in turn is utilized in the synthesis of starch.

We have previously reported the distribution of radioactivity in kernel carbohydrates at various times after treatment of intact *Zea mays* plants with $^{14}CO_2$ (14, 15). In order to interpret best such *in vivo* studies we need to know the rate and pattern of sugar movement into and throughout the kernel. The rate of movement of photosynthetically fixed carbon from maize leaves to developing kernels has been reported (3). Earlier I showed (14) that sucrose was cleaved to glucose and fructose prior to or during its movement from the terminal phloem elements into the endosperm and that radioactive sucrose was rapidly synthesized in the endosperm. In this paper I report the results of studies designed to characterize more completely the movement of sugars from the base of the kernel throughout the endosperm.

MATERIALS AND METHODS

Administration of $^{14}CO_2$ and Sampling. *Zea mays* L. plants (single cross hybrid, H49 × B37) with ears 14 to 41 days after pollination were used. Plants were field or greenhouse grown. The ear leaves of all plants, except where noted, were exposed to 250 to 400 μc of $^{14}CO_2$ for 30 min as described previously (14). The ear leaves of the plants studied 16 and 18 days after pollination were damaged at the time of pollination, therefore the first leaf above the ear was exposed to $^{14}CO_2$ as above. The ears, when enclosed within the treatment bag, were covered with aluminum foil to inhibit any fixation of $^{14}CO_2$ by the ear husks. One-sixth of each ear was collected at various times after beginning the treatment as described previously (14). The ear pieces were rapidly frozen by either dropping them into liquid nitrogen or Dry Ice. The samples were then freeze-dried. In order to reduce collapse of the kernels during drying, cuts were made through the pericarp at the top or sides of the kernels prior to freezing.

Preparation of Kernel Sections for Radioautography. Six freeze-dried kernels from a plant treated 21 days after pollination were carefully removed from the cob and placed in 60 C paraffin (Paraplast,[3] Scientific Products, Evanston, Ill.). The kernels were vacuum infiltrated for 15 hr at 60 C after which time the original paraffin was replaced with fresh paraffin. Vacuum infiltration was continued for 6 hr prior to embedding in fresh paraffin. The blocks containing the kernels were warmed to 37 C and sliced into sections approximately 0.8 mm thick. Freehand sections were cut with a razor blade using a slicing guide. The embedded sections were arranged on mount paper and placed briefly on a warm plate to melt enough paraffin to fix the sections to the paper. To determine the rate of ^{14}C movement in the kernels, 16 freeze-dried kernels from each sampling time were cut in half along the central axis perpendicular to the face of the kernels. One-half of each kernel was mounted with rubber cement to a sheet of foam plastic. Kodak No-Screen or Kodak Royal Blue x-ray film was placed over the mounts and left for 1 week exposure prior to development by standard procedures. Portions of the x-ray film adjacent to the radioactive areas of the kernel pieces were darkened. By carefully comparing the radioautograph

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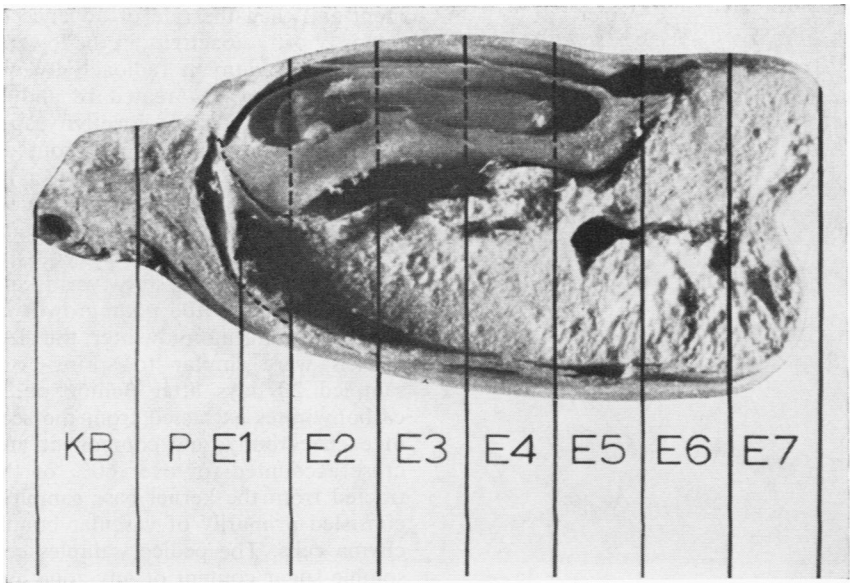

FIG. 1. Longitudinal section through a freeze-dried corn kernel 30 days after pollination showing the zones of subdivision. KB: kernel base (fruit stalk); P: pedicel (maternal tissue in which the pholem terminates); E1: basal endosperm; E2 to E7; starchy endosperm zones.

with the kernel mount it was possible to determine the position on the radioautograph corresponding to the base of the endosperm. Measurements were then made from this point to the uppermost endosperm region having sufficient radioactivity to darken the x-ray film. All measurements were made with a dissecting microscope and a stage-mounted micrometer.

Subdivision of Kernels. Twenty freeze-dried kernels from a plant treated 30 days after pollination were cut in half along the central axis perpendicular to the face of the kernel. One-half of each kernel was used for radioautography, and the remaining kernel-halves were mounted in paraffin and cut into nine zones. Use of the paraffin mount made it easier to hold the kernels and reduced endosperm crumbling during cutting. The kernels were subdivided (as shown in Fig. 1) into the kernel base (KB), pedicel (P), basal endosperm (E1) and six starchy endosperm sections approximately 0.8 mm thick (E2 to E7). The embryo and pericarp tissues were discarded.

Extraction of Kernel Sections. Residual paraffin was removed from all samples by washing with toluene. The dry samples were homogenized in methanol-chloroform-water, 13:4:3 v/v. The kernel base and pedicel samples were ground with a mortar and pestle. The endosperm sections were homogenized with Duall tissue grinders (Kontes Glass Company, Vineland, N. J.). The homogenized samples were serially extracted with methanol-chloroform-water (13:4:3 v/v), 10% ethanol (v/v), and 90% dimethylsulfoxide (v/v) as previously described (13, 14).

Glucose, fructose, and sucrose components were separated on 4 × 50 cm strips of Whatman No. 1 chromatography paper. The sugar zones, located as described earlier (13), were cut out and placed in vials containing 20 ml of scintillation solution (4 g of Omnifluor [New England Nuclear, Boston, Mass.] per liter of toluene). Radioactivity was determined by using a Liquimat 220 (Picker Nuclear, White Plains, N. Y.) scintillation counter. Corrections for paper absorption and counting efficiency were made and results are presented as dpm. After determination of radioactivity, the papers were removed, dipped successively into two beakers containing 100 ml of toluene to remove the bulk of the scintillation fluors, and dried. The sugars were eluted from the papers with water, and the amounts estimated colorimetrically as desecribed earlier (13). Aliquots of the 10% ethanol-soluble polysaccharides were added to counting vials, dried, and then dissolved in 1 ml of water. Ten milliliters of counting solution (8 g of Omnifluor and 50 g of naphthalene per liter of 1,4-dioxane) were added, and radioactivity was determined as before. Aliquots of the dimethylsulfoxide solubilized starch were added to 1 × 4 cm strips of glass fiber paper (Whatman GF-82). The strips were dried under heat lamps, then placed in counting vials containing 20 ml of the toluene-based scintillation fluid, and radioactivity was determined. The amounts of carbohydrates in the WSP[4] and starch fractions were estimated by using the phenol-H_2SO_4 test (7).

RESULTS

Pattern of ^{14}C Movement. Radioautographs were made of serial sections cut from kernels collected 1 to 6 hr after treatment of a plant with $^{14}CO_2$. Examples of sections taken from near the center of kernels 3 hr after treatment are pictured in Figure 2, A, B, and C. At this sampling time the radioactivity was confined to the maternal tissue and the lower one-third of the endosperm (Fig. 2, A and B). Also there was little or no detectable radioactivity in the embryo 3 hr after treatment (Fig. 2, A and C). One to 1.5 hr after treatment, the radioactivity was largely confined to the maternal tissue at the base of the kernel (pedicel). There was no indication, in any section, of carbon-14 transport through the pericarp to the upper part of the kernel prior to its movement into the endosperm.

Rate of ^{14}C Movement. The rate of ^{14}C movement in kernels was determined from radioautographs of kernels cut in half and mounted on foam plastic. As will be shown later, almost all the radioactivity in kernels harvested the first 6 hr after $^{14}CO_2$ treatment was in the soluble sugar fractions. Therefore, differences in the radioactive front in kernels harvested at various times is a result of sugar movement. The distances between the endosperm base (not including the pedicel) and the radioactive front in kernels harvested 1 to 6 hr after exposure to $^{14}CO_2$ are given in Figure 3. Each point represents the average of 16 kernels. The advance of the ^{14}C front (rate of sugar

[4] Abbreviation: WSP: 10% ethanol-soluble polysaccharides.

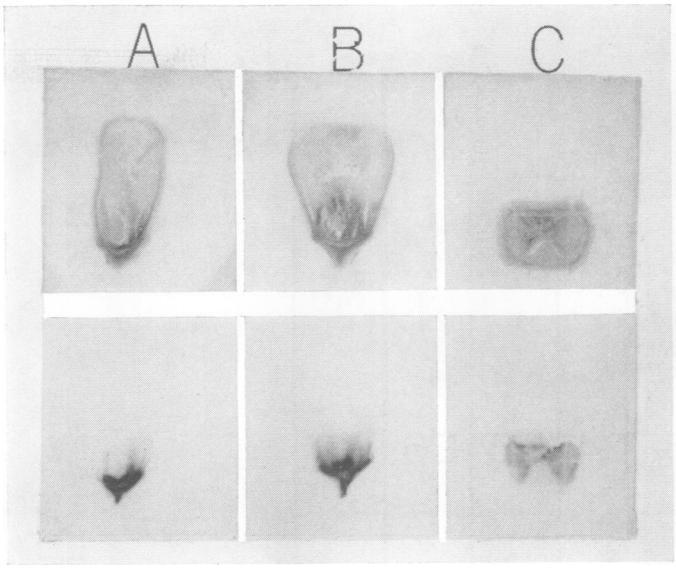

FIG. 2. Radioautographs of kernel sections 3 hr after $^{14}CO_2$ treatment of the ear leaf of a plant 21 days after pollination. The 0.8 mm thick sections were taken from near the center of the kernels. The image on the x-ray film is below the corresponding kernel section. A: Longitudinal section perpendicular to the face of the kernel; B: longitudinal section parallel to the face of the kernel; C: cross section.

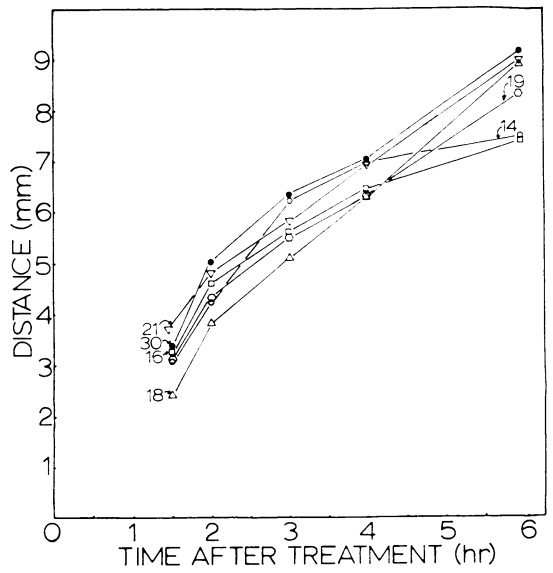

FIG. 3. Movement of radioactivity into kernels of plants treated 14 to 30 days after pollination. All plants, except the one treated 19 days after pollination, were grown in the greenhouse in 5 gallon metal containers. The plants with ears 14, 16, and 18 days after pollination were moved outside the greenhouse 24 hr before treatment and were exposed to the $^{14}CO_2$ from 1:00 to 1:30 PM June 15, June 17, and June 19, 1970 respectively. The plants treated 21 and 30 days after pollination were exposed to the $^{14}CO_2$ in the greenhouse from 10:55 to 11:20 AM on April 23, 1970. The plant treated 19 days after pollination was grown in the field and treated from 11:00 to 11:30 AM on July 28, 1969.

movement) was similar in all age kernels irrespective of the cultural conditions (Fig. 3). The most rapid apparent advance of the ^{14}C front (approximately 2.7 mm/hr) occurred through the lower one-third of the kernel, *i.e.*, the first 2 hr after treatment and then the rate of advance declined (Fig. 3), but the intensity of radioactivity in the lower endosperm increased. By 4 hr after treatment radioactivity was nearing the crown of kernels from plants treated 14 and 16 days after pollination. In the older kernels radioactivity approached the crown after 6 hr, although high concentrations of radioactivity were confined to the lower two-thirds of each kernel.

Sugar Movement. To determine what sugars were moving through the endosperm, kernels from a plant treated 30 days after pollination were subdivided as described earlier (Fig. 1). The plant used in this study was treated with $^{14}CO_2$ on January 13, 1970. Due to the poor growth conditions existing in the greenhouse during midwinter, the size and development of the kernels were similar to spring- or summer-grown kernels sampled 20 days after pollination. The total milligrams of carbohydrates extracted from the sections and the percentage of each carbohydrate component are given in Figure 4. Sucrose accounted for over 60% of the total carbohydrate extracted from the kernel base samples (Fig. 4). These samples consisted primarily of vascular bundles and associated parenchyma cells. The pedicel samples contained the highest total soluble sugar content of any zone and over 65% was sucrose (Table I). Endosperm zones 1, 2, and 3 contained similar quantities of soluble sugar, but above zone 3 sugar content declined (Table I). This decline was particularly evident in the upper half of the endosperm (Table I) which contained a larger proportion of maturing cells (8). In all endosperm sections, sucrose made up over 50% of the total soluble sugar, and the proportion of sucrose increased to a maximum in zone E4 and then declined in the upper three zones (Table I). The lower three endosperm zones were relatively low in starch (Fig. 4). By contrast the upper four zones were high in starch (Fig. 4). Although there was relatively little carbohydrate in the 10% ethanol-soluble fraction (Fig. 4), the highest contents (115 μg/piece and 127 μg/piece in zones E4 and E5, respectively) were in zones most active in starch synthesis.

Radioactivity Distribution. One and one-half hours after treatment there were measurable amounts of radioactivity in

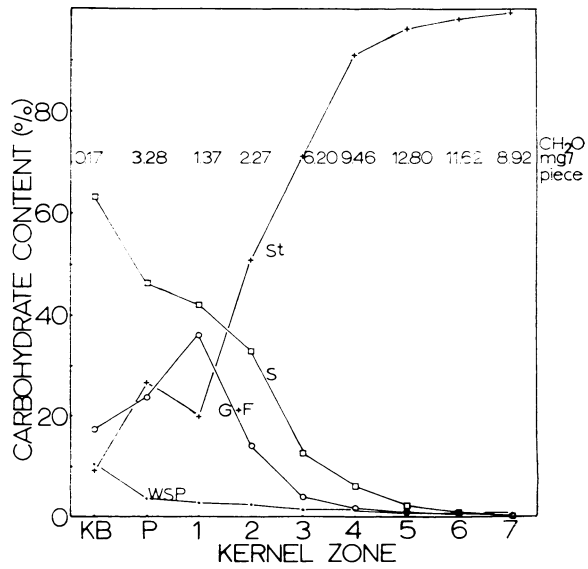

FIG. 4. Percentage of carbohydrates extracted from various zones of a corn kernel. The total carbohydrate extracted from each zone is given. Data are the averages of the six sampling times. Zones are as described in Figure 1. G + F: glucose + fructose; S: sucrose; St: starch; WSP: 10% ethanol-soluble carbohydrate (water-soluble polysaccharides).

the kernel base, pedicel, and lower endosperm (Table II). The distribution of radioactivity among the carbohydrate fractions of the lower three zones 1.5 hr after treatment was the same as that found 2 hr after treatment (Fig. 5). Most of the radioactivity in the kernel base at all sampling times was in sucrose, with the balance in glucose and fructose. This was expected since the radioactivity should have been largely associated with sugars being translocated to the developing kernels via phloem. In the pedicel tissue about one-half of the ^{14}C was in sucrose with the balance in glucose and fructose. At all sampling times, over 65% of the radioactivity in the basal endosperm (E1) was found in glucose and fructose, with the balance in sucrose (Fig. 5). Two hours after beginning the $^{14}CO_2$ treatment significant amounts of radioactivity had reached endosperm zone E4 (Table II) and at this time approximately 70% of the radioactivity in all endosperm zones was recovered in glucose and fructose. Similarly, glucose and fructose retained a high proportion of the endosperm radioactivity 3 hr after treatment. With additional time after treatment there was a decline in the percentage of ^{14}C in the monosaccharides and an increase in the percentage of ^{14}C in sucrose and polysaccharides (Fig. 5).

Little radioactivity was incorporated into the WSP and starch during the 6-hr period studied. But in both fractions incorporation was most active in endosperm zone E3 and above. The WSP fraction appears to become radioactive prior to starch. However, the significance of this observation is questioned because there was only a small amount of radioactivity in the WSP fraction, and also it is possible that this fraction may have been contaminated with soluble sugars.

Similar carbohydrate contents and radioactivity distributions were found in the pedicel, and basal, central, and upper endosperm of kernels treated 19 days after pollination. Therefore, these results need not be included in this paper.

Table I. *Soluble Sugar Content of Kernel Zones*

Data are the averages of the six sampling times. The μg/kernel zone equals the total sugar extracted from the 20 half-kernel samples divided by 10.

Kernel Zone	Sum of Soluble Sugars	Percentage of Sum		
		Glucose	Fructose	Sucrose
	μg/kernel zone			
KB	141	10.6	10.6	78.7
P	2,302	18.0	15.8	66.2
E1	1,062	23.0	22.7	54.3
E2	1,060	15.3	14.4	70.3
E3	1,025	12.7	12.0	75.3
E4	725	10.7	11.2	78.1
E5	359	10.9	13.6	75.5
E6	131	17.6	20.6	61.8
E7	74	24.3	24.3	51.4

Table II. *Total Radioactivity Extracted from Kernel Zones*

Total extracted radioactivity equals the sum of the radioactivity recovered in the glucose, fructose, sucrose, 10% ethanol extract, and starch fractions recovered from the 20 half-kernel samples divided by 10.

Kernel Zone	Time After Treatment (hr)					
	1.0	1.5	2.0	3.0	4.0	6.0
	10^3 dpm/kernel zone					
KB	0.04	0.11	0.13	2.04	2.77	4.53
P	0.05	0.69	1.87	48.09	56.39	103.87
E1	0.03	0.32	3.79	34.96	57.03	79.15
E2	0.02	0.15	0.94	9.50	30.56	68.37
E3	0.01	0.09	0.70	5.70	17.13	46.14
E4	0.03	0.07	0.23	2.80	4.61	39.62
E5	0.01	0.02	0.05	0.91	0.62	10.09
E6	0.05	0.01	0.03	0.09	0.15	4.70
E7	0.01	0.01	0.03	0.10	0.11	0.81

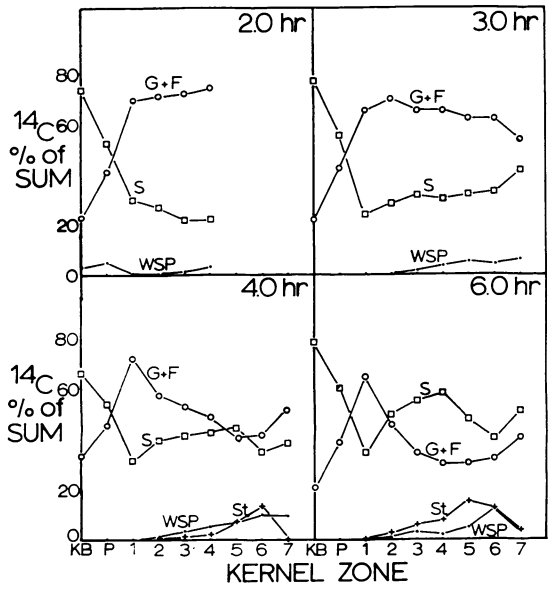

FIG. 5. Distribution of ^{14}C among carbohydrates extracted from various zones of a corn kernel. Kernels were sampled 2, 3, 4, and 6 hr after beginning the $^{14}CO_2$ treatment. Zones are as described in Figure 1 and symbols are as in Figure 4.

DISCUSSION

The vascular elements feeding a particular corn kernel terminate in the pedicel tissue near the base of the endosperm (8, 17, 18). Although no vascular tissue enters the corn endosperm, various workers have described specialized absorbing cells in the basal region of the endosperm (1, 8, 9, 17). This report confirms that sugars translocated to a developing kernel enter the endosperm through this zone of specialized cells. There was no detectable transport of sugars through the pericarp tissue to the upper part of the kernel prior to entry into the endosperm.

I previously suggested (14) that sugar unloading from the phloem and movement into the endosperm involves the hydrolysis of sucrose to glucose and fructose, and the results of this study (Fig. 5) support that suggestion. Sucrose inversion has been shown to be a prerequisite to sugar uptake by some tissues (6, 12) and in part II of this series (16) we report that invertase activity is very high in both the pedicel and the placento-chalazal tissues. The placento-chalazal tissue is located between the pedicel and the specialized cells of the basal endosperm (8). The dense protoplasm containing heavy longitudinal strands and the longitudinal, spiral, and reticulate wall thickenings of basal endosperm cells described by Kiesselbach and Walker (9) are reminiscent of "transfer cells" which are thought to be active in sugar transfer into or out of the sieve cells of the phloem (4, 19). Perhaps sugars move, either actively or passively, into the specialized basal endosperm cells

and then throughout the endosperm via plasmodesmata. However, it is difficult to understand how free glucose and fructose can move through the symplast of the endosperm to the upper endosperm, a process requiring 2 to 3 hr (Fig. 3), without being metabolized. Alternatively, the unusual wall thickenings could favor intercellular transfer through the cell walls. Thus sucrose could be inverted in the pedicel-placento-chalazal tissues and the resulting glucose and fructose could diffuse into and throughout the endosperm via the free space of the cell walls and intercellular spaces. In 1928, Priestley (11) suggested that "organic nutrients" move from the terminal phloem elements to the apex of roots and shoots through intercellular spaces and cell walls. Cormack and Lemay (2) used a radioautographic procedure to show that radioactive sugars transported from the cotyledons of mustard seedlings concentrated in the intercellular spaces of root tips. Also, Kriedemann (10) showed that sugars rapidly moved through the free space of castor bean cotyledons, and Hawker (5) suggested that sugars are transferred through the cell walls of sugar cane parenchyma rather than via the plasmodesmata. The hypothesis that sugars move through the free space of developing corn endosperm is supported by the observations, first, that the monosaccharides can move from the base of the kernel to near the crown without being metabolized (Fig. 3), and, second, the sugars move at a slow rate (2.7 mm/hr), similar to the rate of diffusion. Although the results of these studies favor a hypothesis in which sugars move through the intercellular spaces and cell walls of corn endosperm, proof of this hypothesis must await a microradioautographic demonstration of radioactive sugars in cell walls and intercellular spaces.

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