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Carbon-14 Distribution in Carbohydrates of Immature Zea mays. Kernels Following $^{14}CO_2$ Treatment of Intact Plants[1]

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Abstract. Shortly after *Zea mays* L. plants were exposed to $^{14}CO_2$, most of the radioactivity in the kernel occurred in the free monosaccharides, glucose and fructose. Later the proportion of ^{14}C in sucrose increased and that in the monosaccharides declined. These data have been interpreted as showing that the translocated sugar is hydrolyzed prior to or during its movement into the storage cells of the endosperm. This hydrolysis appears to occur in the "pedicel region" of the kernel. After entry into the endosperm tissue, sucrose was rapidly resynthesized from the monosaccharides prior to its utilization in starch synthesis.

Translocation of ^{14}C from the sites of photosynthesis to developing grain has been reported (2, 3, 13, 15, 17) but the distribution of radioactivity in kernel carbohydrates has not been extensively studied. In an effort to understand the mechanism of starch synthesis *in vivo*, a study of the fate of translocated sugar after arrival at the developing kernel has been made. This paper reports the distribution of radioactivity in kernel carbohydrates at various times after treatment of intact plants with $^{14}CO_2$.

Materials and Methods

Administration of $^{14}CO_2$. Normal inbred corn plants, *Zea mays* L. var. Ohio 43_E with ears 14 to 20 days old were fitted with a $^{14}CO_2$ generating system (8) and covered with a large plastic bag (92 cm × 130 cm). The $^{14}CO_2$ generator consisted of a 10 ml glass vial fitted with a 3 hole rubber stopper. The inlet tube which terminated near the top of the plant was connected to 1 hole in the stopper. The return tube, containing a rubber bulb unidirectional pump, was taped to the plant 30 to 50 cm above the ground and was attached to a second hole in the stopper. A short section of tygon tubing fitted with a screw clamp was connected to the third hole. A foam plastic gasket (24 cm × 7 cm × 2 cm) was wrapped around the plant at the point of attachment of the plastic bag. Portions of the inlet and return tubes were sandwiched between 2 layers of the gasket and tightly tied with string. Immediately before treatment the plastic bag was placed over the plant and tied at the gasket. One mg of barium carbonate-^{14}C containing 129 μc was added to the vial followed by 1 ml of 80 % lactic acid added through the short tube. The liberated $^{14}CO_2$ was swept into the plastic bag by the circulatory pump and distributed by moving the bag in and out creating a turbulence. After a given period of photosynthesis in the presence of $^{14}CO_2$, the bags were removed and the plants allowed to continue normal growth for additional periods.

Field Study. Eight corn plants growing on the Purdue University Agronomy Farm were exposed to $^{14}CO_2$ on August 12, 1966, from 9:00 AM to 10:20 AM. August 12 was cool and sunny with minimum and maximum temperatures of 11.7° and 25°, respectively; August 13 was cloudy and misty. Each plant had 1 ear which had been self-pollinated on July 29. Immediately after removing the plastic bags the first ear was collected (treatment time + 0 hr, *i.e.* Tr + 0). An additional ear was taken at each subsequent sampling time. Duplicate 10 g kernel samples were placed in cold, homogenizing medium and stored in a freezer until assayed. Although the entire ear was not sampled, preliminary studies showed that all kernels in a row, except those at both extremes of the ear, accumulated radioactivity at a similar rate.

Greenhouse Studies. Two plants with ears 18 days old were treated with $^{14}CO_2$ as above. The ear husks were removed from 1 plant on the night of May 30, 1967, and the husked ear was covered with an opaque black cloth bag. Two rows of kernels were removed with a razor blade at 9:20 AM on May 31, and then the entire plant was treated with 129 μc of $^{14}CO_2$ from 9:30 AM to 10:30 AM. The second plant was treated between 9:15 AM and 10:15 AM. The treatment conditions were the same except that the husks were carefully peeled back only enough to remove the 2 rows of kernels and then

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refastened around the ear with rubber bands. The husks served to maintain high humidity around the kernels but did not contribute to photosynthesis since they were also covered with the black bag. Both May 31 and June 1 were overcast days. The natural illumination was supplemented with 4 fluorescent lamps (approximately 800 ft-c). Lights were on from 8:00 AM to 8:00 PM daily. In addition to the initial samples, 1 or 2 rows of kernels were removed at Tr + 0 hr (immediately after removal of the treatment bag) and at various times up to Tr + 36 hr.

In a short-time labeling study, the flag leaf, the ear, and the first leaf above the ear were enclosed in a plastic cylinder. The enclosed portion of the plant was exposed to 155 μc of $^{14}CO_2$ from 9:45 AM to 10:09 AM on November 27, 1967. The rest of the plant was left in normal air. The upper one-fifth of the ear including the cob was removed at 10:54 AM. Four other samples of equal size were collected at various times up to 9:54 PM the same day. These samples were equivalent to Tr + 0, + 1, *etc.* of the preceding experiment.

For the localization of tissue in which sucrose hydrolysis takes place, a single plant with a 19-day-old ear was treated with 129 μc of $^{14}CO_2$ from 9:40 AM to 10:40 AM on July 3, 1967. The entire plant was enclosed within the treatment bag. The first sample (Tr + 0) consisting of the upper one-third of the ear was collected at 10:40 AM. Samples of the middle one-third and basal one-third of the ear were collected at Tr + 2 and Tr + 6 hr, respectively. The ear shank was also removed at Tr + 6 hr. The samples were quick-frozen in dry ice and freeze-dried. The dry kernels were removed from the cob and divided into the maternal tissue (the pedicel and pericarp) and the endosperm. The distribution of radioactivity in the free sugars of each tissue sample was determined.

Extraction of Kernels. The procedure for the stepwise extraction, fractionation, and quantitative measurement of glucose, fructose, sucrose, the water-soluble polysaccharide fraction (WSP), and starch is summarized in figure 1. For the extraction of 20 g samples the volumes given in figure 1 were doubled. To enhance sedimentation of cell debris and protein, larger volumes of dimethylsulfoxide (DMSO) were used for the initial starch extraction from older kernels (18–20 days old). Aliquots of each fraction were plated on 37 mm aluminum planchets and radioactivity was determined with a Picker-Nuclear[2] gas flow detector. The total homogenate radioactivity was corrected for self-absorption.

[2] Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

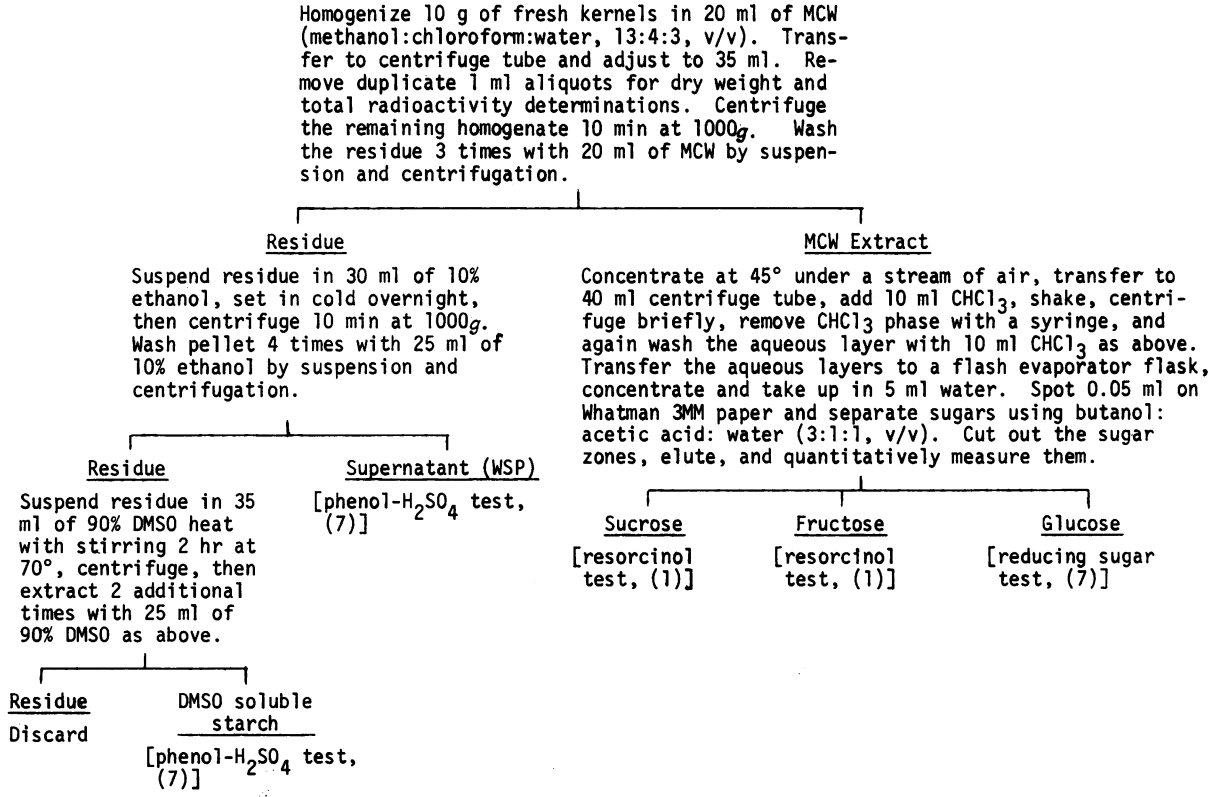


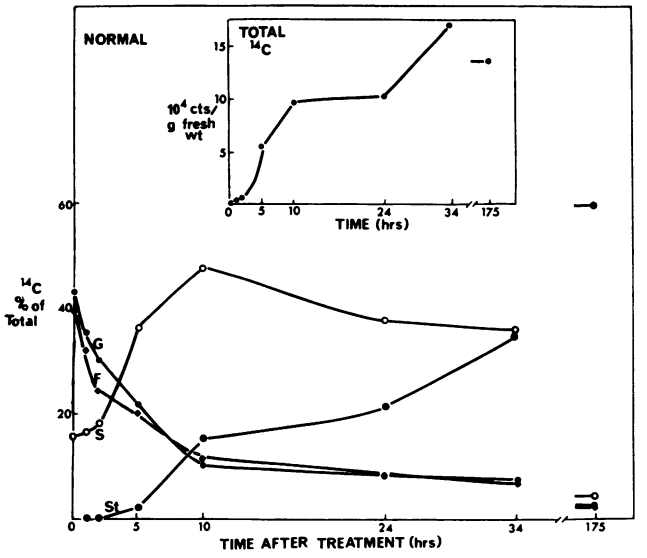
FIG. 1. Flow diagram of the extraction procedure.

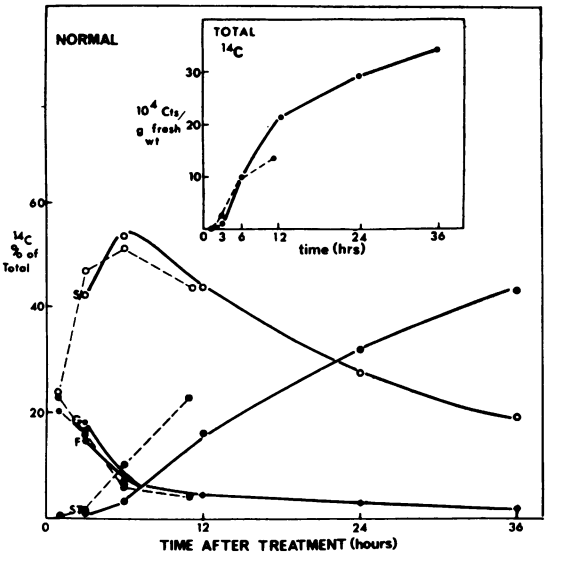
Table I. *Dry Matter and Carbohydrate Content in Corn Kernels*

Ohio 43_E plants with kernels 14 days old were exposed to $^{14}CO_2$ for 80 minutes. The 0, 1, *etc.*, are additional hours before sample collection.

Post-treatment	Dry matter	Glucose	Fructose	Sucrose	WSP	Starch
hr	%	*mM*[1]	*mM*	*mM*	*mg/g fr wt*	*mg/g fr wt*
0	14.9	90.7	71.4	90.1	1.20	17.5
1	14.9	74.1	72.8	66.5	1.03	20.4
2	14.5	82.6	69.8	86.5	1.05	13.2
5	14.8	88.5	74.3	84.3	0.94	15.8
10	15.2	92.8	71.7	81.6	0.95	23.9
24	17.0	85.1	65.4	82.2	0.95	31.8
34	16.1	80.7	63.1	83.9	0.89	36.9
175	28.6	55.9	55.5	80.4	1.50	137.8

[1] Approximate molarity was calculated by dividing the mmoles of sugar in the kernels by the g of water in the kernels.





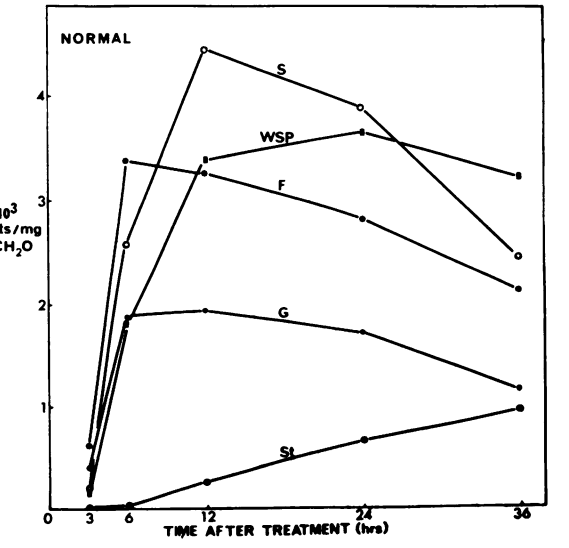


FIG. 2. (Upper left) The percentage distribution of ^{14}C following $^{14}CO_2$ treatment of corn plants. Treatment time and age as in table I. G = glucose, F = fructose, S = sucrose, St = starch, Inset = total radioactivity in kernels.

FIG. 3. (Upper right) The percentage distribution of ^{14}C in samples taken from a single ear. The data represents the average of 2 plants. Ohio 43_E plants with kernels 18 days old were exposed to $^{14}CO_2$ for 60 minutes. Data represented by the dashed lines are from a plant in which 2 leaves were treated for 15 minutes. Symbols as in figure 2.

FIG. 4. (Lower right) The specific activity of carbohydrate fractions at various times after treatment with $^{14}CO_2$. Treatment time and age as in figure 3. Symbols as in figure 2.

Results

Field Study. Individual ears were sampled for each treatment time. Although the plants and ears were all the same age, there was some variability from plant to plant. For example the ears collected at Tr + 2 and Tr + 34 were poorly pollinated.

With the exception of Tr + 175, there was no consistent change in glucose, fructose, or sucrose concentrations (table I). There was considerably less free sugar in samples from the oldest kernels. Sucrose appears to have declined less than the monosaccharides if at all. The DMSO extracted starch increased with age as expected. There was little carbohydrate extracted by the 10 % ethanol.

The total radioactivity and percentage distribution of ^{14}C in the simple sugars and starch are presented in figure 2. Since the WSP fraction never contained more than 1 % of the total radioactivity, it is not presented. There was a rapid transport of ^{14}C to the kernels between Tr + 2 and Tr + 10 hours. The rate of increase in kernel radioactivity decreased during the first night followed by an increase the second day. During the first 2 or 3 hours following treatment, most of the ^{14}C in the kernels was in the free monosaccharides, glucose and fructose. With increasing time after exposure to $^{14}CO_2$ the percentage in sucrose increased whereas that in glucose and fructose declined rapidly. After 175 hours, very little radioactivity was associated with either of the 3 sugars. Rapid accumulation of radioactivity in starch began between 5 and 10 hours after treatment.

Greenhouse Studies. In order to determine whether the reduced uptake and incorporation during the first night (Tr + 10 to Tr + 24) was due to a daily rhythm or to variability among plants, kernels from a single ear were removed at varying times and the distribution of radioactivity determined.

The percent distribution of radioactivity in the various components (fig 3) was similar to that noted in figure 2. In contrast to the field study, the kernel ^{14}C content did not level off at night. The rate of ^{14}C incorporation into starch also appeared to be independent of the time of day. The distribution of radioactivity in the carbohydrates from kernels of the plant in which only 2 leaves were exposed to $^{14}CO_2$ for 15 minutes is presented as the dashed lines in figure 3. These data very closely parallel the data from plants completely enclosed in the plastic bag for 1 hour.

The specific activities (cts/mg carbohydrate) of the different components are given in figure 4. The specific activity of the monosaccharide fractions increased rapidly reaching a maximum about 6 hours after treatment and then declined slowly after 12 hours. Sucrose reached a maximum at Tr + 12 hours. The specific activity of WSP paralleled that of sucrose up to Tr + 12 hours but continued to increase for at least 24 hours. There was a linear increase in the specific activity of starch after an initial lag of 6 hours.

The relatively high percentage of monosaccharide radioactivity at the early sampling times indicates that at least part of the translocated sugar was hydrolyzed prior to or during its movement into the endosperm. In the corn kernel the vascular elements terminate in the pedicel and pericarp tissues (9). Therefore, if sucrose hydrolysis is associated with an unloading of the phloem and uptake by the endosperm, the pedicel and pericarp tissue would be the expected sites of hydrolysis.

Immediately after administering the $^{14}CO_2$ for 1 hour, 95 % of the radioactivity in the endosperm sugars was in the monosaccharides with only 5 % in sucrose (fig 5). In the pedicel and pericarp tissues however, 25 % of the radioactivity at this time was in sucrose. With additional time the

Table II. *Sugar Content and Radioactivity in Endosperm and Maternal Tissues*

An Ohio 43_E plant with kernels 19 days old was exposed to $^{14}CO_2$ for 60 minutes. Sections of the ear were sampled at 0, 2, and 6 hours after removal of the treatment bag. The endosperm, pedicel plus pericarp and cob fractions are represented by E, P, and C respectively.

	Tr + 0			Tr + 2			Tr + 6			
Sugar	E	P	C	E	P	C	E	P	C	Shank
Sucrose										
mg[1]	2.16	5.14	152.2	2.20	5.16	139.8	2.54	5.19	150.0	202.5
counts	60	453	18,000	3743	5753	196,000	12,252	8916	355,000	770,000
Glucose										
mg	0.88	1.98	19.8	0.88	1.88	18.3	0.70	1.84	35.9	38.6
counts	636	710	1000	5466	6443	12,667	4606	3879	14,667	21,000
Fructose										
mg	0.47	0.97	12.0	0.42	1.00	10.1	0.41	0.92	17.7	24.7
counts	510	596	1667	5006	6206	9333	4846	3346	6000	11,333

[1] The sugar content and radioactivity of the endosperm and pedicel plus pericarp tissues are presented as mg or counts per kernel piece respectively. That of the cob and shank tissues are given as mg or counts per g dry weight. The average dry weight of the endosperm and pedicel plus pericarp tissues were 65.8 mg and 15.1 mg per kernel piece, respectively.

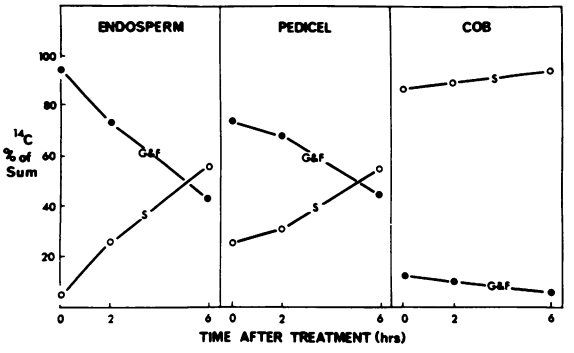


FIG. 5. The percentage distribution of free sugar ^{14}C in endosperm, pedicel, and cob tissues. Treatment time and age as in table II. The monosaccharides were equally labeled and their sum is presented. Symbols as in figure 2.

characteristic decrease in percent ^{14}C in the monosaccharides and increase in sucrose as shown in figures 2 and 3 were evident. Radioactivity in sugars in the shank and in the cob at all sampling times was essentially confined to sucrose; *i.e.*, the sugar of translocation. The sugar content per kernel piece was essentially constant at all sampling times (table II). The pedicel tissue contained approximately twice as much of each sugar as the endosperm. In the shank, cob and pedicel samples, sucrose made up 20 %, 15 % and 34 % of the dry weight respectively. From the high sugar concentration in the pedicel, cob, and shank tissues relative to the endosperm it would appear that either the sugar movement from the phloem endings to the endosperm is a rate limiting step or that the endosperm sugars are utilized in starch synthesis as rapidly as they arrive. The latter possibility does not appear too likely since the specific activity of endosperm sucrose did not reach equilibrium in 6 hours. The specific activity of the monosaccharide sugars in the endosperm reached equilibrium in about 2 hours. The embryo was separated but not analyzed.

Discussion

Sucrose, the translocated sugar in corn (11), appears to be hydrolyzed to glucose and fructose prior to, or during its movement from the terminal phloem elements in the pedicel and pericarp tissues into the storage cells of the endosperm. This is demonstrated by the high proportion of radioactivity in the monosaccharides at the early sampling times (fig 2 and 3). Similar results were obtained when only 2 leaves were enclosed for 15 minutes (fig 3). Therefore, it is unlikely that the hydrolysis of sucrose noted above was an artifact induced by a CO_2 deficit created when the entire plant was enclosed in the plastic bag. The hydrolysis of sucrose may be the result of an active vein unloading similar to that discussed by Geiger (4). This conclusion is supported by the evidence that hydrolysis takes place in the pedicel and pericarp tissues (fig 5). The vascular elements terminate in the pedicel and pericarp tissues, and the basal portion of the endosperm contains elongated cells which are believed to constitute the absorbing tissue for nutrients entering the grain from the parent plant (9). Due to the method used in the separation of the freeze-dried endosperm and maternal tissues, it is possible that a portion of the basal endosperm cells which do not store starch were included with the maternal tissue. Whether hydrolysis was via invertase, similar to the outer space invertase in disks of sugarcane tissue (14), or by an active unloading of the phloem involving phosphorylated intermediates as postulated by Kursanov (10), was not determined in this study. In any event, it appears that sucrose hydrolysis precedes sugar movement into the storage cells of the endosperm.

Once the sugar arrives in the endosperm cells it is rapidly converted into sucrose. Sucrose appears to serve as a temporary storage form of carbohydrate prior to its utilization in starch synthesis. Sucrose synthesis and utilization are in equilibrium, since sucrose rapidly becomes radioactive but does not increase quantitatively.

The accumulation of ^{14}C in kernels from field-grown plants leveled off at night (fig 2). The possibility that this was due to a diurnal rhythm similar to that reported for other plants (5, 12, 17) is unlikely since kernels from greenhouse grown plants rapidly accumulated ^{14}C during both day and night (fig 3). The night plateau in the field study was probably due to plant variability, or to restricted translocation at the low night temperature (11.7°). Low temperatures have been shown to retard translocation in other plants (4, 6, 16). In the greenhouse study the minimum night temperature was 20°.

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