7. KROTKOV, G., V. C. RUNECKLES, and K. V. THIMANN. 1958. Effect of light on the CO_2 absorption and evolution by Kalanchoë, wheat and pea leaves. Plant Physiol. 33: 289–292.
8. KUNITAKE, G., C. STITT, and P. SALTMAN. 1959. Dark fixation of CO_2 by tobacco leaves. Plant Physiol. 34: 123–127.
9. RACUSEN, D. W., and S. ARONOFF. 1952. Metabolism of soy bean leaves. V. The dark reactions following photosynthesis. Arch. Biochem. Biophys. 42: 25–40.
10. SALTMAN, P., G. KUNITAKE, H. SPOLTOR, and C. STITT. 1956. Dark fixation of CO_2 by succulent leaves. Plant Physiol. 31: 464–468.
11. TCHEN, T. T., and B. VENNESLAND. 1955. Enzymatic carbon dioxide fixation in wheat germ. Jour. Biol. Chem. 213: 533–546.
12. THOMAS, M. 1949. Physiological studies on acid metabolism in green plants. I. CO_2 fixation and CO_2 liberation in Crassulacean metabolism. New Phytol. 48: 390–420.
13. THOMAS, M. 1949. Physiological studies on acid metabolism in green plants. II. Evidence of CO_2 fixation in Bryophyllum and the study of diurnal fluctuation of acidity in this genus. New Phytol. 48: 421–447.
14. THOMAS, M. and H. BEEVERS. 1954. Physiological studies on acid metabolism in green plants. III. Further evidence of CO_2 fixation during dark acidification of plants showing Crassulacean acid metabolism. New Phytol. 53: 1–30.
15. WALKER, D. A. 1956. Malate synthesis in a cell free extract from a Crassulacean plant. Nature 178: 593–594.
16. WALKER, D. A. 1957. Physiological studies on acid metabolism. IV. Phosphoenol pyruvate carboxylase activity in extracts of Crassulacean plants. Biochem. Jour. 67: 73–79.

CARBOHYDRATE METABOLISM OF CITRUS FRUITS I. MECHANISMS OF SUCROSE SYNTHESIS IN ORANGES AND LEMONS[1]

R. C. BEAN

DEPARTMENT OF PLANT BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, RIVERSIDE

The basis for controlling the accumulation of metabolites during growth of plants is of special interest in the study of fruit growth since the flavor and quality of the fruit may depend on the stored materials. Oranges and lemons are two fruits having wide differences in characteristic sugar storage patterns. The mature lemon contains little or no sucrose in any of its tissues whereas sucrose constitutes the major storage sugar of the orange. It would be of interest to determine if such variations are reflected in synthetic mechanisms, synthetic capacities, or in utilization of sucrose. It would also be valuable to find the stage of growth where such differences become manifest. The synthesis of sucrose in these fruits has been compared by chemical and radioisotope techniques using enzyme and tissue preparations as well as intact fruits. Results of the study suggest that differences in sucrose storage may not be due to any deficiency of sucrose synthesizing mechanisms within the lemon, since active systems were found to exist in these fruit at all stages of growth. Pronounced differences were found, however, in the synthetic capacities of different tissues of the fruit; it appears that the albedo (white peel layer) has the highest capacity for synthesis in the older fruit.

[1] Manuscript received September 22, 1959.

MATERIALS AND METHODS

Sucrose synthesis was studied in three ways. A. Reaction of fructose or fructose-6-phosphate with uridine diphosphate glucose (UDPG) was determined in vitro with extracts or particulate preparations from the fruit. B. Formation of sucrose in isolated tissue preparations was traced by incorporation of activity from glucose-U-C^{14} or fructose-U-C^{14}. C. The tracer technique was also extended to examine the formation of sucrose from labeled monosaccharides in small, intact fruits. Experimental procedures and materials are given below for each approach.

I. STUDIES WITH EXTRACTS AND HOMOGENATES. Mature Eureka lemon and Valencia orange fruits were obtained fresh from local groves. The lemons and oranges were juiced by hand with a glass reamer. The lemon juice was divided immediately into two parts. One part was neutralized to pH 7 with 1 *N* NaOH before carrying out subsequent steps while the remainder was treated as initially obtained. The juices were filtered through birdseye cloth and centrifuged at approximately 25,000 × G for 20 minutes. The sediments were resuspended in two volumes of 0.01 M phosphate buffer, pH 7, and dialyzed overnight against the same buffer. Supernatants from centrifuging were saturated with ammonium sulfate at pH 5 and the precipitates were collected by centrifuging. This precipitate was redissolved in the pH 7 phos-

phate buffer and dialyzed overnight in Visking sausage casing against the same buffer.

Soluble extracts of lemon and orange peels were also prepared by homogenizing one part peel in ten parts water and treating the homogenates as described above for the juices.

Sucrose synthesis was measured in the soluble preparations by modifications of previously published procedures (2, 10, 11, 12). A 50 µl aliquot of the enzyme solutions was incubated at room temperature (21–23° C) for half an hour with 0.37 µmoles of UDPG and 2.5 µmoles fructose or 2 µmoles of D-fructose-6-phosphate in a total volume of 0.1 ml. Following inactivation by heating in a boiling water bath for one minute, the residual free fructose or fructose-6-phosphate was destroyed by heating in 1 ml 0.1 *N* NaOH for 15 minutes at 100° C. The alkali-stable ketose (keto group protected by glycosidic linkage) was determined by the method of Dische and Borenfreund (8) using a 2-hour incubation rather than the specific 24-hour period. Adequate controls were prepared, omitting UDPG, enzyme or ketose.

For particulate preparations, the procedure was modified by doubling the volumes and quantities used. Following incubation, the suspension was centrifuged to remove the particulate material and an aliquot of 0.1 ml of the supernatant solution taken for analysis as above.

The UDPG was prepared from yeast extracts by a gradient elution modification of the procedure of Cabib, Leloir and Cardini (7). This material contained approximately 25 per cent of uridine diphosphate *N*-acetyl-D-glucosamine and uridine diphosphate. Potassium D-fructose-6-phosphate was obtained from the commercial barium salt by treatment with Dowex-50 (hydrogen form) followed by neutralization with potassium hydroxide.

II. Tracer Studies in Isolated Tissues. Lemons and oranges of varying ages were obtained from the groves. It was possible to obtain almost a full range of growth of the lemon fruit at a single period due to the continuous setting habit of the lemon. The range examined in oranges was limited to relatively young fruit. Each piece of fruit was cut in half and the vesicles were carefully excised. Sections of the flavedo (colored, outer layer of peel) and albedo, approximately 1.5 mm thick, were cut perpendicular to the core of the fruit. Samples in the range of 100 mg of each of these three tissues were weighed into containers and incubated at room temperature, in the dark, with 0.1 ml of a solution of labeled fructose or glucose. The solutions contained 20 µg of the active sugar with a total activity of 800,000 to 900,000 cpm per 0.1 ml aliquot. The sugars were prepared photosynthetically by the method of Putman, et al (14, 15).

Following incubation for 3 to 4 hours, the isolated tissues were inactivated and extracted with boiling ethanol. The ethanol extracts were concentrated and chromatographed by standard procedures. Water saturated phenol, butanol-acetic acid-water (52:13:35) and butanol-ethanol-water (52.5:32:15.5) solvents were used in chromatography. Active sugars were located by radioautography and the activity in each sugar determined directly on paper with a Nuclear D-47, Micro-mil end window, gas-flow counter. Sucrose was eluted from the paper and rechromatographed following hydrolysis with invertase. Activity in each of the resulting monosaccharides was determined as above.

III. Tracer Studies in Intact Fruit. Fruit in the range of 5 g total fresh weight was obtained. The stems and buttons were cut off under water and each piece of fruit was weighed quickly. A small ring of polyethylene was attached around the stem scar with the aid of a little silicone grease. The solution of carbon 14 labeled glucose or fructose was placed in the cup thus formed. An aliquot of 0.1 ml containing 1.1 mg of the sugar was used. Total activity with glucose was 9.3×10^6 cpm. For the fructose experiments 8.0×10^6 cpm was used with oranges and 16×10^6 cpm for lemons. Activity was measured with the equipment mentioned above. The fruit was allowed to take up the major portion of this solution in the dark. This required a period of about 90 minutes for the lemons and 150 minutes for the oranges. The residual solution was washed into boiling ethanol. The flavedo was rapidly cut off into hot ethanol. The fruit was then cut in half and the juice sacs excised into hot ethanol. The remaining albedo material was also inactivated in boiling ethanol after slicing it into thinner sections. After extraction with ethanol, the extracts were treated as outlined above for analysis of the isolated tissue extracts. The approximate amount of each sugar on the paper chromatograms was determined by a densitometric technique, using the color developed by reaction of the sugars with benzidine dihydrochloride (4). Density was measured with a variable intensity light source and an International Rectifier Company photocell. The signal from the photocell was amplified through a transistorized amplifier and recorded with a recording milliammeter. The amount of sugar present was determined by comparing peak heights with those peak heights found for known quantities of sugars. Using this technique, it was possible to get approximate specific activities of the sugars directly on paper.

Results

Results for sucrose synthesis by enzyme preparations from oranges and lemons are given in table I. Data on unprocessed juices and extracts could not be obtained directly since chromogenic materials in the original preparations interfered with determination of the small amounts of sucrose formed. In the soluble extracts, this difficulty could be overcome by ammonium sulfate precipitation of the enzyme except in orange peel preparations. No difficulty was met with the resuspended particulate fractions.

TABLE I

FORMATION OF SUCROSE BY PREPARATIONS FROM LEMON AND ORANGE FRUITS

PREPARATION	SUCROSE SYNTHESIZED	
	FRUCTOSE REACTION	FRUCTOSE-6-PHOSPHATE REACTION
	μmoles	*μmoles*
Orange Juice		
Ammonium sulfate fraction	0.12	0
Particulate sediment	0	0
Lemon Juice		
Ammonium sulfate fractions		
pH 2.5 juice	0	0
pH 7 juice	0	0
Particulate sediment		
pH 2.5 juice	0.17	0
pH 7 juice	0.10	0
Small Lemon (15 g size) homogenates		
Ammonium sulfate fraction	0	0
Particulate sediment	0.15	0

Conditions: 50 μl. Enzyme preparation incubated 30 minutes with 0.37 μmoles UDPG and 2.5 μmoles fructose or fructose-6-phosphate in total of 0.1 ml. Volumes and quantities doubled for analysis of particulate material and 0.1 ml of supernatant withdrawn for analysis after incubation.

The data for the juices indicate that the soluble portion of the orange juice contains an active system for the synthesis of sucrose from free fructose and UDPG. On the other hand, the lemon has no activity in the soluble fraction; instead, the particulate material shows a good capacity for sucrose synthesis. Rather than being an integral part of the particulate system, the sucrose synthesizing enzyme in lemons might be a soluble protein which is adsorbed on the particles during juicing operations due to the low pH. To test this possibility, a suspension of particles sedimented at pH 7 was divided into four portions. One part was retained for control; the other parts were divided into supernatant and sediment fractions by centrifugation. The sediments were suspended in phosphate-citrate buffer, one at pH 3.5, another at pH 5, and the third at pH 7. Each suspension was thoroughly rehomogenized in a Potter-Elvehjem apparatus, allowed to stand at 4° C for several hours with occasional shaking, and finally recentrifuged. All sediments were resuspended in phosphate buffer, pH 7, to give the original starting volume. All the final suspensions as well as the supernatant fractions were then analyzed for activity. No significant activity was found in any of the supernatants and practically all the activity (69–81 % of the original) was recovered on the particles. Thus, although this does not completely exclude the possibility that the enzyme might have been irreversibly adsorbed during processing, it tends to indicate a rather firm bond between the particles and the enzyme, in contrast to the readily soluble enzyme of the orange juice. The probability that the enzyme actually is normally associated with the particles is strengthened by the fact that it is also found in the sedimentable fraction in the preparations from the young lemons which do not have an abnormally low pH. Other enzymes, normally found in the soluble form in other organisms, have also been found in citrus associated with the particulate material (3).

Lemon tissue preparations showed an incorporation of activity into sucrose with either glucose-C^{14} or fructose-C^{14} at all ages of the fruit. The results of pertinent experiments are given in figure 1A, B. With glucose-C^{14} as substrate no activity was found in the free fructose. Similarly, no labeling of the free glucose occurred when fructose-C^{14} was used. Detectable amounts of activity were always found in the hexose phosphate although concentrations of these compounds in the citrus tissues are very low. It is evident that the albedo had a much higher capacity for sucrose synthesis per unit of fresh weight than either the juice sacs or the flavedo under these conditions. The capacity for synthesis by the albedo remained surprisingly constant throughout the fruit's growth. Activity in the flavedo and vesicles dropped off greatly in the larger fruit.

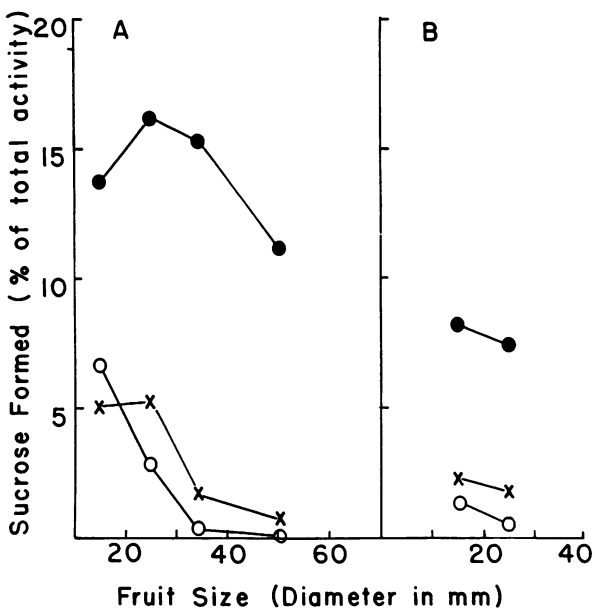
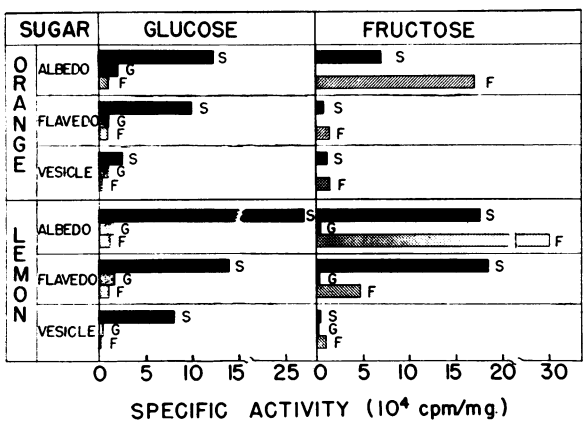


FIG. 1. A. Formation of sucrose from glucose in isolated tissues from lemons of various ages. B. Formation of sucrose from fructose in tissue preparations from lemons of two ages. ●, Sucrose formed in albedo tissue slices; ○, Sucrose formed in flavedo tissue slices; X, sucrose formed in excised vesicles. No activity was found in free fructose with glucose-C^{14} as substrate nor in free glucose when fructose-C^{14} was used. Total free fructose was higher than free glucose in smallest fruits approximately equal in larger fruits.

When young orange tissues were incubated with labeled glucose or fructose, no significant labeling of sucrose occurred. Instead some anomolous reactions were found (3).

Table II presents the results of the studies with intact fruits in terms of the total activity found in each sugar of the various tissues of the fruit. These data were also used to calculate the values for specific activities of the sugars given graphically in figure 2. With glucose as substrate, little activity remained in the monosaccharides; sucrose had the greatest total activity as well as the highest specific activity. On the other hand, when fructose was given, it was converted much less extensively to sucrose in both fruits. A great deal of activity remained in fructose at the end of the experimental period in all tissues. With the exception of lemon flavedo, the specific activity of fructose exceeded that of sucrose in all tissues.

Sucrose from each of the labeling experiments was subjected to hydrolysis and rechromatographed to determine the ratio of activity in glucose to activity in fructose. To determine the accuracy of the method and to furnish a control, experiments were also performed in which uniformly labeled sucrose was fed to the fruits. The resulting labeled sucrose in the fruit was hydrolyzed and measured in the same manner as the other samples. Results of this work are included in figure 3. Here it may be seen that activity was found in both components of the sucrose no matter which monosaccharide was supplied. A ratio which closely approaches unity would indicate complete equilibration of the two monosaccharides or derivatives entering into sucrose synthesis. When the sugar supplied enters into sucrose faster than it equilibrates with the other hexose precursor to sucrose a ratio greater than unity would be expected. In all these experiments an excess of the labeled monosaccharide was present through the duration of the experiment so that one might expect a higher activity in the sucrose moiety corresponding to the sugar fed unless equilibration between the two precursors to sucrose were extremely rapid. The data shows that activity was found in both components of the sucrose no matter which monosaccharide was supplied. In most cases, the monosaccharides moiety corresponding to the sugar supplied still retained a somewhat higher degree of labeling. The close approach of ratios to unity in the orange albedo indicates that a very rapid equilibration occurred between both sugars in the tissue. Lemon tissues consistently show a higher activity in the sugar which was fed than in the other sugar but even here, the activity ratios are low enough to indicate a relatively high rate of equilibration of the sucrose precursors.



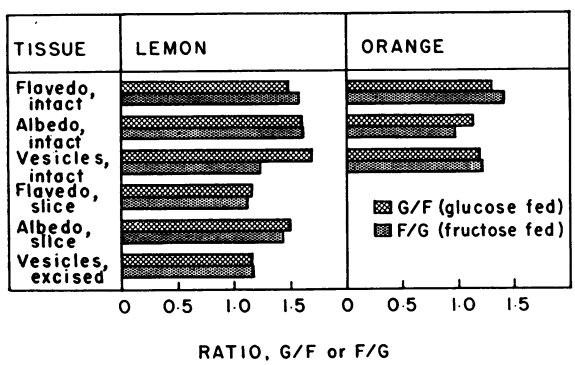


FIG. 2 (*Top*). Specific activity of sugars in the different tissues of intact lemons and oranges after uptake of glucose-C^{14} or fructose-C^{14}, Solid bars, specific activity of sucrose; cross-hatched bars, specific activity of fructose; speckled bars, specific activity of glucose.

FIG. 3 (*Bottom*). Ratios of activities of monosaccharide components of sucrose synthesized from glucose-C^{14} and fructose-C^{14} in tissue preparations or intact lemon and orange fruits. Cross-hatched bars, ratio of activity of glucose to fructose from tissues given glucose-C^{14}, speckled bars, ratio of activity of fructose activity to glucose in tissues given fructose-C^{14}. Control experiments in which the ratios were determined after giving uniformly labeled sucrose indicated variability of 5 per cent in final ratios.

DISCUSSION

Two mechanisms have been established for the synthesis of sucrose in plant materials (10, 11, 12). One mechanism involves the reaction of UDPG with free fructose to form sucrose directly while the other forms sucrose phosphate from the reaction of UDPG with fructose phosphate. Evidence presented by a number of workers (5, 6, 13) makes it appear that photosynthetic leaves use the latter reaction, exclusively. Etiolated wheat seedlings also seem to make use largely of the phosphorylated form of fructose in sucrose synthesis. Fresh, green peas have been shown to contain enzymes for synthesis of sucrose from free fructose as well as from fructose phosphate (2). In the present study it has been established that cell-free preparations from citrus fruits are capable of forming sucrose from free fructose and UDPG. No activity could be detected in these systems for the reaction of fructose-6-phosphate with

TABLE II

DISTRIBUTION OF ACTIVITY IN SUGARS FROM INTACT YOUNG LEMON AND ORANGE FRUITS*

LABELED SUBSTRATE	SUGAR	ACTIVITY IN TISSUE/MG OF TISSUE DRY WT					
		ORANGE FLAVEDO	ORANGE ALBEDO	ORANGE VESICLE	LEMON FLAVEDO	LEMON ALBEDO	LEMON VESICLE
Glucose	Glucose	171	536	295	154	293	171
	Fructose	105	105	47	26	118	54
	Sucrose	249	1,420	1,470	720	2,810	3,580
Fructose	Glucose	...	...	...	204	360	102
	Fructose	162	1,560	490	692	6,380	1,710
	Sucrose	388	814	1,150	4,070	4,420	1,870

* Total activity in each sugar per unit of dry weight.
Fruits were all in the range of 5 g total weight. Labeled sugar solutions (0.1 ml) contained 1.1 mg of sugar. Glucose activity, 9.3×10^6 cpm in both experiments. Fructose activity 8.0×10^6 cpm in the orange and 16×10^6 cpm in the lemon.

UDPG. The more indirect evidence of the tracer studies does indicate, however, that a mechanism must exist for the formation of sucrose without the utilization of free fructose and this path may be more important in the intact cell than the free fructose mechanism.

As in previous studies in intact tissues (13) the conclusion that a phosphorylated form of fructose participates in sucrose synthesis in citrus must rest mainly on the fact that the monosaccharide components within sucrose become rapidly equilibrated without similar equilibration of the free hexoses. In peel slices and intact vesicles sucrose was rapidly labeled by either glucose-C^{14} or fructose-C^{14}. Examination of the sucrose formed showed that both hexose moieties of sucrose were labeled (fig 3). No labeling of the free fructose pool occurred with glucose-C^{14} as the substrate. It is obvious from the sucrose degradation that rapid isomerization of glucose to fructose did occur before synthesis of the sucrose. Since such an isomerization is most likely to occur through the phosphorylated forms it would be safe to assume that fructose phosphate would be available for sucrose synthesis. This is supported by the fact that activity was always found in the area corresponding to the hexose phosphates. It would then seem most likely that the mechanism of synthesis of sucrose in these experiments would be phosphorylation of glucose followed by simultaneous isomerization and formation of UDPG; condensation of UDPG, and fructose phosphate could then occur with subsequent dephosphorylation of the sucrose. The observations cannot completely eliminate the possibility that free fructose might have participated in the formation of sucrose in these isolated tissue experiments but several unlikely assumptions would have to be made to support this possibility. If the free fructose is participating, the pool involved must be very small in order to account for the lack of any apparent activity in the free fructose. Since the most likely path of isomerization of glucose and fructose is through phosphorylated intermediates, an added dephosphorylation step would be required to form the active free fructose pool. This added step would tend to increase the probability of a wide spread in the activity of the two hexose moieties of the sucrose. It should also be noted that if formation of sucrose were occurring through reaction of UDPG with a small sequestered pool of free fructose the feeding of fructose-C^{14} might be expected to give sucrose with a much higher activity in the fructose than the glucose moiety. This is not the case since glucose and fructose each give very similar labeling patterns in the sucrose molecule, indicating very close relationships in the pathways followed by the two sugars in entering the sucrose. Thus it would appear that the condensation of fructose phosphate with UDPG is the most likely reaction for the formation of sucrose from sugars supplied externally to isolated tissues of citrus fruits.

In the intact fruits the free fructose pool does become labeled when glucose-C^{14} is the substrate so that evidence for the participation of the fructose phosphate pathway is not so unequivocal. The high specific activity of sucrose relative to fructose in the glucose utilization experiments does make it clear that the normally observed main endogenous pool of fructose cannot contribute greatly to the synthesis of sucrose. This does not exclude possible utilization of isolated small pools of free fructose which could have a high specific activity.

Results of the tissue slice experiments indicate that the albedo is the most active site of sucrose synthesis in lemons. Interfering reactions prevented similar comparisons in orange tissues. The participation of a transport system makes it difficult to assess the contribution that each tissue makes to sucrose synthesis in the intact fruit. The sugars must pass first through a vascular network in the albedo before reaching the other tissues so that many transformations could occur during the transport phase. The differences in the distribution of activity in the hexose components of sucrose isolated from the dif-

ferent tissues indicates that each tissue must make some contribution to the synthesis of sucrose. The fructose to glucose activity ratio in sucrose from the albedo of oranges fed fructose-C^{14} was 1.0 (fig 3). This ratio was 1.4 in the flavedo and 1.2 in the vesicles in the same experiment indicating some dilution of the glucose portion of the sucrose may have occurred after the sugars left the albedo or that independent synthesis took place in the other tissues. Similarly, when glucose was fed, the glucose to fructose activity ratio was 1.1 in the albedo but 1.3 and 1.2, respectively, in the flavedo and juice sacs indicating changes occurring in these tissues. Intact lemons do not show the same wide differences between activity ratios in different tissues but the work with isolated tissues shows that different tissues may carry out independent synthesis of sucrose.

Axelrod and Seegmiller (1) have investigated the formation of sucrose in apple tissues and found that sucrose became labeled in both hexose moieties although not to the same extent as has been observed here.

Summary

Formation of sucrose in oranges and lemons has been studied using tracer techniques with isolated tissues and with intact small fruit. Sucrose synthesis has also been followed by normal analytical procedures with extracts and particulate preparations.

Soluble preparations from the juice of mature oranges catalyze the reaction between uridine diphosphate glucose (UDPG) and free fructose to form sucrose. Soluble material from the juice of lemons is inactive, but particulate preparations were found to activate the same reaction. No activity could be found in these systems for catalyzing the reaction between fructose-6-phosphate and UDPG to form sucrose phosphate.

In isolated tissues (peel slices and excised vesicles) from lemons of all ages, label from glucose-C^{14} or fructose-C^{14} was incorporated into sucrose. Sucrose was labeled in both hexose components no matter which monosaccharide was supplied. No labeling of the free fructose occurred when glucose was given. This suggests formation of sucrose from a phosphorylated form of fructose.

Albedo tissue from lemons carried out a relatively constant rate of incorporation of glucose activity into sucrose throughout the growth of the fruit, but the rate of incorporation in the flavedo and juice sacs fell off rapidly with increase in age.

In the intact fruit, glucose is incorporated into sucrose much more rapidly than fructose. When glucose is fed, the free fructose pool also becomes labeled in both lemons and oranges. When fructose is the labeled sugar imbibed, a small amount of label appears in the free glucose of lemons but no conversion to free glucose appears in oranges.

Literature Cited

1. Axelrod, B., and C. G. Seegmiller. 1954. Conversion of glucose to sucrose in apple tissue. Jour. Agr. Food Chem. 2: 1329.
2. Bean, R. C., and W. Z. Hassid. 1955. Synthesis of disaccharides with pea preparations. Jour. Amer. Chem. Soc. 77: 5737.
3. Bean, R. C. 1959. Glucose aerodehydrogenase from young oranges. Fed. Proc. 18: 188.
4. Bean, R. C. and G. G. Porter. 1959. Detection and differentiation of sugars and polyols on single paper chromatograms. Anal. Chem. 31: 1929.
5. Buchanan, J. G. 1953. Path of carbon in photosynthesis. XIX. The identification of sucrose phosphate in sugar beet leaves. Arch. Biochem. Biophys. 44: 140.
6. Burma, D. P. and D. C. Mortimer. 1956. The biosynthesis of uridine diphosphate glucose and sucrose in sugar beet leaves. Arch. Biochem. Biophys. 62: 16.
7. Cabib, E., L. F. Leloir, and C. E. Cardini. 1953. Uridine diphosphate acetylglucosamine. Jour. Biol. Chem. 203: 1055.
8. Dische, Z., and E. Borenfreund. 1951. A new method for the detection and determination of keto sugars. Jour. Biol. Chem. 192: 583.
9. Edelman, J., V. Ginsburg, and W. Z. Hassid. 1955. Conversion of monosaccharides to sucrose and cellulose in wheat seedlings. Jour. Biol. Chem. 213: 843.
10. Leloir, L. F. and C. E. Cardini. 1953. Biosynthesis of sucrose. Jour. Amer. Chem. Soc. 75: 6084.
11. Leloir, L. F. and C. E. Cardini 1955. The biosynthesis of sucrose phosphate. Jour. Biol. Chem. 214: 157.
12. Leloir, L. F., C. E. Cardini, and J. Chiriboga. 1955. The biosynthesis of sucrose. Jour. Biol. Chem. 214: 149.
13. Putman, E. W. and W. Z. Hassid. 1954. Sugar transformations in leaves of *Canna indica*. I. Synthesis and inversion of sucrose. Jour. Biol. Chem. 207: 885.
14. Putman, E. W. and W. Z. Hassid. 1954. Isolation and purification of radioactive sugars by means of paper chromatography. Jour. Biol. Chem. 196: 749.
15. Putman, E. W., W. Z. Hassid. G. Krotkov, and H. A. Barker. 1948. Preparation of radioactive carbon-labeled sugars by photosynthesis. Jour. Biol. Chem. 173: 785.