SUCROSE BREAKDOWN AND SYNTHESIS IN THE RIPENING GRAPE BERRY*

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Sucrose is the major translocated sugar in the grapevine (Swanson and ElShishiny 1958), but in the berry glucose and fructose together make up the bulk of the sugar content at all stages of development (Kliewer 1965*a*). The inversion of sucrose to glucose and fructose in ripening berries is suggested by the presence of invertase (Arnold 1965) and by the occurrence of approximately equal amounts of glucose and fructose (Kliewer 1965*a*). During the early stages of berry development, glucose and fructose concentrations are low, but at the onset of ripening there begins a phase of rapid glucose and fructose accumulation (Kliewer 1965*a*). Besides sugar, the other major soluble constituents of grapes are malate and tartrate. The concentrations of both substances increase rapidly in the immature berry, but when ripening commences sharp decreases occur, particularly in the concentration of malate (Kliewer 1965*b*). Some workers believe that malate and tartrate are translocated from the leaves (Amerine 1956; Peynaud and Maurié 1958) but there is evidence that some at least of the malate and tartrate is synthesized in the berry (Hale 1962). The mechanisms of the syntheses of malate and tartrate in the berry are not known.

In an experiment designed to give information on the fate of sucrose in the ripening berry, individual excised sultana (cv. Sultanina, Thompson Seedless) berries were presented with radioactive sucrose, glucose, or fructose and the distribution of radioactivity in sugars and organic acids was examined after 7·5 and 24 hr. Each treatment was duplicated.

Materials and Methods

Uniformly ^{14}C-labelled sucrose (specific activity 22·8 μc/μmole), glucose (specific activity 87 μc/μmole), and fructose (specific activity 86·2 μc/μmole) were obtained from the Radiochemical Centre, Amersham, England, and were used without altering the specific activities. Samples for counting were dried on glass planchets and counted at infinite thinness at 35% efficiency using a thin end-window gas-flow detector. A Nuclear Chicago Actigraph III instrument was used, with a 3 mm slit width and set at 1000 counts/min for full-scale deflection.

Twelve sultana berries (approximately 1·1 g fresh weight) were selected from a bunch of grapes and cut off leaving a 5-mm length of pedicel attached. 20-mm lengths of Tygon plastic tubing (internal diameter 1·6 mm) were fitted over the pedicel stumps and 2 μc ($1{\cdot}5 \times 10^6$ counts/min) of radioactive sugar in 10 μl distilled water was placed in each tube in contact with the cut end of the pedicel. The experiment was carried out at room temperature. The solutions were completely taken up by the berries in between 1 and 3 hr, after which the plastic tubes were kept full of distilled water. At 7·5 or 24 hr after administration of the radioactive sugars, the

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pedicel stumps were cut off at the base and the berries placed in boiling 80% ethanol. The berries were later extracted in a further four changes of 5 ml of 80% ethanol for a total of 100 min. The extracts were evaporated down to 2 or 3 ml and cleared by centrifugation. The aqueous extracts were made up to 10 ml with water and 0·5 ml of each was passed down a 10 by 1 cm column of Dowex 50W, X10, hydrogen form, 20–50-mesh resin to remove basic compounds which were afterwards eluted with 100 ml 4N NH_4OH. Recovery from this resin averaged $96 \cdot 75 \pm 3 \cdot 15\%$. The Dowex 50 effluent, containing sugars and organic acids, was chromatographed on Whatman 3MM paper using as solvent the organic phase of a 24-hr aged mixture of n-butanol–acetic acid–water (4 : 1 : 5 v/v) until the solvent reached the bottom of the paper. An automatic strip scanner was employed to examine the distribution of radioactivity and the positions of individual compounds were located by reference to standards. Aliquots of the original extracts were used for determination of total reducing sugar (Shallenberger and Moores 1957) and ketose (Ashwell 1957). Sucrose

TABLE 1

RADIOACTIVITY IN SUGARS AND ORGANIC ACIDS FROM INDIVIDUAL GRAPE BERRIES SUPPLIED WITH [^{14}C]SUCROSE, [^{14}C]GLUCOSE, OR [^{14}C]FRUCTOSE

The values for both duplicates are given

Duration of Feeding (hr)	Radioactivity (10^{-3} × counts/min) in Sugars and Organic Acids		
	Berry Supplied with 2μc* [^{14}C]Sucrose	Berry Supplied with 2μc* [^{14}C]Glucose	Berry Supplied with 2μc* [^{14}C]Fructose
7·5	824	196	516
	1117	302	685
24	570	308	292
	404	410	449

* $1 \cdot 5 \times 10^6$ counts/min.

determinations, made after alkaline destruction of fructose (Pontis and Leloir 1962), revealed that the sucrose concentration in each berry was less than 0·03 g/100 g fresh weight, and the ketose values were therefore assumed to be due to fructose. Glucose was determined by difference.

Results

The mean fresh weight of the 12 berries was $1 \cdot 106 \pm 0 \cdot 10$ g. The mean glucose and fructose concentrations were $3 \cdot 34 \pm 0 \cdot 32$ g and $3 \cdot 31 \pm 0 \cdot 31$ g per 100 g fresh weight, respectively. These values place the berries at the stage of development when glucose and fructose concentrations are increasing rapidly, and when malate and tartrate concentrations are on the decline (Kliewer 1965*a*, 1965*b*).

The radioactivity in basic compounds (amino acids) from all berries was small and will not be discussed further. The total radioactivity in sugars and organic acids for each berry is shown in Table 1. At 7·5 hr, the radioactivity in sugars and

organic acids from [^{14}C]sucrose-treated berries represented about two-thirds of the total supplied radioactivity, but in the berries treated with [^{14}C]glucose or [^{14}C]fructose considerably less radioactivity was present at this time, suggesting that sucrose was the least readily metabolized of the three. More radioactivity was found in the sugars and organic acids of [^{14}C]fructose-treated berries at 7·5 hr than of [^{14}C]glucose-treated berries, suggesting that glucose was the more readily metabolized of the two exogenously supplied monosaccharides. After 24 hr, decreases had occurred in the radioactivity isolated from [^{14}C]sucrose- and [^{14}C]fructose-treated berries, especially in the former case. No such decrease occurred in the berries presented with [^{14}C]glucose.

In Table 1 it can be seen that there were considerable differences between duplicates in regard to total radioactivity isolated. The patterns of distribution of radioactivity [Figs. 1(*a*)–1(*c*)] in the duplicates were, however, remarkably uniform. In all instances radioactivity was found in malate showing that this compound can be synthesized from sugar in the berry. However, no radioactivity was detected in tartrate. When either [^{14}C]glucose or [^{14}C]fructose were supplied, both monosaccharides became labelled, demonstrating the presence of a mechanism for the interconversion of these sugars. Sucrose synthesis occurred in all berries presented with radioactive monosaccharides.

7·5 hr after administration of [^{14}C]sucrose [Fig. 1(*a*)] fructose was the predominantly labelled monosaccharide, suggesting that the glucose moiety of the exogenously supplied sucrose was preferentially metabolized. When [^{14}C]glucose was supplied, glucose and fructose were found to be equally labelled at 7·5 hr [Fig. 1(*b*)] but when [^{14}C]fructose was supplied, the extracted monosaccharides were labelled mainly in fructose at this time [Fig. 1(*c*)]. At 7·5 hr, a somewhat greater proportion of the radioactivity was found in malate from [^{14}C]glucose- than from [^{14}C]fructose-treated berries. These observations suggest that glucose was the more readily metabolized of the two exogenously supplied monosaccharides.

In Figures 1(*a*) and 1(*b*) it can be seen that at 7·5 hr after berries were presented with [^{14}C]sucrose or [^{14}C]fructose, the predominantly labelled monosaccharide was fructose in both instances. After 24 hr, fructose remained as the predominantly labelled monosaccharide in berries treated with [^{14}C]fructose, but in the [^{14}C]sucrose-treated berries, glucose and fructose had become equally labelled. The total radioactivity was also reduced to a greater extent between 7·5 and 24 hr in the [^{14}C]sucrose-treated berries. These findings indicate that at 7·5 hr a proportion of the exogenously supplied [^{14}C]fructose had penetrated to a site where it could not subsequently be metabolized or converted to glucose, whereas in the [^{14}C]sucrose-treated berries, much of the fructose which had become labelled at 7·5 hr was later metabolized.

Discussion

The results indicated that exogenously supplied glucose was more readily metabolized in the berries than was fructose. This could result from the preferential phosphorylation of glucose or the more rapid penetration of glucose to metabolic sites in the berry. The predominant labelling of fructose 7·5 hr after administration of [^{14}C]sucrose could therefore be the result of the preferential utilization of glucose

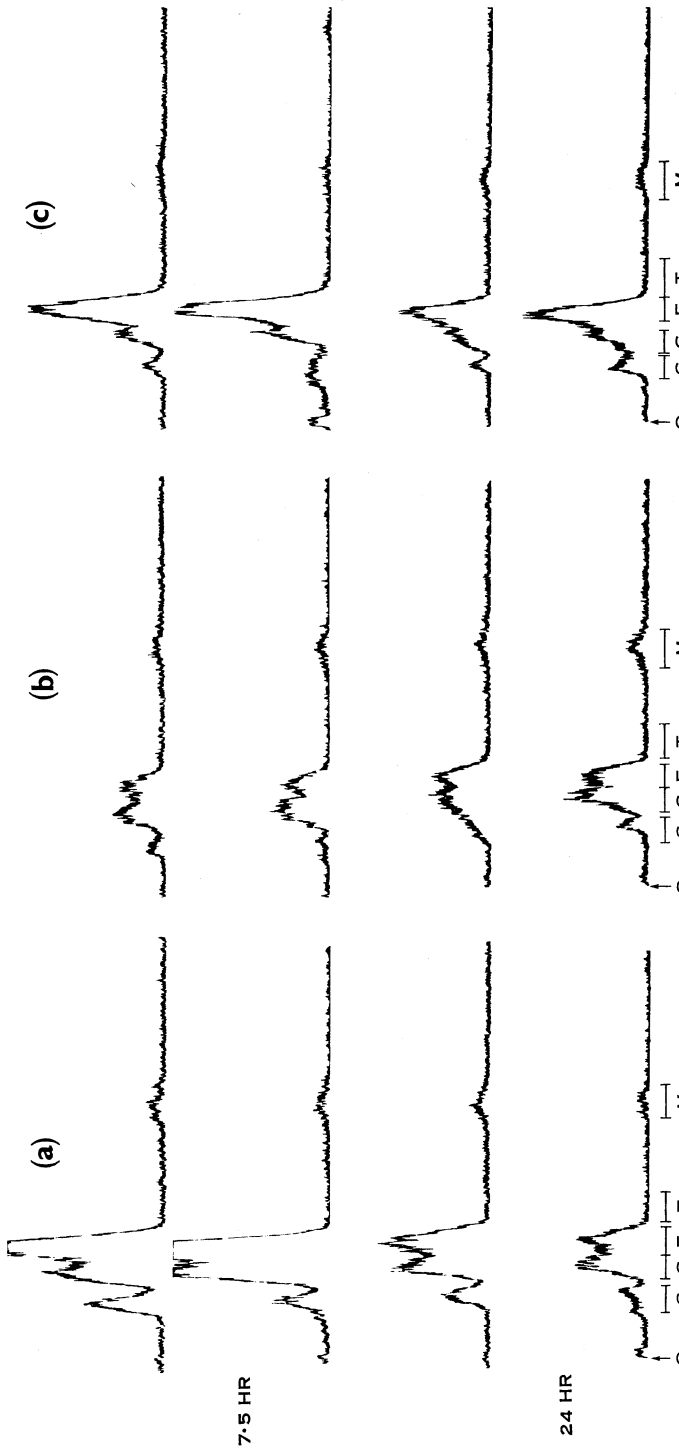

Fig. 1.—Distribution of radioactivity (in duplicate) in sugars and organic acids from individual grape berries 7·5 or 24 hr after administration of [^{14}C]sucrose (*a*), [^{14}C]glucose (*b*), or [^{14}C]fructose (*c*). *O*, origin; *S*, *G*, *F*, *T*, *M*: positions of sucrose, glucose, fructose, tartaric acid, and malic acid, respectively.

following inversion of sucrose. The synthesis of sucrose from exogenously supplied monosaccharides, however, indicates the activity of one or both of the two sucrose (or sucrose phosphate) synthesizing enzymes known to occur in plants: UDPglucose–fructose transglycosylase or UDPglucose–fructose-6-phosphate transglycosylase (UDP = uridine diphosphate). The former enzyme catalyses a freely reversible reaction (Cardini, Leloir, and Chiriboga 1955):

$$\text{UDPglucose} + \text{fructose} \rightleftharpoons \text{UDP} + \text{sucrose},$$

and it could conceivably participate in sucrose breakdown provided that UDPglucose can be utilized. This reaction could therefore account for the predominant labelling of fructose on administration of [^{14}C]sucrose [Fig. 1(*a*)].

The results of the present work probably apply only to sultana berries at the stage of rapid sugar accumulation: at earlier stages of development, the concentration of glucose may be up to five times that of fructose (Kliewer 1965*a*). When $^{14}CO_2$ was presented to the leaves of sultana vines bearing clusters of immature grapes, the monosaccharides afterwards extracted from the berries were found to be labelled predominantly in glucose (Kliewer 1964). These results suggest that the changes in sugar content at different stages of berry development may be associated with changing mechanisms of sucrose utilization.

7·5 hr following the administration of [^{14}C]glucose and [^{14}C]fructose, the isolated radioactivity represented less than one-half of the total supplied radioactivity. Most of the radioactivity remaining at 7·5 hr was still present at 24 hr. The patterns of distribution of radioactivity in sugars and organic acids were the same at 24 hr as at 7·5 hr. This indicates that a substantial proportion of the exogenously supplied glucose and fructose was rapidly metabolized whilst the remainder, after undergoing interconversion, was transported to a metabolically inactive site. That the exogenously supplied monosaccharides which were metabolized did not first equilibrate with the endogenous glucose and fructose is suggested by the apparent preferential utilization of glucose whilst the total contents of glucose and fructose in the berries remained equal. When either [^{14}C]glucose or [^{14}C]fructose was supplied, radioactivity appeared in sucrose. Since the total content of either glucose or fructose was at least 100 times that of sucrose, the specific activity of the extracted sucrose must have been considerably higher than that of either monosaccharide in all cases. Thus the bulk of the endogenous glucose and fructose did not take part in sucrose synthesis.

These results suggest that glucose and fructose accumulate in pools separate from the sites where sugar interconversion and breakdown take place, and that the sugar utilized in metabolic processes is probably derived primarily from sugar transported into the berry. The present results do not show whether these separate regions of sugar metabolism and sugar storage represent different regions of the berry or separate intracellular compartments.

Further studies are presently being undertaken to examine the fate of sucrose in berries at different stages of development, employing sucrose labelled only in the fructose moiety.

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