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Metabolism of Sugars and Organic Acids in Immature Grape Berries

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Abstract. **Individual intact excised immature Sultana berries were supplied through the cut pedicel with ^{14}C-sugars and organic acids. When ^{14}C-hexoses were supplied malic and tartaric acids accounted for 25 % and 10 % of the total activity extracted after 24 hours, and sucrose was synthesized. It is proposed that the changes in the levels of organic acids during ripening are related to changes in the ability of the berry to synthesize them. Although administration of uniformly labeled sucrose resulted in the unequal labeling of glucose and fructose, the results indicate breakdown of sucrose by invertase. It is suggested that the route of entry of the pedicel-fed sugars into the berry may be different from the route taken by sugar translocated from the leaf.**

Sucrose is the major translocated sugar in grapevines (12) but glucose and fructose make up the bulk of the sugar in the grape berry at all stages of development (9). During the early stages of berry development the total sugar concentration is low and glucose exceeds fructose by up to 5 times. At the onset of ripening the concentrations of glucose and fructose increase rapidly and soon become equal (9). Malic and tartaric acids increase in concentration in the immature berry but decline during ripening (8). Both acids can be synthesized in the immature berry (4). Radioactive malic and tartaric acids have been isolated from grape berries following administration of $^{14}CO_2$ to the leaves, malic acid being formed more rapidly than tartaric (4, 6, 7). The labeling of organic acids in the berry is greater where $^{14}CO_2$ is supplied to leaves of vines bearing immature fruit than nearly ripe fruit (6) and the labeling of organic acids is also influenced by temperature (6) and light intensity (7). In these experiments it is unclear whether the compounds becoming labeled in the berry are translocated from the leaves or are formed from sucrose in the berry. Thus the changes in the relative amounts of sugars and organic acids at different stages of berry development may be due either to changes in the composition of the translocated material or to changes in the metabolism of translocated sucrose in the berry. The use of individual excised intact grape berries permits the study of the utilization of various ^{14}C-metabolites over short time intervals. When ^{14}C-sucrose, glucose or fructose were supplied to excised ripening berries, small amounts of radioactivity appeared in malic acid but none in tartaric acid (5).

The presence of invertase (1, 2) and equal amounts of glucose and fructose in mature berries (9) suggests that the translocated sucrose may be inverted. When ^{14}C-sucrose was presented to ripening berries however, fructose became more heavily labeled than glucose (5), which seemed inconsistent with inversion, and evidence for sucrose synthesizing enzymes was obtained (5). Thus it is conceivable that sucrose is broken down by a reversal of the sucrose synthetase reaction, resulting in the production of UDP-glucose and fructose.

The present paper describes experiments on the utilization of ^{14}C-sugars and organic acids by immature grape berries.

Materials and Methods

Grape berries were taken from fully grown vines of *Vitis vinifera* L., cv. Sultanina (syn. Sultana, Thompson Seedless) growing in a vineyard near Adelaide, South Australia. The berries used in an experiment on ^{14}C-sucrose utilization were harvested 3 weeks after flowering when the fresh weight was 0.22 g/berry. For the experiment on ^{14}C-glucose, fructose, malic and tartaric acid utilization the berries were taken 4 weeks after flowering when the fresh weight was 0.37 g/berry.

Uniformly ^{14}C-labeled sucrose (22.8 μc/μmole), glucose (87 μc/μmole), fructose (86.2 μc/μmole), L-malic acid (15.1 μc/μmole), and D,L-tartaric acid-1,4-^{14}C (5.1 μc/μmole) were obtained from the Radiochemical Centre, Amersham, U.K. and were used without altering the specific activities. Sucrose labeled only in the fructose moiety was prepared by means of UDP-glucose-fructose glucosyl transferase from wheat germ. The enzyme was prepared according to the procedure of Cardini *et al.* (3) and the purification was taken as far as the first ammonium sulfate fractionation. The reaction medium contained 2.0 M tris buffer (pH 7.2),

20 μl; 0.05 M UDP-glucose, 40 μl; enzyme, 40 μl and 200 μc of the ^{14}C-fructose in a total volume of 3.0 ml. The mixture was incubated 1 hour at 37°. Sucrose was isolated by paper chromatography. No radioactivity was detected in glucose after invertase treatment of a sample of the sucrose and separation by paper chromatography.

Radioactive compounds were administered to individual excised immature berries via the cut pedicel by a method described previously (5) in the dark at 25° in a dry atmosphere. In all experiments, for each sampling, 2 berries were used and extracted and analyzed separately. Each result is the mean of the 2 replicates. Most of the berries absorbed the 10 μl of water containing 2 μc radioactive compound in 70 minutes. At this time the plastic tubes and pedicels were removed and the berries were placed in test tubes in the dark at 25°. The tubes were closed with stoppers bearing filter paper strips soaked in 0.15 ml 10 % KOH. The alkali papers were changed at intervals and dropped into test tubes containing 5 ml distilled water. Aliquots of the solutions were dried on glass planchets using an infra red lamp. Each sample was counted as soon as it was dry using a gas-flow detector with 37 % efficiency. Self absorption was less than 5 % and no correction was made.

In an experiment on the utilization of uniformly-labeled sucrose and sucrose labeled only in the fructose moiety, berries were killed at 0, 1, 3, 6 or 12 hours after they were placed in the tubes with alkali papers. The utilization of ^{14}C-glucose, fructose, malic and tartaric acids was studied over periods of 2 or 24 hours. The berries were killed, extracted and the soluble compounds analyzed for ^{14}C distribution as previously described (5) with the following modifications. The effluent from the Dowex 50 column was run directly through a 5 × 1 cm column of Amberlite IR4B resin in the hydroxyl form. Anions were eluted with 50 ml 4N NH_4OH, and after washing with water the resin was ready to be used again. Neither decomposition nor absorption of glucose or fructose occurred on the resin and malic and tartaric acids were recovered quantitatively. The IR4B eluates were evaporated to dryness in a rotary evaporator. Volatile ammonium salts were removed by dissolving the residue in a few ml water and evaporating to dryness again. The free organic acids were obtained by passing a solution of the ammonium salts down a 3 cm × 1 cm column of Dowex 50 resin in the hydrogen form. Organic acids were chromatographed on paper using the organic layer of a mixture of *n*-butyl alcohol, formic acid and water (1:1:1, v/v) which had been allowed to stand overnight, or of a mixture of *n*-butyl alcohol, acetic acid and water (4:1:5, v/v). Sugars were separated on paper using a mixture of ethyl acetate, pyridine and water (8:2:1, v/v). Chromatograms were scanned using a Nuclear Chicago Actigraph II instrument and the peak areas measured with a planimeter to give the percentages of total extracted ^{14}C in different components of the extracts.

Results

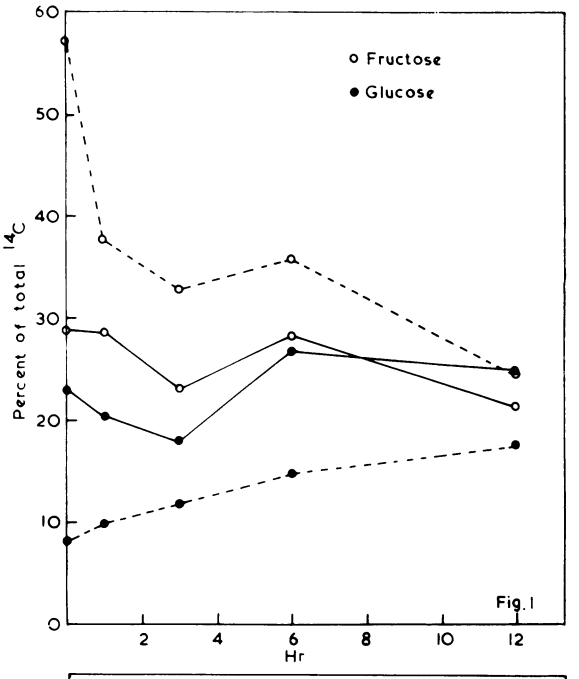
Determinations of fresh weight and concentrations of glucose, fructose, malic and tartaric acids were made throughout the 1966-67 season. The changes during the course of berry development were in general accord with those reported by Kliewer for the same cultivar (8, 9). Sugar concentrations began to rise during the seventh week after flowering and the greatest rate of increase in fresh weight and sugar concentrations was during the ninth and tenth weeks. The decline in organic acids began in the eighth week.

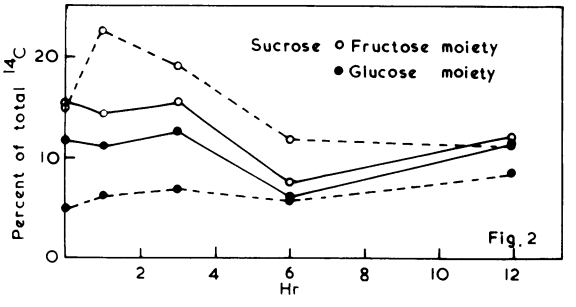
Utilization of Uniformly-labeled Sucrose and Sucrose Labeled Only in the Fructose Moiety. The mean recovery of ^{14}C (extract plus CO_2) from all berries supplied with radioactive sucrose was 43.9 ± 7.8 % of the total ^{14}C supplied. Figures 1 and 2 show that most of the ^{14}C-sucrose absorbed through the pedicel was immediately broken down; only 20 to 25 % of total ^{14}C remained in sucrose while glucose and fructose together accounted for 50 to 60 % of the extracted ^{14}C at this time. During the following 12 hours there was little further decrease in the percentage of total ^{14}C in sucrose. Changes occurred in the ratio of label in the glucose and fructose moieties of sucrose which were paralleled by the changes in the ratio of labeling of the free glucose and fructose (table I). When sucrose labeled only in the fructose moiety was supplied the ^{14}C-glucose/^{14}C-fructose ratios increased towards unity during the 12 hour incubation showing that the radioactive sucrose remaining in the berries was being broken down and resynthesized.

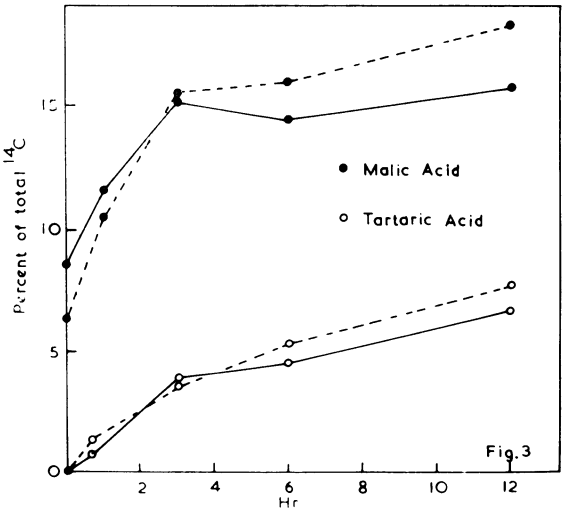
When uniformly labeled sucrose was supplied the labeling of fructose was initially slightly greater than that of glucose (fig 1) but on administration of sucrose labeled only in the fructose moiety nearly all of the hexose labeling was in fructose in the early stages. These results show that the radioactive glucose formed from uniformly labeled sucrose came from the glucose moiety of sucrose and not from conversion of fructose to glucose following breakdown of sucrose to UDP-glucose and fructose.

Figure 3 shows the time course of the percentages of total ^{14}C in malic and tartaric acids. A rapid transformation of sucrose to malic acid occurred such that malic acid accounted for 15 % of total ^{14}C in 3 hours. Tartaric acid accounted for 7 % of total ^{14}C in 24 hours. There was no significant difference between the contributions of the glucose and fructose moieties of sucrose to organic acid synthesis.

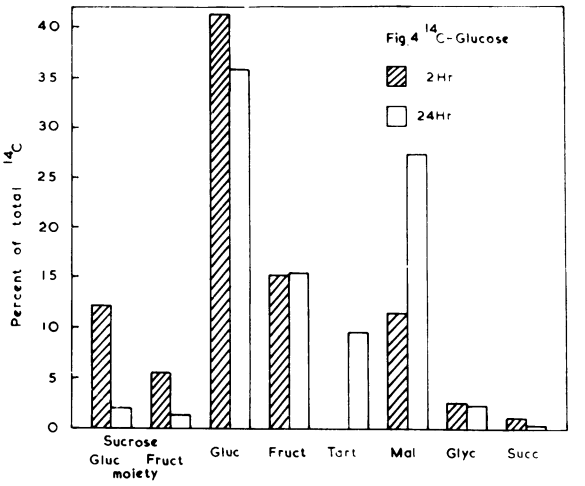
Small amounts of radioactivity were detected in amino acids and in other organic acids which

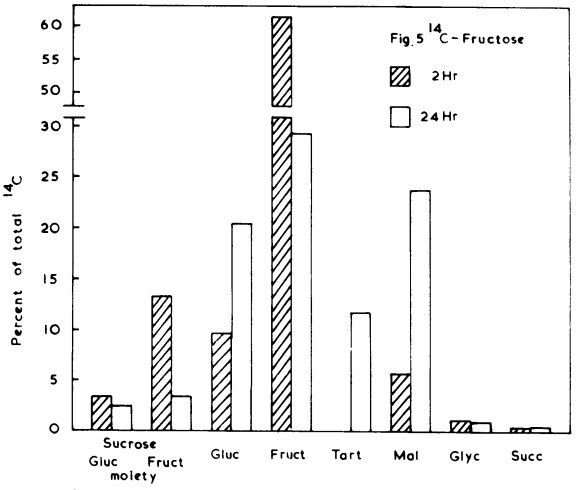


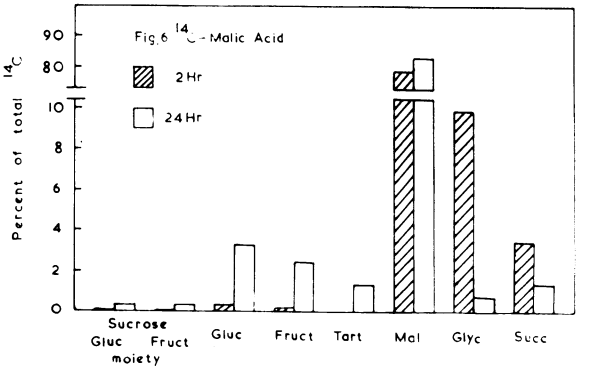




FIGS. 1, 2, 3. Changes in the percentage of total extracted ^{14}C in glucose and fructose (fig 1), the glucose and fructose moieties of sucrose (fig 2), and malic and tartaric acids (fig 3) at various times after uptake of uniformly labeled sucrose (solid lines) or sucrose labeled only in the fructose moiety (broken lines) by immature grape berries. Pedicels were removed at 0 time which was 70 minutes after administration of the radioactive solutions.







FIGS. 4, 5, 6. Percentage distribution of total extracted ^{14}C in sugars and organic acids from immature grape berries 2 or 24 hours after uptake of ^{14}C-glucose, fructose or L-malic acid.

Table I. *Ratio of ^{14}C-Glucose to ^{14}C-Fructose in Free Hexoses and in Sucrose Extracted from Immature Berries at Different Times after Administration of Uniformly Labeled Sucrose or Sucrose Labeled only in the Fructose Moiety*

Hr	Uniformly labeled sucrose		Sucrose labeled in fructose moiety only	
	Glucose / *Fructose*	*G moiety of sucrose* / *F moiety of sucrose*	*Glucose* / *Fructose*	*G moiety of sucrose* / *F moiety of sucrose*
0	0.80	0.75	0.14	0.32
1	0.72	0.75	0.26	0.27
3	0.78	0.82	0.36	0.36
6	0.94	0.82	0.42	0.48
12	1.19	0.95	0.71	0.67

corresponded to phosphate esters, citric, glycolic, succinic and fumaric acids on the paper chromatograms. The percentage of the total ^{14}C in these acids was highest at the beginning of the experiment and declined with time.

The contributions of the glucose and fructose moieties of sucrose to respiratory CO_2 were similar. The total ^{14}C recovered in CO_2 during the 12 hour incubations represented 20 to 25 % of the radioactivity extracted from the berries at 12 hours.

Utilization of ^{14}C-Glucose, Fructose, L-*Malic Acid and* D,L-*Tartaric Acid-1,4-^{14}C.* Figures 4, 5 and 6 show the percentage distribution of ^{14}C in the sugars and organic acids isolated at 2 or 24 hours from berries treated with ^{14}C-glucose, fructose or malic acid. ^{14}C-Glucose was more rapidly metabolized than ^{14}C-fructose in the first 2 hours. This is shown by the relatively heavier labeling of malic, glycolic and succinic acids and by the more rapid conversion of ^{14}C-glucose to fructose than of ^{14}C-fructose to glucose. Both ^{14}C-hexoses were converted to sucrose and the ratios of label in the glucose to fructose moieties again were similar to those in the free hexoses. Both ^{14}C-hexoses were converted to organic acids. At 24 hours malic and tartaric acids accounted for approximately 25 % and 10 % of the total ^{14}C respectively in both cases. Two hours after supplying ^{14}C-L-malic acid, most of the radioactivity in compounds other than malic acid was in the organic acids corresponding to glycolic and succinic. Radioactivity was also found in areas of the chromatograms corresponding to citric and fumaric acids. The percentage of the total ^{14}C in these acids decreased during the following 22 hours and some appeared in tartaric acid and sugars. D,L-Tartaric acid-1,4-^{14}C was metabolized very slowly. Traces of ^{14}C were detected in compounds corresponding to glycolic and malic acids but over 99 % of the ^{14}C extracted at 24 hours remained in tartaric acid.

The relative contributions of ^{14}C-glucose, fructose, malic and tartaric acids to respiratory CO_2 are shown in figure 7. Malic acid was the most rapidly respired substrate. ^{14}C-Glucose was respired more rapidly than ^{14}C-fructose in the first 5 hours, but afterwards the rates of $^{14}CO_2$ release per unit of total extracted ^{14}C were the same. In

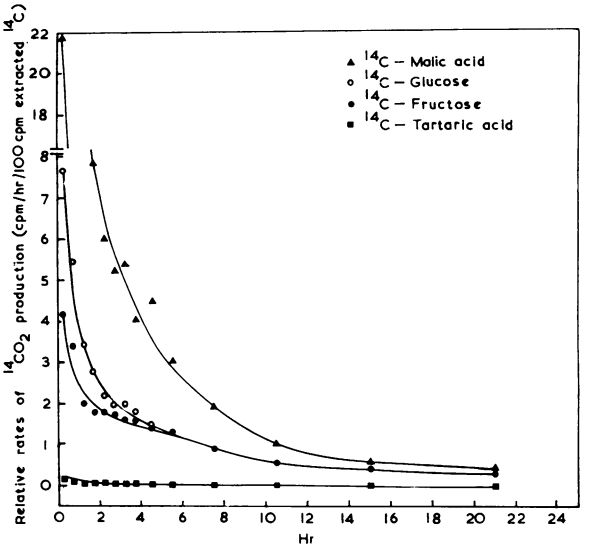


FIG. 7. Comparison of rates of $^{14}CO_2$ production by immature berries after uptake of uniformly-^{14}C-labeled glucose, fructose, L-malic acid, or D,L-tartaric acid-1,4-^{14}C.

each case the rate of $^{14}CO_2$ release fell sharply from the time CO_2 collection was started and leveled off at a rate which was a small fraction of the initial rate. The total $^{14}CO_2$ collected over the whole 24 hour period from berries treated with ^{14}C-glucose, fructose, malic and tartaric acids represented respectively 25, 20, 59, and 0.5 % of the total ^{14}C extracted from the berries at 24 hours.

Discussion

The high percentages of total extracted ^{14}C in organic acids a few hours after administration of ^{14}C sugar suggests that most of the malate and tartrate in immature berries is formed from glucose and fructose in the berry. However the possibility that some of the organic acid is also translocated as such from the leaves still cannot be dismissed. The present results, taken together with previous work on ripening berries (5) show that towards the onset of ripening, changes occur in the berry causing a marked reduction in the conversion of glucose and fructose to malic acid and a cessation

of tartaric acid biosynthesis. Tartaric acid content per berry remains approximately constant because it is not broken down but malic acid declines both on a concentration and on an absolute basis as it is utilized in respiration and possibly to some extent in conversion to sugar. The results show that malic and tartaric acids are not closely related biochemically. The small amount of radioactivity in tartaric acid in the berries treated with ^{14}C-L malic acid was probably formed after conversion of malic acid to sugar.

The presence of separate regions for metabolism and storage of sugars as evidenced in ripening berries (4) is also shown by the present results, applying also to organic acids. When radioactive malic acid was supplied the rate of $^{14}CO_2$ production in relation to the amount of radioactive malic acid in the berry decreased to one fiftieth of the initial rate during 24 hours. A similar trend occurred when ^{14}C hexoses were supplied showing that these also became sequestered at sites where they were unavailable for respiration.

When uniformly labeled sucrose was supplied to immature berries, fructose was initially more heavily labeled than glucose. This result was also obtained with ripening berries (5) and 1 possible explanation suggested was that sucrose may be broken down by a reversal of the reaction catalyzed by UDP-glucose-fructose glucosyl transferase (5).

$$\text{sucrose} + \text{UDP} \leftrightarrows \text{UDP-glucose} + \text{fructose}$$

This possibility is unlikely in view of the present results obtained with sucrose labeled only in the fructose moiety, in which it was shown that the radioactive glucose formed from uniformly labeled sucrose came from the glucose moiety of sucrose and not from the conversion of fructose to glucose. The possibility that UDP-glucose is an intermediate in the formation of glucose from sucrose:

$$\text{UDP-glucose} \xleftarrow[\text{UTP}]{\text{pyrophosphate}} \text{glucose-1-P} \rightarrow \text{glucose}$$

is unlikely because of the instability of inorganic pyrophosphate (11) and the wide distribution of pyrophosphatase in plants (10) which is considered to make the UDP-glucose pyrophosphorylase reaction essentially a 1 way reaction in the direction of UDP-glucose synthesis (11).

Alternative explanations for the predominant labeling of fructose following administration of uniformly labeled sucrose to grape berries were suggested (5). After inversion of sucrose, glucose may penetrate more rapidly than fructose to metabolic sites in the berry or may be more readily phosphorylated and utilized (5). The present results would seem to favor the former explanation. When ^{14}C hexoses were supplied ^{14}C-glucose was metabolized faster than ^{14}C-fructose. In contrast, administration of $^{14}CO_2$ to leaves of grapevines results in the predominant labeling of glucose (6, 7) and the disparity increases with time from $^{14}CO_2$ administration and increasing temperature (6). These differences in labeling patterns when ^{14}C-sugars are administered via the cut pedicel as opposed to $^{14}CO_2$ leaf feeding experiments may reflect different routes of entry of labeled sugar into the berry: transpiration stream as opposed to phloem transport. That the pedicel-fed sugars enter via the transpiration stream is evidenced by the inhibition of uptake which occurs when the berries are placed in a humid atmosphere, and it was noteworthy that while ripening berries took approximately 3 hours to absorb 10 μl of solution (5) immature berries took up the same amount in little more than 1 hour. The exogenous sugars are probably taken passively into the berry intercellular spaces whence they are absorbed by the surrounding cells. The more rapid absorption of ^{14}C-glucose than ^{14}C-fructose could therefore account for the apparently more rapid utilization of ^{14}C glucose. On administration of ^{14}C-sucrose, hydrolysis of some of the sucrose in the intercellular spaces by insoluble invertase (2) may be followed by the preferential uptake of glucose.

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