Distribution of Solutes within the Developing Grape Berry in Relation to Its Morphology

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Grape berries taken at four stages of ripeness (from veraison to overripeness) were dissected to give 19 segments representing divergent tissues and their content of eight solutes measured. Each solute was found to have distinctive space and time patterns of variation in concentration. In general, solutes that did not accumulate massively (sucrose, phenols, potassium, inorganic anions, and tartrate) were in low concentration in the flesh and in higher concentrations in the skin and brush. Those that did accumulate massively (malate at veraison and glucose and fructose thereafter) had higher concentrations in the flesh than in the skin and brush. Tartrate was high in peripheral flesh at veraison, and potassium was low in peripheral and adjacent flesh at all stages. High levels of sucrose and inorganic anions were found in central flesh of overripe berries. Gradients in malate were created by the low concentrations in segments containing vascular tissue, both peripheral and central, presumably due to malate respiration by this tissue. In one year, but not in the next, a large zone of the flesh of overripe berries extending around the seed from the skin to the opposite empty locule was found to have abnormally high concentrations of many solutes, possibly caused by a localized dehydration of these tissues.

The composition of grape berries and grape juice is of great importance in the making of wine, and much effort is expended in grape analysis. However, nearly all of this effort is directed to the analysis of whole berry or crop samples, prepared in various ways, and surprisingly little to within-berry analysis. There are good reasons why more should be known about the distribution of compounds within the berry: *(1)* The methods of vinification can be chosen better when the influence of inequalities in distribution are known; and *(2)* the basic study of the source, metabolism, and compartmentation of the chemicals in the grape berry depends on such knowledge.

Possner and Kliewer (9) have recently commented on this lack of information and have reported on measurements of two sugars, two organic acids, and two metal cations in concentric zones of Chardonnay berries at frequent intervals from 23 to 122 days after anthesis. Their results showed significant gradients in the concentrations of malate, tartrate, potassium, and calcium across the berry and also that these gradients varied during development; unfortunately, some of these comparisons may be confounded by the mixture of tissues within each zone. They also compared longitudinal zones, but again, their comparisons may be confounded by the gradients across the concentric zones.

The work in this paper had a similar aim. However, a different variety was used, and dissections were made into smaller regions, using less frequent samplings and extending to the overripe stage of development. In addition, the effects of seed presence were examined by selecting one-seeded berries and arranging the dissections to permit comparison with the non-seeded side.

Materials and Methods

The methods were designed to learn as much as possible about differences in distribution using fine dissection of samples and microanalysis. The large number of segments necessitated minimizing numbers of replicates; this was compensated by the large degree of internal replication and the greater care possible in selecting uniform berries at each sampling time (within-sample variation was < 0.5°Brix).

Berries were selected from two mature Muscat Gordo blanco vines (in the Clarement Orchard of the Waite Agricultural Research Institute, Glen Osmond, S.A., Australia), free of surface blemish, turgid, of average to large size, and with one seed (determined by candling and asymmetry in longitudinal shape). Three berries were taken at each of four developmental stages: *(1)* close to veraison, berries hard and green, 6.2°Brix; *(2)* berries ripening, 10.2°Brix; *(3)* early ripe stage, 17.4°Brix; and *(4)* overripe, 26.4°Brix. Intervals between these stages would be about one, four, and six weeks (3).

A disc of tissue *ca* 3 mm wide was removed by two parallel cuts made on either side of the central longitudinal axis and passing though the position of the seed (Fig. 1). The °Brix of juice from the remaining tissue was measured with a hand refractometer and a decision then made about the suitability of that berry. The pedicel and seed were removed from each chosen disc and 19 segments dissected as shown in Figure 1 using a low-power dissecting microscope. Segments 1, 12, 13, and 17 were of peeled skin, without flesh or vascular tissue. The adjacent peripheral flesh segments 2, 11, and 14 contained dorsal vascular bundles. Segments 16, 7, and 19 (central tissue) contained ventral vascular bundles, the latter including the tissue known as the brush. Segments 4, 5, 8, 9, and in some cases 15 had one locular face, those of 8 and 9 being appressed to the seed.

Each segment was blotted lightly, weighed quickly to within 10 μg on an Alfoil "boat," and washed into an Eppendorf tube with sufficient water to make the total water volume, including that in the tissue, 200 μL. Segment weights ranged from 1.5 to 22 mg. The tubes were sealed, held in a boiling water bath for 15 minutes, and then frozen until assayed. An assumption implicit in this method is that the concentrations in the tissues within the tubes were the same as those in the tissues before dissection, *i.e.*, that juice lost during dissection had

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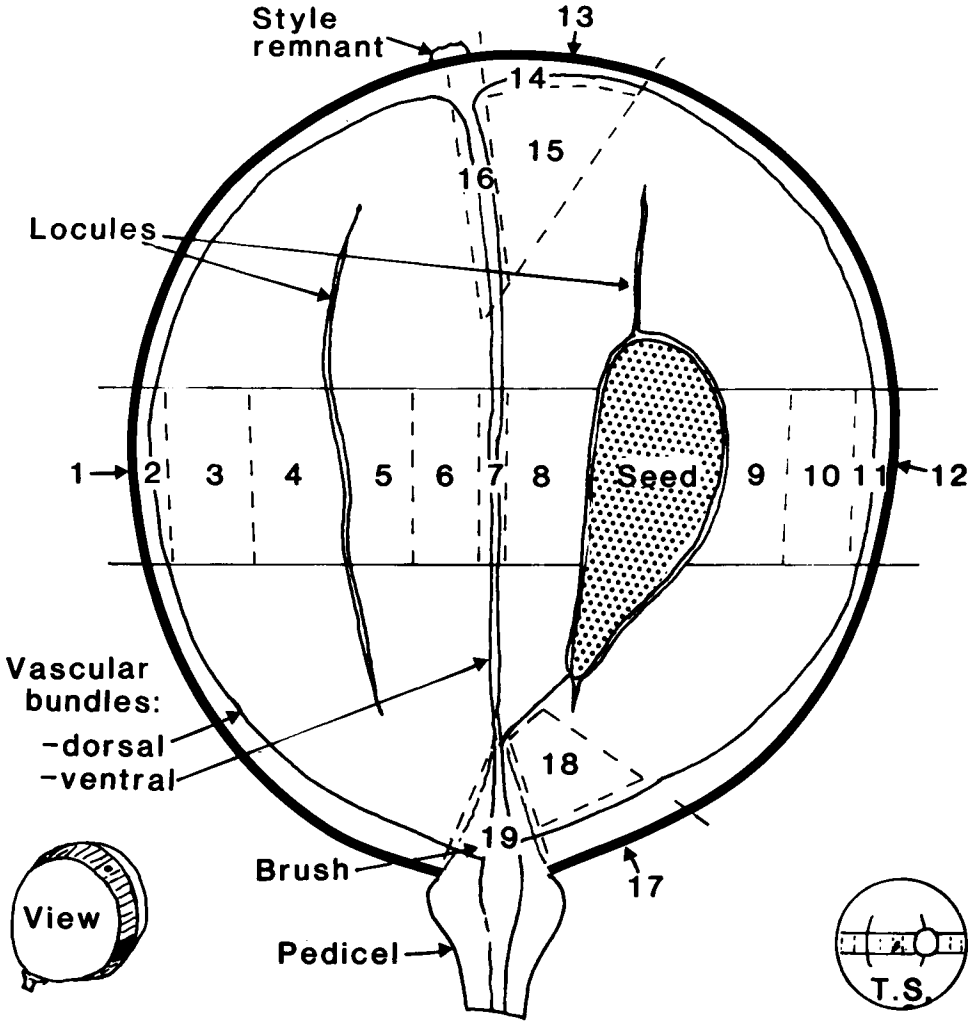

Fig. 1. Central longitudinal section of a one-seeded grape berry showing the position of the cuts made to give 19 segments. The inset drawings show the position of the slice taken from each berry. The results from segments 1 through 12 are shown in Figures 2 through 9 and from the other segments in Tables 1 through 4.

the same concentration as the whole segment.

After thawing and centrifuging, duplicate aliquots were assayed for glucose, fructose, and sucrose by the Boehringer method (1), potassium by flame emission spectrometry, phenols by the Folin-Ciocalteu reagent (11), and malate and tartrate by the ion exclusion liquid chromatography method of Monk and Iland (7). The latter traces showed an additional single peak corresponding to the elution points of several inorganic anions such as sulfate, phosphate, chloride, and nitrate; these were quantified collectively as tartrate equivalents. Where duplicate assays differed from each other by more than 10%, assays were repeated.

Results

Concentrations were calculated per gram fresh weight of tissue and the data analyzed by analysis of variance. Where differences were significant, the collective least significant difference at $p < 0.05$ is indicated on Figures 2 through 9 and Tables 1 through 4.

Concentrations of each solute at four stages of development are shown in two groups: Those of segments lying laterally across the berry (segments 1 - 12) are shown in Figures 2 through 9 and those showing longitudinal values in Tables 1 through 4. Segments 7, 10, 11, and 12 appear in both sets. In the lateral segments (Fig. 2 - 9), there was reasonable agreement between left and right of the center with one exception: segments 5 through 11 of 26°Brix (overripe) berries had exceptionally high glucose and fructose values. Because of these unusual results, this part of the experiment was repeated on the same vines during the next season, and the new results are included in Figures 2 and 3 and Table 1. They form the basis of the presentation which follows, and the comparison with the Year 1 results is taken up at the end of the **Discussion**.

Glucose concentrations in unripe (6°Brix) grapes were double those of fructose, and with development, both hexoses increased at about the same rate to very high values (to between 0.5 and over 1.0 *M* of each hexose) in 26°Brix berries (Fig. 2, 3). Concentrations in skin were generally lower than those in flesh, but otherwise, values within each developmental stage were fairly uniform (except for the above-mentioned 26°Brix data). There were longitudinal differences in the levels of both sugars in the outer and central flesh tissues. The values increased from low near the brush to high at the stylar end (Table 1).

Sucrose concentrations were less than 2 mg/g (mostly 0 - 1 mg/g) in all segments at 6 and 10°Brix. By 17°Brix, sucrose had increased in skin segments and in the extremities of the central tissue, *i.e.*, near the style and in

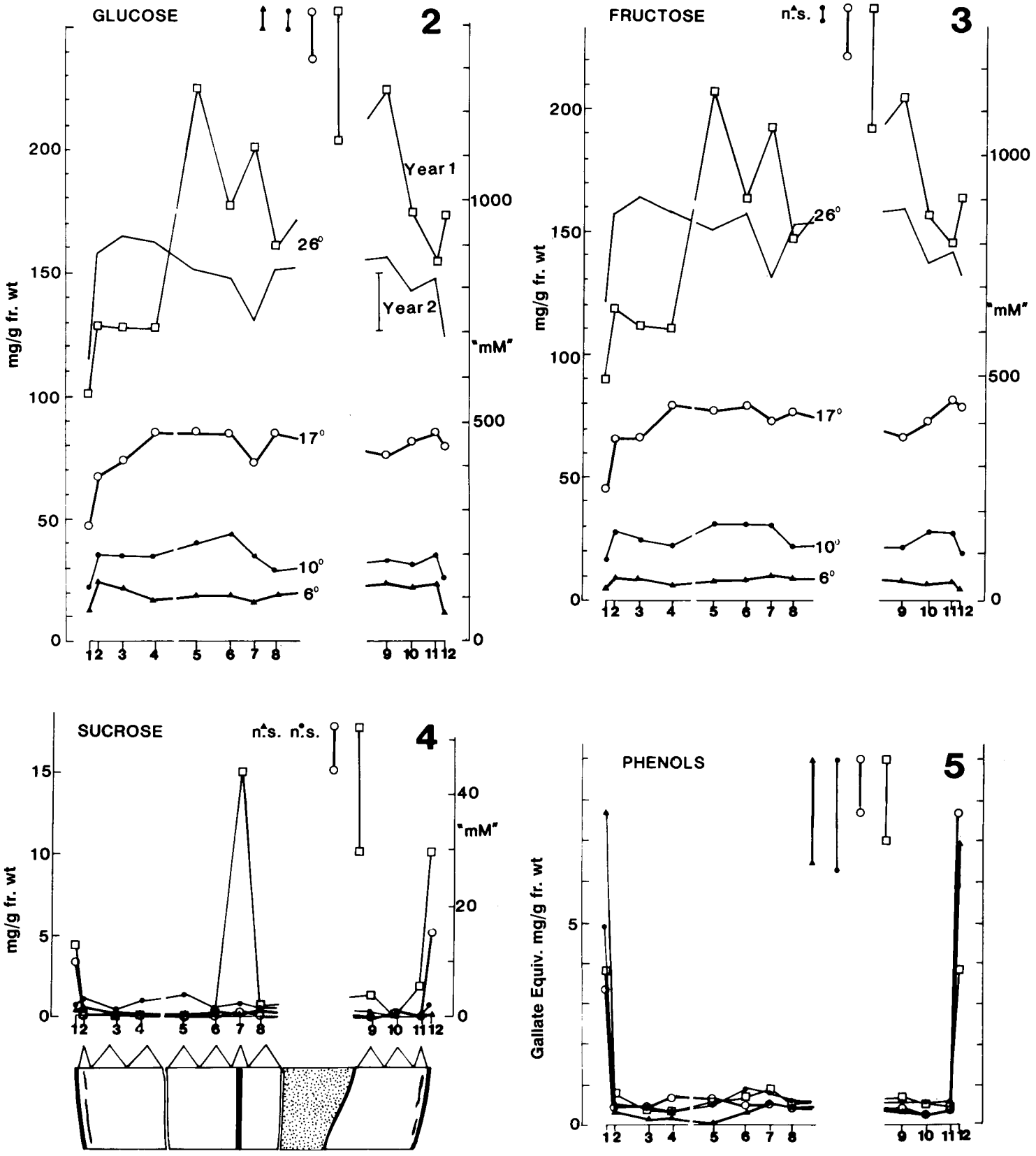

Fig. 2, 3, 4, 5. Concentration of glucose, fructose, sucrose, and phenols in segments 1 through 12 from grape berries at four stages of development (6, 10, 17, and 26°Brix). The sketch at bottom left is to assist recognition of segment positions. The results of statistical analysis are shown at the top of each figure.

Table 1. Glucose and fructose concentrations (mg/g fr. wt) in grape berry segments forming longitudinal sequences from stylar to pedicel end (in the same direction on the page as in Fig. 1).

		Glucose					Fructose				
Tissue	Segment	6°	10°	17°	26°	26° Yr. 2	6°	10°	17°	26°	26° Yr. 2
Skin	13 (nr. style)	10	25	46	142	124	6	21	48	131	128
	12	12	26	81	174	125	5	20	80	164	133
	17 (nr. pedicel)	18	19	85	101	126	6	13	55	98	132
Peripheral	14	19	38	81	140	153	10	27	85	129	153
(dvb)*	11	23	35	85	155	147	8	30	83	146	143
Outer	15	21	39	80	243	150	7	28	77	223	148
flesh	10	22	31	81	175	142	7	28	74	156	138
	18	24	28	61	118	148	11	20	58	106	149
Central	16	19	41	85	198	166	11	36	86	185	157
(vvb)*	7	16	34	73	201	129	10	31	74	192	132
	19 (brush)	17	17	45	78	134	13	15	45	81	134
LSD p = 0.05		8	8	20	51	26	ns	5	18	49	ns

*dvb and vvb signify dorsal and ventral vascular bundles, respectively.

Table 2. Sucrose and phenols (Folin-Ciocalteu reactives as gallic acid equivalents) as mg/g fresh weight in grape berry segments forming longitudinal sequences from stylar to pecidel end.

		Sucrose				Phenols			
Tissue	Segment	6°	10°	17°	26°	6°	10°	17°	26°
Skin	13	0.8	0.3	6.4	10.9	12.3	7.5	5.6	4.5
	12	0.1	0.7	5.2	12.2	6.9	5.9	8.7	3.8
	17	0.3	0.8	4.3	13.4	13.5	10.5	10.3	5.7
Peripheral	14	0.3	0.8	0.4	5.5	1.7	0.9	0.7	0.9
(dvb)*	11	0.2	0	0.4	1.9	0.3	0.6	0.4	0.5
Outer	15	0.4	0.5	0	5.2	0.5	0.6	0.3	1.0
flesh	10	0.1	0	0.3	0	0.2	0.5	0.2	0.4
	18	1.1	0.2	0	5.7	1.1	1.0	0.7	0.9
Central	16	0.4	0.3	3.7	12.8	1.1	0.9	0.8	1.2
(vvb)*	7	0.1	0.8	0.3	15.4	0.5	0.7	0.5	0.9
	19 (brush)	0.7	0	6.8	28.2	4.9	6.9	6.4	2.5
LSD p = 0.05		ns	ns	2.4	7.7	2.6	2.7	1.3	2.0

* dvb and vvb signify dorsal and ventral vascular bundles, respectively.

Table 3. Tartrate and malate concentrations (mg/g fr. wt) in grape berry segments forming longitudinal sequences from stylar to pecidel end.

		Tartrate				Malate			
Tissue	Segment	6°	10°	17°	26°	6°	10°	17°	26°
Skin	13	23	15	11	17	11	12	18	10
	12	27	16	11	13	24	17	15	8
	17	26	14	13	14	18	15	6	7
Peripheral	14	32	13	5	8	24	12	1	3
(dvb)*	11	25	12	8	8	30	17	2	2
Outer	15	21	11	6	9	38	19	4	3
flesh	10	14	11	6	11	43	30	9	6
	18	22	11	7	9	33	21	7	5
Central	16	20	9	5	9	33	14	5	2
(vvb)*	7	14	9	4	9	28	18	5	2
	19 (brush)	22	15	13	22	15	14	6	6
LSD p = 0.05		7	5	3	5	11	9	5	4

* dvb and vvb signify dorsal and ventral vascular bundles, respectively.

the brush. By the 26°Brix stage, sucrose concentration had increased in all of these segments and in the flesh neighboring the extremities of the central strip and in its middle portion.

The levels of phenols were consistently high in the skin and brush and consistently low in all other tissues (Fig. 5, Table 2). The levels in the skin decreased with berry development and distance from the base.

Tartrate concentrations were highest in 6°Brix berries and declined with development to about one-half, much of the decline occurring between 6 and 10°Brix (Fig. 6, Table 3). The distribution of tartrate in 6°Brix berries was distinctive in that high levels occurred in both skin and the adjacent flesh containing dorsal vascular bundles (in basal, mid, and distal positions) and also in outer flesh and central tissues at the pedicel and stylar

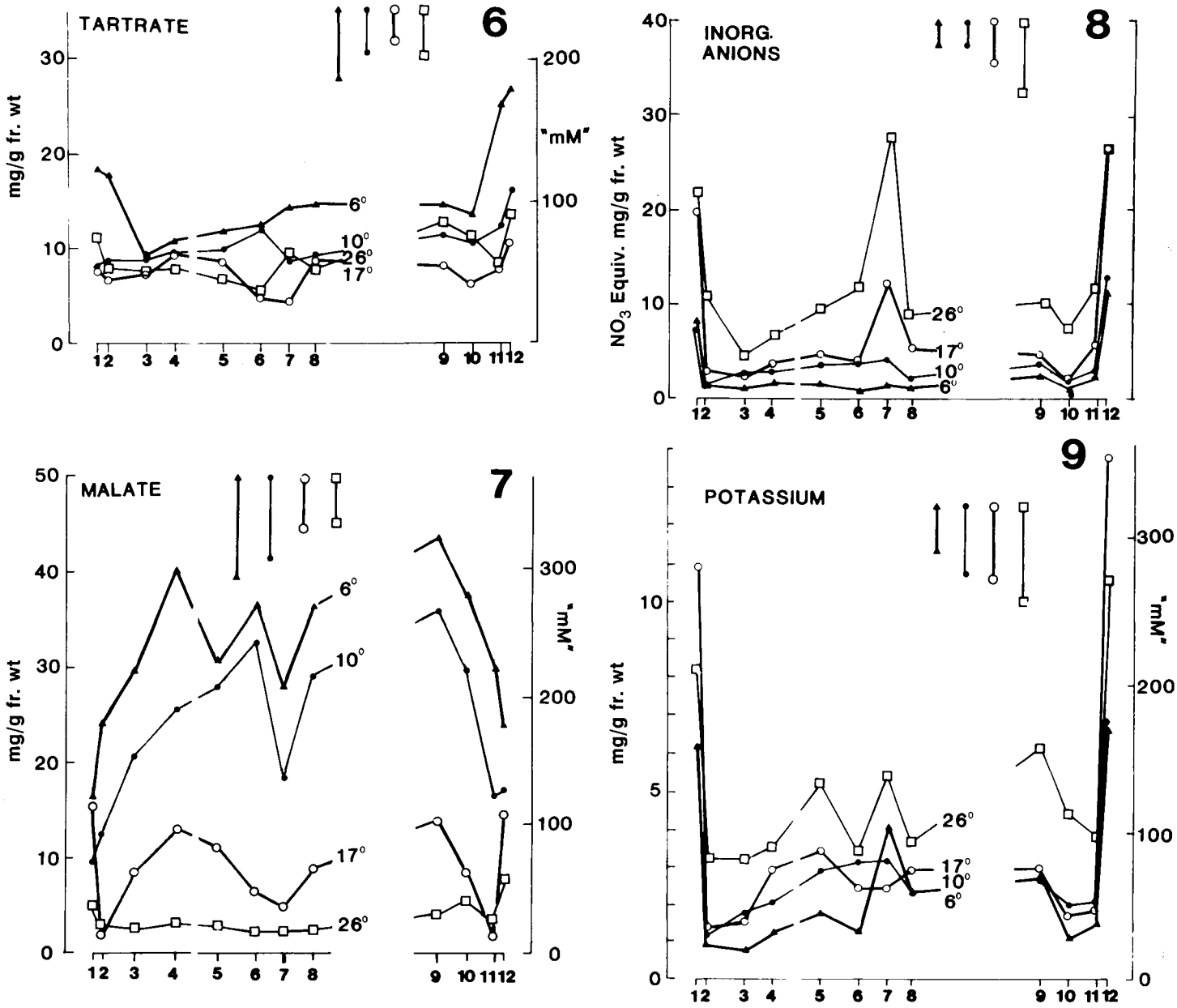

Fig. 6, 7, 8, 9. Concentrations of tartrate, malate, inorganic anions, and potassium in segments 1 through 12 from grape berries at four stages of development (6, 10, 17, and 26°Brix). The results of statistical analysis are shown at the top of each figure.

Table 4. Inorganic anions and potassium concentrations (mg/g fr. wt) in grape berry segments forming longitudinal sequences from stylar to pedicel end.

		Inorganic anions				Potassium			
Tissue	Segment	6°	10°	17°	26°	6°	10°	17°	26°
Skin	13	13	8	18	25	8	7	12	11
	12	11	13	27	26	7	7	14	11
	17	6	8	24	41	6	8	9	12
Peripheral	14	2	2	3	12	2	2	1	3
(dvb)*	11	2	3	6	12	1	2	2	4
Outer	15	2	3	3	10	1	1	2	3
flesh	10	1	2	2	7	1	2	2	4
	18	1	3	5	13	1	2	2	5
Central	16	3	4	10	22	3	3	3	4
(vvb)*	7	1	4	12	27	4	3	3	5
	19 (brush)	4	8	22	39	4	4	5	9
LSD p = 0.05		2	2	4	8	1	2	2	3

* dvb and vvb signify dorsal and ventral vascular bundles, respectively.

ends. With development, these differences declined or disappeared except that tartrate levels remained high in the skin and brush.

Malate was the most abundant solute in the flesh of unripe berries but declined during development by proportions comparable with the degree of increase that occurred in glucose and fructose concentrations (Fig. 7, Table 3). The decline was most rapid between 10 and 17°Brix. Levels in the skin and brush showed smaller changes as berries developed, being initially lower than in flesh and changing less. Decline in the skin occurred earlier near to the pedicel, but levels in the skin adjacent to the style remained constant at all times examined. Within flesh, lowest malate concentrations were found where vascular bundles occurred (both dorsal and ventral), with downward gradients towards these zones. These gradients were evident at all stages except overripe berries, wherein malate levels were uniformly low.

Levels of inorganic anions were highest in the skin and increased with development, especially later (Fig. 8, Table 4). In 17 and 26°Brix berries, the levels were considerable, especially in the skin and central strip; the brush and the skin near the pedicel had particularly high values (Table 4).

Potassium concentrations were highest in the skin and lowest in the flesh tissues adjacent to the skin. They increased with development in all tissues, especially in the skin (Fig. 9, Table 4), but the increase was slow in outer flesh tissue (segments 2, 3, 10, and 11). Longitudinal gradients were small except that the brush developed concentrations in ripe and overripe berries midway between those of skin and flesh tissues.

Discussion

The variations that may occur in the composition of must from crushed grapes, and hence of wine, are readily apparent when the within-berry variations in concentration of this small number of solutes are considered. The graphs of the distribution of each solute had distinctive idiomorphisms, suggesting differences between solutes in the factors controlling their concentration. These are discussed for each tissue in relation to the relevant literature.

Skin: The largest discrepancies in solute concentrations between tissues within the berry were due to skin/flesh differences. Concentrations in skin exceeded those in flesh (on a fresh weight basis), with potassium, inorganic anions, phenols, and tartrate at most stages of development and with sucrose and malate at 17 and 26°Brix. The tartrate differences were the smallest and were probably associated mainly with the greater percentage dry weight of skin tissues. These relationships accord with the literature (1,6,9). The variations are not surprising when one considers the anatomical differences between skin and flesh cells, the former being smaller, thick-walled, and more cytoplasmic (2,4,8).

There were instances where skin concentrations were less than flesh levels; all of these were associated with solutes that had recently accumulated in the flesh in large amounts or were in the process of so doing, *viz.* malate at 6°Brix and hexoses at all stages examined. Both of these groups are regarded as deriving from sucrose transported into the berry via phloem (10).

There were also some instances of longitudinal gradients within skin, the most significant being due to greater decreases in malate and increases in inorganic anions at the pedicel end and decreases in phenols at the stylar end.

The brush: The next largest discrepancy in concentrations was due to the brush tissue (segment 19, Tables 1 - 4). All of the "non-accumulating" solutes showed high levels in the brush, *viz.* sucrose, inorganic anions, phenols, tartrate, and potassium. The largest increases in the first two solutes occurred late in ripening. A high concentration of phenol was also shown to occur in this tissue by Hawker *et al.* (5) and Nii and Coombe (8).

Flesh segments and vascular bundle zones: Many variations in solute concentrations within the flesh were attributable to the presence of the vascular bundles. High concentrations of sucrose were found in central tissue (including the brush) in overripe berries and in inorganic anions in central and peripheral flesh during the latter half of ripening. These differences are probably associated with the nature of phloem unloading in senescing organs.

Tartrate levels were high in the peripheral flesh at the 6°Brix stage, but not thereafter (Fig. 6). Potassium concentrations were lower in the peripheral zone and in the adjacent flesh compared with all more central tissues; these results agree with those of Possner and Kliewer (9).

A striking difference associated with the vascular tissue was in the level of malate (Fig. 7). In both peripheral (dorsal vascular bundles) and central (ventral vascular bundles) flesh zones, malate concentrations were lower than in other flesh segments. Possner and Kliewer (9) describe a malate gradient increasing from the skin towards the center and refer to other papers describing this phenomenon but do not attribute it to a specific effect of the vascular tissue. Steffan and Rapp (12) showed high respiratory losses of $^{14}CO_2$ after perfusing ^{14}C-malic acid through the pedicel of ripening berries and low losses after injecting solutions into the berry center. They interpreted their results as showing an inactive malate pool in the interior flesh from which, following permeability changes associated with ripening, malate was transported to the periphery and there dissimilated. The present results suggest an alternative explanation: that metabolism (respiration) of malic acid is rapid at vascular bundles during ripening, and probably before, and that consequent gradients lead to malate movement towards these zones.

A seed effect: The unusually high concentrations of glucose and fructose in some segments of 26°Brix berries in Year 1 and the absence of such gradients in Year 2 was an unexpected result. The sweeter tissues extended from the skin on the seed side to the inside edge of the empty locule on the opposite side (*i.e.*, all of the flesh surrounding the seed) but did not extend to the proximal or distal parts other than in central flesh near the style (segments 15 and 16). Closer examination of concentrations of other solutes in 26°Brix berries in Year 1 showed that the effect

Table 5. Solute concentrations in segments adjacent to the two locules, empty (left) and seeded (right), of 26°Brix berries expressed as a percentage of those in segments 3 and 4. Sucrose is excluded since its base value was zero.

		Empty		With Seed	
	Solute	3 & 4	5 & 6	8	9 & 10
Year 2	Glucose	100	91	92	91
	Fructose	100	96	95	93
Year 1	Glucose	100	158	127	158
	Fructose	100	165	131	160
	Phenols	100	150	125	138
	Tartrate	100	83	79	176
	Malate	100	128	111	157
	Inorganic anions	100	79	100	153
	Potassium	100	192	162	161

was not confined to hexoses (Table 5). Phenol and potassium differences were similar, and increases also occurred in segments 9 and 10 in tartrate, malate, and inorganic anions. These results suggest that the flesh surrounding the seed (segments 5 through 10) lost *ca* 35% of its water in Year 1, possibly due to local heating associated with the body of the seed. Other tissues did not dehydrate that year, and none dehydrated in Year 2. The increases that followed dehydration did not occur with anions (inorganic anions, tartrate, and possibly malate) in flesh bordering the central vascular strands (segments 5, 6, and 8; Table 5). The reason for this is not known.

Conclusions

An increase in concentration of a solute within a tissue may be caused by transport into the tissue, synthesis within the tissue, and a loss of the solvent (water) from the tissue. A decrease may be caused by transport from the tissue, metabolism to other compounds, and an increase in the volume of water within the tissue. Comparisons of concentrations are inadequate methods for determining the causes of change in concentration, but they do provide bases for speculation. It seems likely that the high levels of malate at veraison and the large increases in the levels of hexoses that occur throughout ripening are due to transportation into and synthesis within the tissue. The lowering of the concentration of malic acid as ripening progresses may be due to metabolism to other compounds at the vascular tissue and a consequent transportation from neighboring flesh tissues. The smaller decline that is seen in the levels of tartrate in the flesh is probably due to an increase in the volume of water within the tissue. The increases in the concentration of several solutes in the flesh around the seed in overripe grapes in Year 1 are probably due to a loss of water from the tissue.

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Appendix

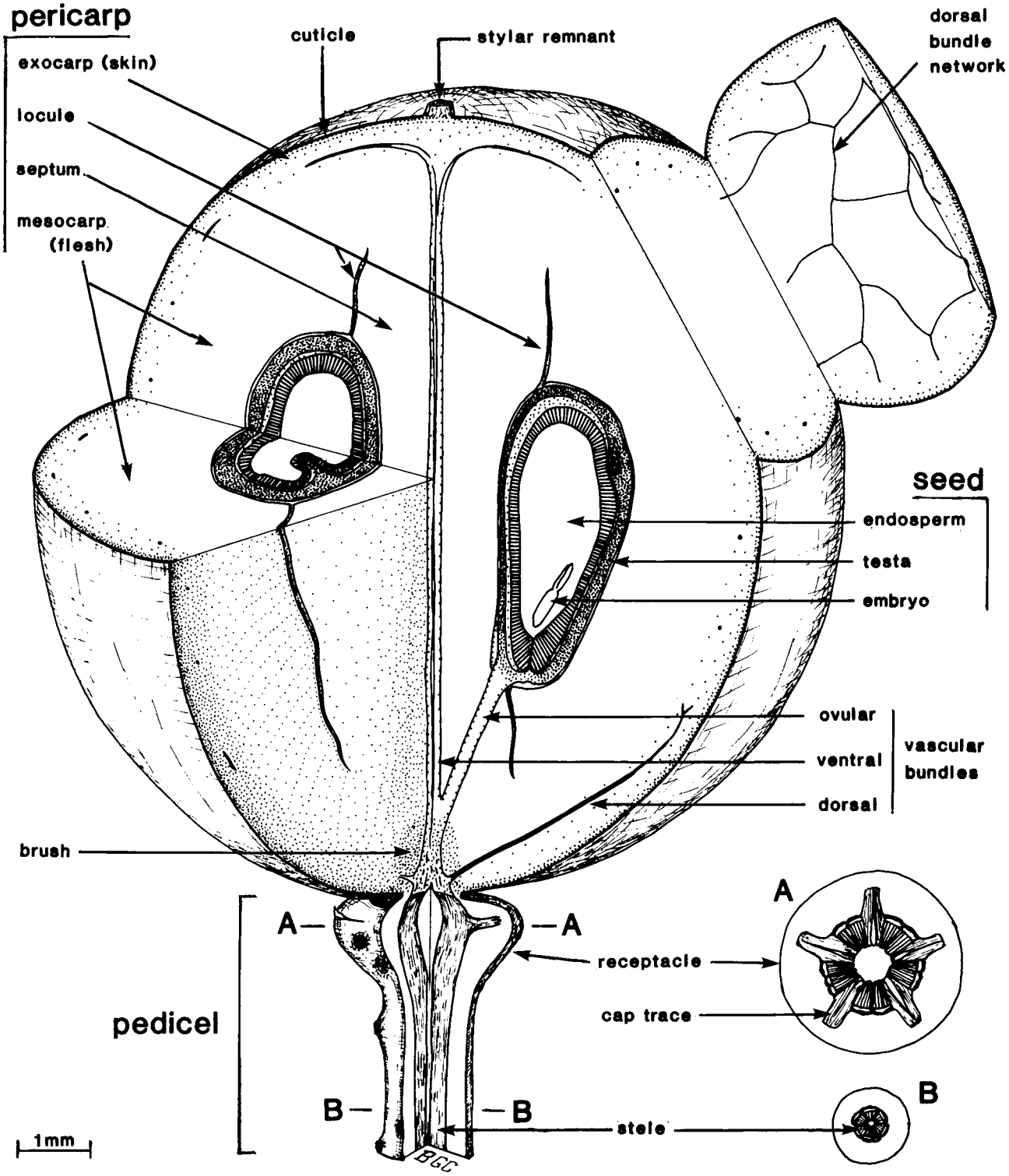

Diagram of a grape berry