Relationship of Solute Leakage to Solution Tonicity in Fruits and Other Plant Tissues [1]

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Although essentially all plant parts and especially thin sections derived from them have been observed to leach solute upon immersion in water (17), fruits have attracted particular attention because of the concept that an increased permeability of the tonoplast may accompany or be a prerequisite of fruit ripening (3, 19, 27). Previously it was shown (9) that exposure to water causes sections of apple tissue to lose solute and water, decrease in their ability to produce ethylene, and undergo an extensive browning reaction. The changes in water content, ethylene production, and polypenolase activity were prevented by inclusion of a high concentration of various organic or inorganic materials in the soaking media, and the present communication demonstrates that solute leakage also is restricted by this condition. In fact, leakage probably is the indirect cause of many of these effects, and it occurs to a significant extent and in a closely similar manner not only in several fruits, but also in other plant tissues.

Materials and Methods

Bananas (var. *Gros Michel*) were donated by the Banana Supply Co. of Miami, who obtained the fruit from southern Ecuador, and apples (post climacteric), potatoes and pears were purchased in local markets. Peas (var. Alaska) were dark grown in moist vermiculite at 23° for 8 days, at which time 10 cm subapical sections were cut under dim red illumination ($> 580\ \mu$).

Assay of reducing sugar was by the Nelson-Somogyi method (23), and this same procedure was used for sucrose by repeating the determination after hydrolyzing the sample with invertase. Sugar was quantitatively recovered from pea sections by shaking them with 50% acetone for 60 minutes, after which the acetone was removed by boiling for 5 minutes with the sections still present, and the resultant solution directly analyzed. However, extracts of fruit segments, prepared by grinding the tissue with 50% methanol, had to be clarified with neutral lead acetate and deleaded with dibasic sodium phosphate before they could be analyzed for sugar content. Respiration rates were determined with contant pressure respirometers (35), except that a Warburg manometer was used for pea sections and a Grubb-Parsons Infra Red Gas Analyzer for whole fruits and for rapid measurements with apple slices. Ethylene was assayed by means of gas chromatography as previously described (8).

In a typical experiment, 1-cm diameter plugs were cut from a fruit or potato with a cork borer and sliced with a razor to yield disks approximately 1 mm thick. These were blotted twice on absorbent towel, and equivalent lots were weighed and floated for 60 minutes on 25 ml of solution contained in a petri dish. The tissue was then blotted and reweighed, and the reducing sugar content of the solution assayed. The total reducing sugar content of the untreated tissue was determined on a duplicate sample of sections.

Results

Experiments with McIntosh Apples. In figure 1 the 2 major curves illustrate the rates at which reducing sugar is lost from apple slices suspended respectively in water and concentrated glycerol solution. Invariably the outflow is largely prevented by 0.3 to 1 M glycerol, and partially prevented by lower concentrations. When sections which have been soaked in water are transferred to glycerol the exit of sugar ceases, and conversely leakage starts anew when tissue which has been soaked in glycerol is transferred to water. Slices soaked consecutively in glycerol and water lose approximately the same total amount of sugar regardless of which solution is first applied, provided that no time elapses between the soakings. This is shown in table I for successive 10 minute treatments in each of the 2 solutions. However, after slices which have been incubated in water are dried and preserved in a moist atmosphere for 60 minutes, a substantial quantity of solute is again able to escape into a glycerol solution (table I). Since this does not occur in the reciprocal experiment, the total loss of solute is greater if the tissue initially was exposed to water.

That the requirement for glycerol is based upon its osmotic properties is shown by the ability of many

[1] Received June 28, 1963.

[2] This work was supported by a research grant (EF-21403) from the United States Public Health Service.

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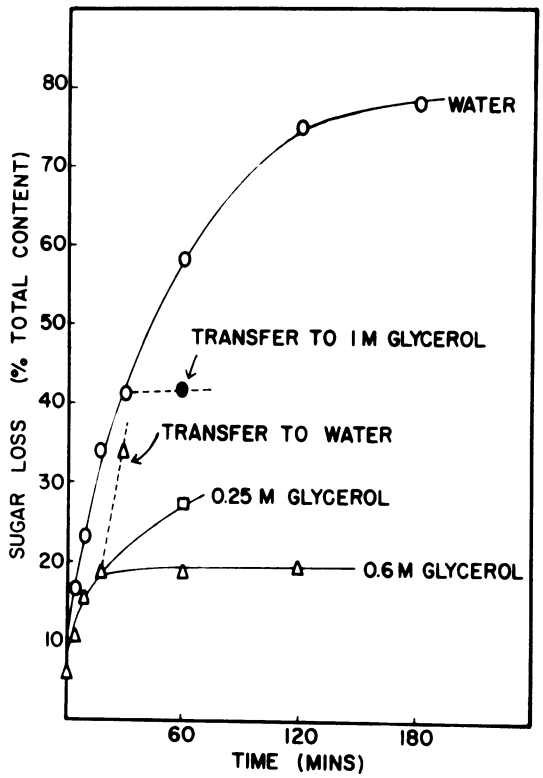
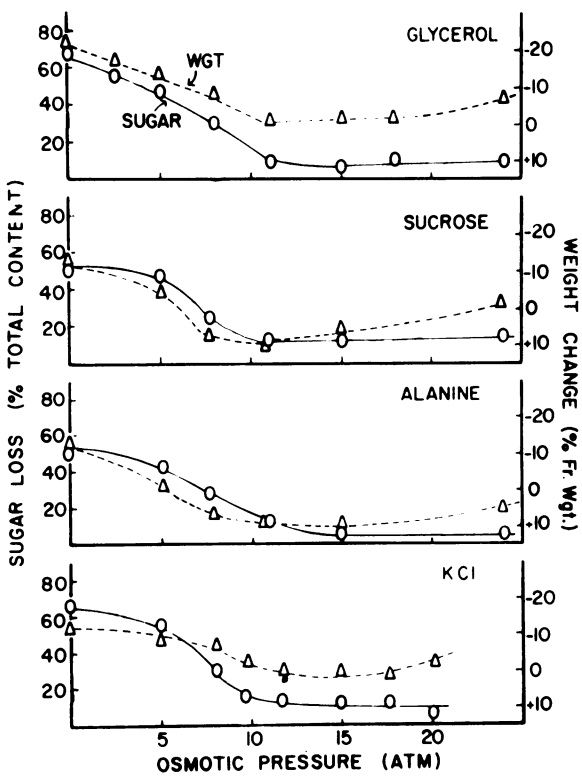




other organic and inorganic substances to partially or wholly substitute for it. For example, equal tonicities of KCl, sucrose, alanine, and glycerol behave in a closely similar fashion (fig 2). On the other hand formamide, acetamide, and ethylene glycol were only partially effective, and such highly permeable substances as ethylene glycol monomethyl ether, ethanol, methanol, urethane, and acetone had no effect whatsoever. Since the first 3 compounds usually penetrate cells relatively rapidly (12), and the remaining 5 with great ease, it is concluded that the cell must exhibit semipermeability with respect to a material before an effect on solute leakage may be manifest. Figure 2 also demonstrates that differing concentrations of glycerol and other substances prevent weight loss to about the same extent that they retard the outflow of reducing sugar. In addition, mannitol, glucose, and potassium fluoride also yielded closely similar results, and since the tissue plasmolyzed at the same tonicity in each case and did not deplasmolyze even after prolonged exposures to hypertonic concentrations of these materials, it can be concluded that they did not readily penetrate into the vacuole. Apple tissue contains approximately 10.8% of its fresh weight as reducing sugar and 88% as water (2), so in figure 2 the observed loss of solute which occurred in the absence of glycerol could not account for even one-third of the weight change. The remainder necessarily must be due to water escape. A plot of the weight change as a function of tonicity during a 1 hour soaking interval (fig 2) is not atypical of a semi-permeable cell except at osmotic pressures less than 7 atm, and the derived value of approximately 13.5 atm for the water potential of briefly rinsed, turgid tissue is compatible with the sugar content of the expressed juice. Tissue incubated in water deviated from expected behavior after only 3 seconds when following an initial transient water uptake amounting to approximately a 3% increase in fresh weight, water outflow began. It is important to note that the water loss which then ensued is different in nature from that induced by a hypertonic solution or wilting in dry air because the latter 2 do not entail any loss of solute. Consequently, as shown in table II, plasmolyzed or wilted tissue is able to regain most of its initial fresh weight when it is transferred to 0.5M glycerol, whereas sections which have been soaked in water have lost this ability.

It was found previously (9) that a decreased

FIG. 1 (*upper*). Outflow of reducing sugar from McIntosh apple tissue sections incubated in water, 0.25 M or 0.6 M glycerol solution. Broken curves indicate the pattern of sugar loss in sections transferred from glycerol to water or vice versa at the indicated times.

FIG. 2 (*lower*). Relationship between osmotic pressure of the soaking solution and the loss of fresh weight and reducing sugar from McIntosh apple tissue sections incubated for 60 minutes in either glycerol, sucrose, alanine, or KCl solutions of varied tonicities.

Table I

Leakage of Reducing Sugar from Apple Slices during Reciprocal Soaks in Water and Glycerol

Initial soak (10 min)	Time in air (min)	Reducing sugar loss (mg/g)			
		10 min Initial soak	10 more min Water	or Glycerol (M)	Total loss to* reciprocal solutions
Water	0	23.6	17.0	4.5	28.1
"	60	23.5	18.6	11.7	35.2
"	120	22.7	17.7	13.7	36.4
Glycerol (1M)	0	18.5	8.2	2.2	26.7
"	60	18.4	8.8	1.7	27.2
"	120	17.4	10.5	3.3	27.9

* Total sugar loss during 10 minutes initial soak plus 10 minutes soak in reciprocal solution, at 24°.

ability to produce ethylene parallels the loss in weight induced by soaking cylinders of apple tissue in water. This inhibition did not occur if the tissue was soaked in a high tonicity solution, and it could be reversed by transferring the tissue to molar glycerol, provided the incubation in water did not exceed 80 to 90 minutes. The behavior of tissue slices (fig 3) is closely similar to that reported for tissue cylinders, except that they respond more swiftly, but it should be noted that the rate of loss of solute and weight from tissue slices is also considerably more rapid than from tissue cylinders.

To investigate the nature of the response to glycerol, the substance was administered at various pH's and temperatures, and in conjunction with several metabolic inhibitors, hormones, and narcotics. Rates of respiration are included in table III to indicate that an inhibitory concentration of 2,4-dinitrophenol, fluoride, and iodoacetamide penetrated the tissue. It is clear that this in no way interfered with the action of glycerol. Saturation of glycerol solution with either toluene or ether, inclusion of molar acetone, and between 5 and 500 μM 2,4-dichlorophenoxyacetic, IAA, or napthalene acetic acid also had no influence on the efflux of solute into either water or glycerol solution. While changes in the pH did not alter the rate of loss of solute from tissue incubated in water or dilute buffer, below pH 2.0 the action of glycerol was impeded and at approximately pH 0.3 it was abolished (fig 4). Surprisingly, when tissue which had been soaked in glycerol at pH 0.5 was transferred to glycerol at pH 5, it consistently leached sugar at a substantially increased rate. For example, during 20 minutes at pH 0.5, reducing sugar loss in glycerol amounted to 7.9 mg/g, and during an additional 20 minutes 11.4 mg/g escaped, whereas if the pH was

Table II

Changes in Fresh Weight Induced by Consecutive Incubations in Water, Glycerol Solution, or Air

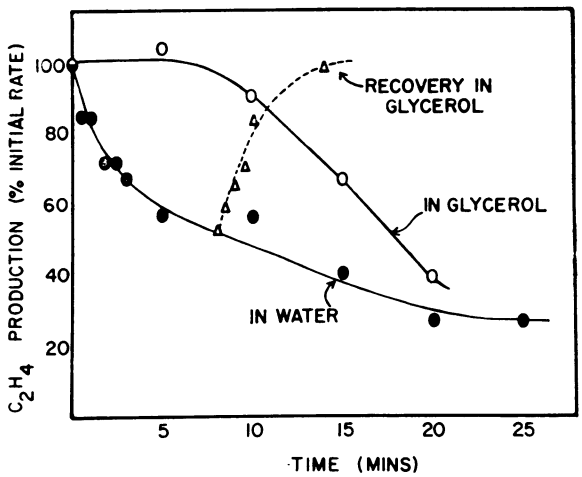
Initial treatment	Final wt (as % Loss of initial fr wt)			
	After 60 min Initial treatment	After 60 more min in Water	or	0.5 M Glycerol
Water	—10.9	—16.9		—14.7
Glycerol (2 M)	—12.7	— 8.5		— 4.1
Wilt in air	—27.5	— 7.3		— 2.0

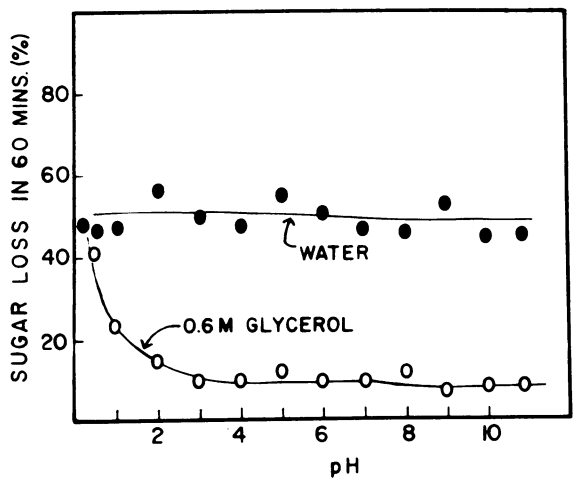
Table III

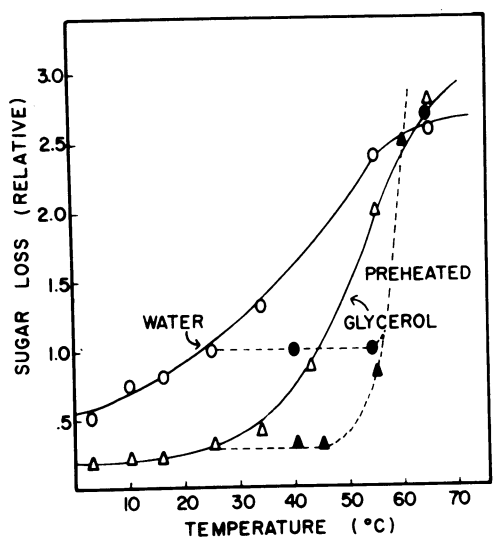
Effect of Metabolic Inhibitors and Anaesthetics on Solute Leakage from Apple Sections

Content	Conc (M)	Loss of reducing sugar (mg/g fr wt)*	Change in fr wt (%)*	QO_2 (μl/g/hr)
Water	...	54.7	—12.1	20.5
glycerol	5.5×10^{-1}	9.8	+ 2.7	25.0
" + 2,4 dinitrophenol	10^{-4}	8.7	+ 1.8	7.0
" + fluoride	10^{-3}	10.9	+ 0.5	12.2
" + iodoacetamide	10^{-2}	10.6	+ 3.0	8.0
" + chloroform	(sat)	8.5	+ 3.4	27.0
" + toluene	(sat)	10.9	+ 4.2	25.8
" + ether	(sat)	8.1	+ 2.7	8.0
" + acetone	1	8.9	+ 1.0	...

* Loss or change during 60 minutes incubation in solution at 24°.







raised to 5.0 for the latter period the sugar loss was 31.3 mg/g. The solid curves in figure 5 compare leakage in the presence and absence of glycerol during 1 hour incubations at various temperatures. For reasons germane to a later discussion, the leakage in glycerol at 5 to 10° must be subtracted from each value on both the water and glycerol curves before the temperature coefficient can be calculated. The Q_{10} in water throughout the range 10 to 45° is 1.5, which is only slightly higher than that which might be expected for the process of diffusion. In 0.55 M glycerol solution, the coefficient could not be accurately determined below 20°, but between 25 and 45° it is approximately 2.8, typical of a chemical reaction. The broken curve labeled *preheated* shows that below 45° no permanent damage is afforded tissue when it is preheated in air to the indicated temperatures for 60 minutes, and then cooled to 24° and incubated in water or glycerol. However, above 55°, all semipermeability is irreversibly lost, and a high rate of leakage is noted even when the tissue is placed in glycerol at 24°.

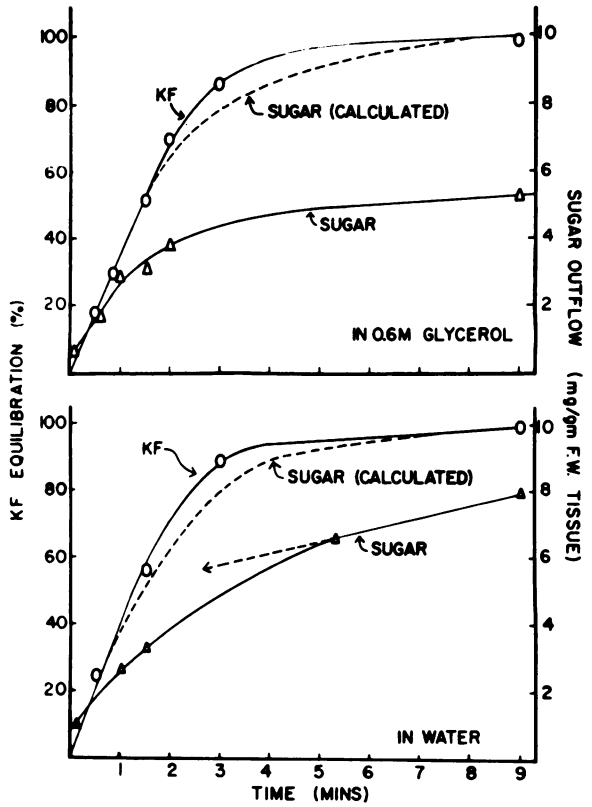
The vacuole of the mature apple cell appears microscopically to occupy at least 90 to 95% of the cellular volume. Gross weight changes which occur in the tissue as a result of exposure to solutions of various tonicities reflect the status of this vacuole and the permeability of its membrane, but do not necessarily provide information about the plasma membrane unless it constitutes the main permeation barrier. To investigate the plasomolemma use was

FIG. 3 (*upper*). Inhibition of ethylene production caused by incubating McIntosh apple tissue slices in water, and recovery after transfer to glycerol. Soaking in water for the indicated periods of time reduces the rate of ethylene production measured during a subsequent half-hour to the indicated value. If the tissue is transferred to 1 M glycerol for 30 minutes before the rate measurement is taken it recovers to the values shown in the upper curve labeled in glycerol. The kinetics of the recovery are shown in the broken curve for tissue which has been soaked for 8 minutes in water and then transferred for the indicated time intervals to molar glycerol before the rate was determined.

FIG. 4 (*middle*). Effect of pH on the exosmosis of reducing sugar from McIntosh apple tissue slices incubated for 60 minutes in either water or 0.6 M glycerol. Below pH 5 the acidity was adjusted with HCl, between 5 and 8 with 0.01 potassium phosphate buffer, and between 8 and 10.5 with 0.01 M carbonate-bicarbonate buffer. The basicity of solutions of pH greater than 8 decreased by as much as 1 or 2 units during the incubation, but only the starting value is shown on the graph.

FIG. 5 (*lower*). Effect of temperature on the leakage of reducing sugar from McIntosh apple tissue sections during a 60 minute incubation in either water or 0.6 M glycerol. Leakage at 24° has arbitrarily been assigned a relative value of unity. The broken curves labeled *preheated* indicate the amount of leakage which occurs when tissue which has been incubated in air for 60 minutes at the specified temperature is replaced in water or glycerol for 60 minutes at 24°.

made of the fact that fluoride causes an inhibition of respiration when it is present in the soluble portion of the cytoplasm, due to its effects on the enzyme enolase and certain phosphatases. A curve was established relating the extent of the respiratory inhibition, measured as a reduced O_2 consumption, to the log concentration of fluoride applied. After removal from a fluoride solution, each lot of tissue was blotted dry and temperature equilibrated in the moist atmosphere of the respirometer vessel, so that a total of 20 to 30 minutes elapsed before the first measurements of respiratory rate were completed. By that time the rate had stabilized at a value which was maintained for at least 2 hours, and since the results were unaltered by decreasing the soaking time from 90 to 10 minutes it can be concluded that equilibration between the external solution and cytoplasm had been completed in all cases. Tissue was exposed to 10^{-3} M fluoride for intervals of time varying from 0 to 10 minutes and the QO_2 determined as above. In each case the rate was found to be constant, but with the shorter exposures inhibition was less complete. From the QO_2 value the final concentration of inhibitor in the cytoplasm was calculated by reading from the fluoride concentration curve the molarity which at equilibrium with the cytoplasm yielded the same QO_2. In this manner a curve was constructed (fig 6) describing the relationship between time of soaking and the final cytoplasmic fluoride content both in the presence and absence of glycerol. Coincident with these measurements, the rate of exit of reducing sugar was determined and this data is included for comparison. In each case the half-time for fluoride entry is approximately 80 seconds, which is nearly identical to the half-time for sugar escape in the presence of 0.6 M glycerol. If the free space of this tissue were small relative to the volume of the cytoplasm, this half-time would be largely descriptive of the kinetics of fluoride



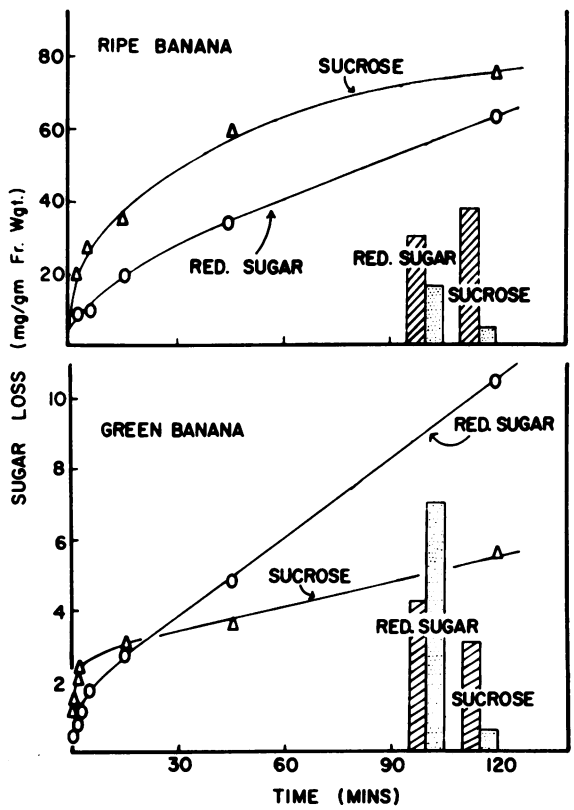
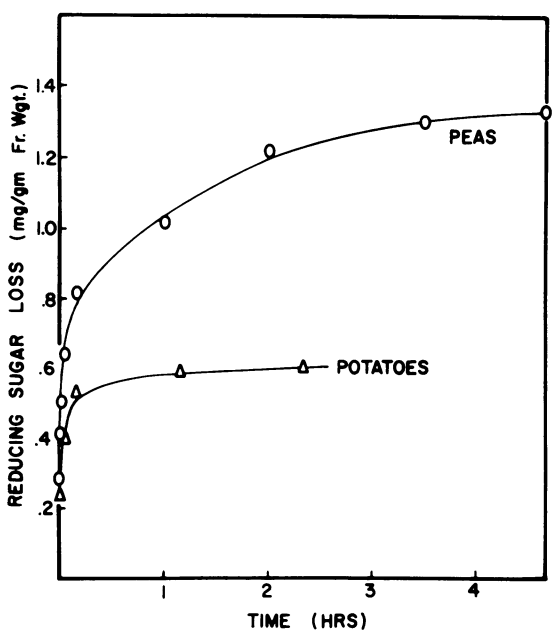


FIG. 6 (*upper*). Kinetics of fluoride penetration and reducing sugar exit during the incubation of McIntosh apple tissue sections in water and 0.6 M glycerol. Fluoride entry has been calculated from respiratory data (see text). The calculated sugar outflow curve in 0.6 M glycerol, expressed as percent equilibration, is corrected for the rapid outflow of sugar which occurs during the first few seconds (probably due to surface sugar originating from damaged cells of the unrinsed tissue) by subtracting this amount from each determination. The calculated outflow curve in water has, in addition, been corrected by the method of Briggs and Robertson (4) to discriminate against emptying of sugar from a second compartment. A tangent to the curve, shown as a dotted arrow, intersects the ordinate at 5 mg/g, which is subtracted from the ordinate value of the tangent at each particular time to yield a correction factor which must be subtracted from the outflow curve at that same time.

FIG. 7 (*lower*). Exosmosis of sucrose and reducing sugar from ripe and green banana pulp slices incubated in water. The barred histograms depict the sugar content of the untreated tissue, speckled histograms the sugar content after 120 minutes incubation.

penetration into the cytoplasm. However, there is reason to suppose that the free space of apple tissue is large in comparison to the cytoplasmic volume, for intercellular air space amounts to about 30% of the tissue volume (24) whereas the cytoplasm is very restricted in extent. After tissue is removed from a soaking solution, substances in the free space should penetrate into the cytoplasm, for example during the period of temperature equilibration, and under these conditions the half-time measured in the fluoride entry experiment might describe diffusion into free space rather than permeation into the cytoplasm. That this is the case was demonstrated by following short term changes in CO_2 evolution, using an infrared gas analyzer, after tissue was exposed to 5×10^{-3} M fluoride or 5×10^{-2} M iodoacetamide for 2 minutes and blotted dry. With both treatments there was a lag of about 2 minutes between the conclusion of the soaking and the appearance of a detectable respiratory inhibition. Subsequently, as judged from the relationship between inhibitor concentration and respiration rate, fluoride reached half its final concentration in the cytoplasm in approximately an additional 2.2 minutes, whereas iodoacetamide required nearly 20 minutes. Maximal inhibition occurred after 6 and 36 minutes respectively. It is significant that the results with iodoacetamide and fluoride were not appreciably different in the presence and absence of 2% KCl.

Leakage in other Fruits, Potatoes and Pea Sections. In slices of Baldwin and Red Delicious apples and Bartlett and Bosc pears, solute leakage, weight loss, inhibition of ethylene production, and a browning reaction are all induced by water and prevented by concentrated solutions. Similarly, leakage from both green and ripe bananas (fig 7) can be reduced approximately 50% during a 1 hour period by addition of a sufficiently high molarity of glycerol, mannitol, or KCl to the soaking solution. However, slices of this fruit, unlike sections of apples and pears, gain weight when soaked in water. Not only different fruits, but also various other tissues such as potato disks and sections of dark grown Alaska peas follow a similar pattern of sugar exosmosis (fig 8). All phases of these leakage curves show a dependence upon external tonicity (fig 9; upper and lower) which is in marked contrast to the situation with apples in which outflow during the first 10 minutes is totally independent of external solute concentration. However, with pea segments the total amount



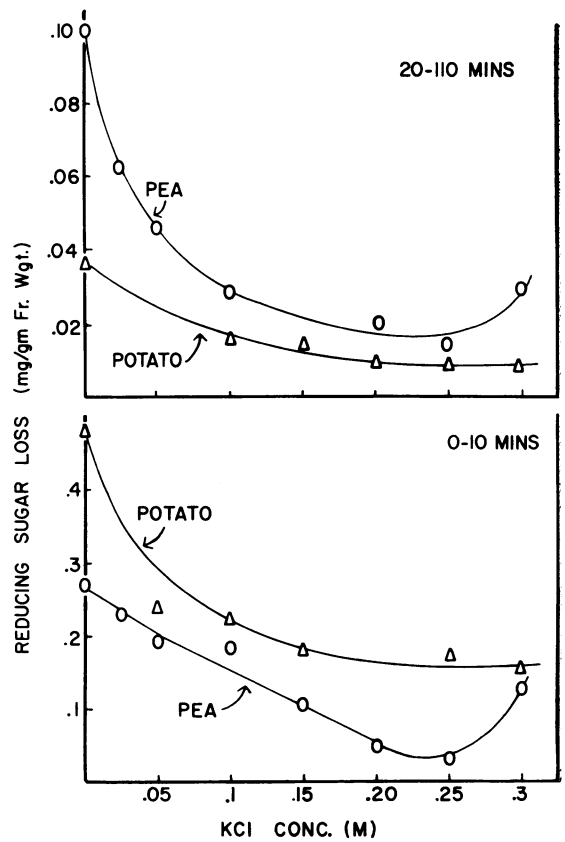


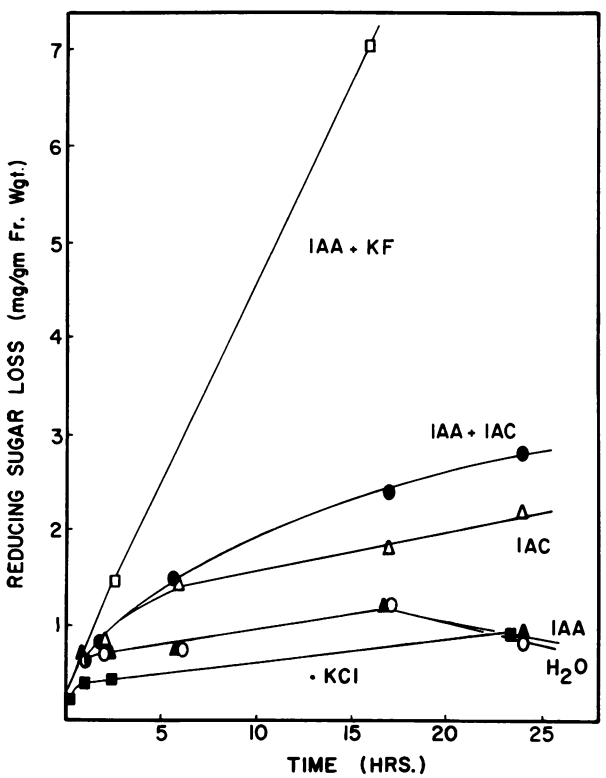
FIG. 8 (*left*). Exosmosis of reducing sugar from pea sections and potato slices incubated in water at 24°.

FIG. 9 (*right*). Effect of solution tonicity on the exosmosis of reducing sugar from pea sections and potato slices. Lower graph (0–10), unrinsed tissue soaked for 10 minutes; upper graph (20–110), tissue rinsed in flowing water for 20 minutes before being incubated in solution for 90 minutes.

of sugar which ultimately escapes is not altered by inclusion of 0.1 M KCl (fig 10). The entry of fluoride, measured as respiratory inhibition following a brief soak and a period of temperature equilibration, had a halftime of 70 seconds in potato slices, and 40 to 120 seconds with pea segments. By this same method it was found that half-penetration of iodoacetamide into the cytoplasm of potato tissue occurred in a somewhat longer time, 9 minutes. It is noteworthy that in potato slices the kinetics of the tonicity sensitive escape of sugar, and the rate of entry of fluoride, are closely similar to the 0.8 to 1.5 minute half-times reported by Thimann et al. both for equilibration of this tissue with tritiated water and for escape of C^{14}-mannitol from a compartment postulated to be apparent free space (33).

Neither IAA nor ethylene gas altered the pattern of leakage from pea sections, although an optimal growth acceleration resulted with 10^{-6} M auxin, and ethylene in excess of 0.02 ppm markedly retarded elongation and caused thickening of the tissue. No concentration of fluoride or iodoacetate affected sugar outflow during the first few hours, but in the presence of 6×10^{-4} M iodoacetate or 5×10^{-2} M fluoride, exosmosis did not abate as usually occurs, but rather continued for the duration of a 24-hour incubation (fig 10). The iodoacetate response was enhanced by auxin, but even when IAA was present, 5×10^{-3} M fluoride hardly affected leakage, in spite of the fact that both it and 6×10^{-4} M iodoacetate half-inhibited the growth of the tissue. That leakage occurs exclusively through the cut ends was demonstrated by an experiment in which 3-cm segments were immersed in a downcurved position with only the cut tips held above the surface of the solution. No leakage occurred, whereas in a comparable experiment with the sections completely submerged, the expected exosmosis resulted.

Relation of Leakage to Fruit Ripening. Recently Sacher (*27*) demonstrated that the leakage of solute from bananas increases in intensity as the fruit ripens, and he also mentioned that avocados behave in a



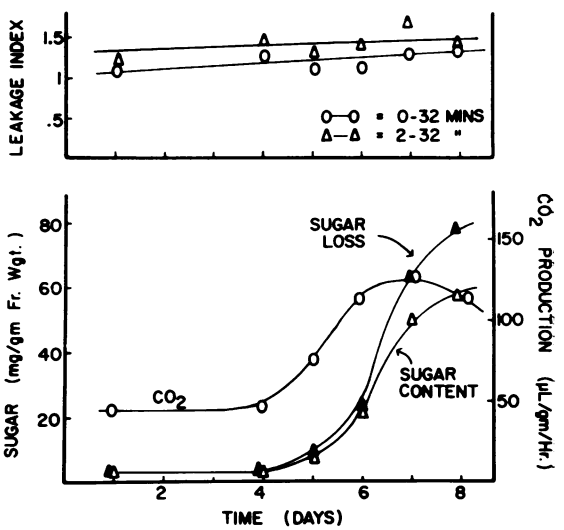


FIG. 10 (*left*). Reducing sugar level of the external solution during pea straight growth assay, using as an incubation medium water or 10^{-6} M IAA plus 6×10^{-4} M iodoacetate (IAc), 10^{-1} M KCl or 5×10^{-2} M KF as indicated. Ten 1 cm sections weighing about 0.25 g have been floated on 20 ml of fluid, and results are expressed as mg sugar in the fluid/g initial fresh weight of tissue.

FIG. 11 (*right*). Relationship between sugar exosmosis and sugar content in bananas during ripening. The respiratory climacteric, measured as CO_2 output, indicates the stage of ripeness. Sugar loss is the total outflow of reducing sugar plus sucrose during 32 minutes incubation of slices in water. This figure is compared in the upper graph to the sugar content to yield a leakage index for 0 to 32 minutes = mg sugar lost in 32 minutes per g fr wt tissue/sugar content (mg/g fr wt). The leakage index for 2 to 32 minutes is calculated in the same manner except that the tissue has been prerinsed for 2 minutes, and the sugar content is considered to be the average between the value determined at 2 minutes and that at 32 minutes.

similar manner. In these experiments the total weight of filterable material (the bulk of which must be sugar) which escaped from fruit sections during several hours of incubation in water was used as a measure of solute loss. In the present investigation the escape of sugar was used as an index of leakage, and the results with bananas (fig 11) are in accord with those reported by Sacher. However, such data alone does not show that leakage is enhanced as the climacteric progresses, because it is well established that sugars accumulate as the banana ripens (38), as shown in figure 11, where it can be seen that there is a close parallel between the amount of sugar present in the tissue and the apparent intensity of leakage at any particular stage of development. The situation is further complicated by the fact that this tissue rapidly replaces leached sugar, presumably by starch hydrolysis, with the result that more sugar is reclaimed from the external solution after a 120 minute water soak than originally was present within the slices, and at that time the endogenous total sugar content is frequently closely similar to the initial sugar content (fig 11). In fact, the shape of the sucrose and reducing sugar outflow curves in figure 11 is largely a reflection of the relative ability of the tissue to maintain an internal-external concentration gradient by replacing the specific sugar which has been lost. A useful index of leakage was found to be the escape of sugar between 2 and 32 minutes immersion in water. By the second minute, as shown in figure 7, the initial rapid outflow of sugar has been completed and the sum of the sugar which has escaped plus that remaining within the tissue was found to be closely similar to the initial sugar content. Leakage can then be expressed as mg sugar lost/ 30 minutes per average mg sugar/g fresh weight tissue in the period between 2 and 32 minutes. This is the derivation of the 2 to 32 minute leakage index included in figure 11. It is evident that the overall tendency for sugar leakage is not very much altered during the ripening of the banana, and it is especially constant during the first few days of the climacteric. The same conclusion also arises from a comparison of the total mg/g lost in 32 minutes with the initial content of sugar (fig 11).

Discussion and Conclusions

The exosmosis of substances from plant cells once was considered to be a process equal in importance to endosmosis or uptake [for example c.f. Stiles and Jorgensen (1915–1917) and the review by Helder (17)], but with the establishment of De Vries and Pfeffer's theory of the semipermeable membrane as an explanation of plasmolysis and deplasmolysis, and the recognition of the importance of active uptake, gradually the mechanisms by which plants excrete substances have been relegated to a lesser position. To the extent that exosmosis often is an artifact induced by various unusual treatments this view is justified. For example, Brooks (5) suggested that leakage occurs when tissues which are not normally exposed to water are placed in contact with it, and various authors maintain that exosmosis is enhanced at a cut surface or in tissue which has been narcotized or poisoned (29, 30, 36), electrically stimulated (28), or exposed to extremes of pH or temperature (31). In general the results here reported support this conclusion, and in particular the finding that pea sections leak entirely through their cut ends is significant, but nevertheless, in the case of roots (1, 6, 20) and leaves (22, 32) there is fairly convincing evidence that solute leakage is a normal phenomenon. Moreover, if we hold to the view that exosmosis is principally an artifact induced by cutting tissue or altering its natural environment, then we must accept the consequence that this condition is specifically produced in many laboratory experiments employing tissue slices or stem sections. Brooks (5) recognized this problem and in 1916 cautioned that exosmosis could affect the time course of plasmolysis-deplasmolysis experiments, although it should be noted that these studies are necessarily carried out with hypertonic solutions in which leakage may be minimized. Reference has also been made in the present communication to various effects, for example tissue browning, loss of ability to produce ethylene, and water outflow, which probably arise secondarily as a result of induced solute outflow. These examples serve to indicate that an element of caution must be interposed before equating results obtained with tissue sections incubated in aqueous media to the situation in an intact structure.

Most studies of the effects of ions and other substances upon exosmosis have been concerned with long term responses to dilute solutions, but in at least a few cases it has been observed that a high tonicity solution prevents leakage. Wachter (37) reported that strips of onion bulb scales give off sugars to distilled water, but less readily to 0.1 to 0.4 M KCl, NaCl, or KNO_3, and Iljin (18) noted that as much as 2% of the initial fresh weight of onion tissue may be lost as sugar. Possibly a similar case is that reported by Stiles and Jorgensen (29) who found that 0.2N NaCl or $CaCl_2$, or a combination of the 2, prevented exosmosis from potato slices as measured by changes in the conductivity of the external solution, but the authors explained this result in terms of enhanced uptake due to the high applied concentration. In both cases the concentrations involved are closely similar to those found effective in the present study.

The curve describing leakage of reducing sugar from McIntosh apple tissue slices immersed in water is compatible with a 2 compartment system. As shown in figure 1, the first phase lasts for approximately 10 minutes, and is followed by the emptying of a very much less permeable compartment during the course of several hours. The kinetics of escape from the first compartment have been approximated in figure 6 by correcting the curve for sugar outflow in water after the fashion of Briggs and Robertson (4), which yields a 60 to 80 second half-time for equilibration. From figure 1 it can be calculated that the second compartment half-empties to water in

approximately 45 minutes. When glycerol is added to the soaking solution in sufficient concentration, leakage from the first compartment is unaffected but the second fails to empty (fig 1, 6), whereas if the concentration of glycerol is only partially effective, for example 0.25 M (fig 1), leakage continues at a reduced rate. The kinetics of escape of sugar during the first phase can be very exactly determined in the presence of glycerol since no concomitant loss occurs from other areas of the cell, and figure 6 shows that under these conditions half-equilibration occurs in 81 seconds. This escape is so rapid that it reaches completion within an hour regardless of the temperature, causing the curve for leakage in glycerol during 1 hour (fig 5) to be flat in the 0 to 25° range. At higher temperatures, leakage from the second compartment into glycerol solution is discernible and the Q_{10} for this process can be calculated after subtracting the constant value in the 0 to 15° range. The nature of the first compartment is indirectly revealed by experiments in which it was found that fluoride applied externally for 75 to 87 seconds enters the cytoplasm to cause a respiratory inhibition after several minutes lag, ultimately bringing the cytoplasmic fluoride content to half the applied concentration. The simplest explanation of this observation is that diffusive entry into an extensive free space system occurs with a half-time of approximately 80 seconds, and the small cytoplasmic compartment subsequently fills from this source even after the tissue has been removed from the inhibitor solution. Accordingly, the similarity in the kinetics of fluoride entry and reducing sugar exit (fig 6) indicates that the reducing sugar originates in free space. Since the vacuole comprises most of the cellular volume and almost all of the sugar contained in the cell escapes during the emptying of the second compartment, the vacuole and second compartment must be synonomous. When the tissue is placed in water, the free space is reduced to a low tonicity because solute rapidly diffuses to the external water, and this condition leads to a reversible loss of differential permeability in the vacuolar membrane. If solute is present in the free space, outward passage of sugar from the vacuole occurs with the Q_{10} of a chemical reaction, whereas in the absence of solute sugar flows out with a Q_{10} typical of a diffusive process. However, both at very low pH's and very high temperatures irreversible changes in membrane structure occur even in the presence of glycerol, rendering the membrane totally permeable to the diffusive passage of sugar. These observations, and the lack of sensitivity of the leakage process to narcotics, suggest that an alteration of the protein structure of the membrane may be involved in the tonicity response. The contribution of the cytoplasmic compartment to the leakage process apparently is too small to be discerned in the outflow curve, and since as indicated by the fluoride experiments this barrier is far more permeable than the vacuolar membrane, it is not clear whether its structural properties are affected by a tonicity change. As solute escape continues, water is squeezed from the cells by the walls as they revert to a lower value of wall pressure compatible with the reduced osmotic pressure of the cell contents. If the external volume of water is sufficiently large, the escape of water and solute theoretically could continue until all soluble matter of low molecular weight has been dialyzed from the cell. On the other hand, if the external tonicity is kept high by added solute, the osmoticum may enter as rapidly as the free space solute escapes, with the result that the tonicity in the vicinity of the plasma membrane is not appreciably altered. In such a situation, escape of sugar from the free space would be complete, but the cell would fail to lose its semipermeability. This interpretation predicts that the free space will refill with solute if the tissue is first soaked in water and then replaced in air, but not if the initial exposure is to a hypertonic solution. The data in table I show that this expectation is fulfilled, for with 10 minute reciprocal soaks in water and glycerol separated by 120 minutes in air, 30% more sugar escapes if the first exposure of the tissue is to water than when the first exposure is to glycerol.

When account is taken of solute leakage, the seemingly anomalous water relations of apples and pears (fig 2) become clarified. At first there occurs a transient water uptake lasting only a few seconds as the tissue approaches full turgor, but even before this is reached, water outflow, induced as a consequence of, or in parallel with solute leakage leads to a progressively intensifying weight loss if the tissue is suspended in water or a low molarity solution. But if the tonicity is increased (fig 2), leakage is arrested and at a tonicity slightly lower than that which must have initially existed in the free space a slight water uptake occurs. Above this value, the cells behave as typical semipermeable osmometers. This interpretation is in complete accord with the results included in table II, where it can be seen that those conditions which lead to water loss without solute loss leave the tissue still able to regain water from a 0.5 M glycerol solution. Bananas differ from the above case in that an outflow of water, measured as a weight loss, does not result from a severe leakage of solute, but rather the tissue gains weight in spite of losing its entire content of sugar within a few hours. This behavior is explained by the fact that freshly cut tissue is able to replace its sugar by hydrolytic processes at about the same rate that it escapes into the external solution.

The effect of solution tonicity on ethylene production is probably an indirect consequence of solute leakage. Since the ethylene production can be reinstated by a high tonicity solution after either cylinders (9) or slices of tissue (fig 3) have been inhibited by incubation in water, hypertonicity initially does not prevent the escape of a material vital for ethylene evolution, but rather it must act directly on the mechanism of production of ethylene. The time required to half-inhibit or half-reinstate ethylene production by soaking tissue slices in water and glycerol respectively (fig 3) is similar to the half-time for both fluoride inhibition and sugar outflow from the first compartment (fig 6). This indicates the in-

volvement of a mass movement of solute from or into the free space, and suggests that the production of ethylene may be dependent upon the state of the cytoplasmic membrane, or the integrity of semipermeable structures in the cytoplasm, perhaps the mitochondria, which swell and become inactivated when the tonicity of the cytoplasm is lowered due to leakage (9). The inhibition caused by soaking in water may become irreversible after a short time either because the supply of an essential material is eventually exhausted by exosmosis or because the osmotic bodies have swollen and become permanently distorted or burst. It should be noted that this system is considerably more susceptible to osmotic damage than is the respiratory mechanism located in the mitochondria, and attempts to demonstrate ethylene production by mitochondria have so far failed to yield conclusive results (7,21).

Leakage in pea sections and potatoes differs from that in apple tissue in at least 2 important aspects: A) their initial rate of leakage is dependent upon the tonicity of the external solution, and B) metabolic inhibitors may increase the final quantity of exosmosed material. Inhibitor studies with potato slices are not included in the present communication, but Stiles and Jorgensen (29, 30, 31) and Thoday (36) demonstrated that about one-fourth of the electrolyte is lost from this tissue to distilled water in 48 hours, and the amount is considerably augmented by narcotics and heavy metal poisons. The maximum rate of leakage from pea sections is the initial rate regardless of the treatment, but a sufficiently high concentration of fluoride or iodoacetate extends the duration of leakage. That loss of solute is not correlated with growth inhibition per se is indicated by the marked difference in the effects on leakage of concentrations of fluoride (5×10^{-3} M) and iodoacetate (6×10^{-4} M) which both cause a 50% reduction in the growth rate (fig 10). Christiansen (10), who analyzed the exudate from pea sections grown in iodoacetate and auxin, concluded that it was composed predominantly of reducing sugar (32.2%), and that it was not formed in significant quantity in the absence of auxin. Although his observation that auxin enhances the formation of exudate in the presence of iodoacetate is confirmed in figure 10, the results differ in that here iodoacetate alone significantly enhances exomosis. Contrary to all these findings, leakage from apple tissue, both in the presence and absence of a high tonicity external solution, is insensitive to inhibitors and narcotics. Since in the continued presence of hypertonic glycerol and an inhibitor, exosmosis is arrested whilst the capacity to leak is preserved, it must be concluded that in apple slices neither the cause nor the prevention of leakage is dependent upon an anabolic cellular process.

Exit of materials, especially sugar, from pea sections may be a factor contributing to the growth promoting ability of high sugar concentrations in the pea straight growth assay (13). Subapical third internode sections from etiolated pea plants contain 1.2% reducing sugar and 0.4% sucrose on a fresh weight basis (11), and assuming that the sugar is equally distributed throughout the cell, the molarity is equivalent to a 2.7% sucrose solution. This is very nearly the quantity of sucrose reported to be optimal for growth (14), and it would be required to maintain the free space and/or the cytoplasm at its original sugar concentration by compensating for the 10% loss in sugar content which occurs by exosmosis during the initial phase of incubation.

In contrast to several cases demonstrating an effect of auxin on membrane properties (34, 25, 26) no change in the efflux of sugar from apple disks or pea sections could be detected following treatment with the hormone. In the case of pea sections (fig 10), which are increased in growth and respiration rate almost immediately after application of IAA, it is clear that hormone action can occur without any increase in permeability. Similarly, in spite of the fact that ethylene has been shown to increase the permeability of tissue (15, 16), it is not likely that this is a requisite mechanism in the biological response to the gas because ethylene is able to produce a growth reaction in pea sections without hastening exosmosis. In this connection it is important to note that the recent demonstration of an increased efflux of solute during the ripening of bananas (27) has been interpreted to mean that leakage is increased by endogenously produced ethylene, but in point of fact the reverse argument is more valid, for the total amount of sugar outflow increases in direct proportion to the sugar content, which (from Fick's law) means that the permeability of the tonoplast to sugar remains constant in spite of the presence of a biologically active quantity of ethylene gas (fig 11).

Summary

During incubation in water, sections cut from potato tubers, apples, bananas, pears, and the third internode of etiolated pea seedlings leak sugar with a basically similar pattern; a rapid initial outflow followed by a slower second phase of exosmosis. In apples, the rapid phase of sugar loss is caused by the emptying of free space, but the bulk of the sugar which subsequently escapes originates in the vacuole. A high concentration of glycerol or other suitable osmotic agents prevents exosmosis through the vacuolar membrane of apples, but does not retard the emptying of the apparent free space, whereas with pea and potato sections all phases of the leakage process are tonicity dependent. Water loss, tissue browning, and reduction in the rate of ethylene formation are secondary results of exosmosis from fruit sections, and therefore these processes are dependent upon solution tonicity in a closely similar manner. The effect on leakage of temperature, pH, auxins, narcotics, enzyme inhibitors, and ethylene is discussed. Experiments with bananas indicate that at all stages of ripeness, leakage is proportional to the sugar content so that the propensity to leak is constant in spite of a large increase in the ethylene content. Similarly, in the pea straight growth assay neither auxin nor

ethylene alter the exosmosis pattern while both affect the growth of the tissue.

Acknowledgment

The authors express their sincere appreciation to Dr. K. V. Thimann for his counsel during the investigation, and for his assistance in the preparation of this manuscript.

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