THE INITIATION OF DEVELOPMENTAL DRIFTS IN EXCISED PLANT TISSUES*

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Excising plant tissue initiates changes in respiration and other biochemical processes, potential solute accumulation, and fine structure, which continue over several days (Van Steveninck 1964; Jackman and Van Steveninck 1967; Laties 1967; ap Rees and Royston 1971; Bryant and ap Rees 1971; Kahl 1971). These may all be part of a single developmental process, as is suggested by the observation that low temperature, a nitrogen atmosphere, and certain metabolic inhibitors slow down the changes in both respiration and solute accumulation (Rees 1949; ap Rees 1966; MacDonald *et al.* 1966). It is not known how these changes, which take place in a constant environment, are regulated, and in particular it is not known what factor associated with excision initiates them. Comparisons of intact and freshly excised tissues have shown numerous biochemical and fine structural changes (Laties 1962; Jackman and Van Steveninck 1967; Jacobson *et al.* 1970; Adams and Rowan 1971; Kahl 1971; Rungie and Wiskich 1972). One can conclude from these initial changes that the initiating event(s) must be fairly rapid.

There are several types of rapid changes which could follow excising tissue into aerated water: (1) a wound response; (2) diffusion of some substance from the extracellular spaces, or of a very permeable substance from within the cells; (3) diffusion of water or oxygen into the tissue.

A wound response can be ruled out, since potato disks put together and replaced in the tuber do not change in the way that isolated disks do (Laties 1962). This observation would be consistent with the second or third alternatives. The partial pressure of oxygen has been shown not to limit respiration in intact or freshly excised potato tuber tissue (Laties 1962). On the other hand, an inflow of water to freshly excised tissue and the consequent increase in internal hydrostatic pressure and stretching of the cytoplasm (Briggs 1971) could occur fast enough (15 min—Kohn and Dainty 1966) to be the initiating factor. Some rapid effects of "osmotic shock" are known in plant cells (Laties 1954) and bacterial cells (e.g. Heppel 1969). The possibility that osmotic shock initiates developmental changes in excised tissue is examined in this paper. Briggs (1971) has also recently considered this possibility.

Methods and Results

An increase in internal hydrostatic pressure on excision could be prevented by maintaining a high enough external osmotic pressure with a non-penetrating solute.

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Over short periods sucrose was used as the osmoticum. Over longer periods mannitol was used as it does not enter excised potato tissue at a significant rate, and is not metabolized by the tissue (Thimann, Loos, and Samuel 1960).

In carrot tissue chloride influx increases 10–20 times on washing in water for 3 days after excision. The change taking place on excision might be a rapid loss of accumulating capacity which is recovered during development. In this case if a rise in internal hydrostatic pressure was the initiating signal then maintaining a high external osmotic pressure would prevent the loss of accumulating capacity. There would be an apparent 10–20 times stimulation of chloride influx to freshly excised tissue by a high external osmotic pressure. Alternatively, excision might initiate a rise from a normal low accumulating activity in intact and freshly excised tissue. In this case a high external osmotic pressure would prevent the development rise, or there would be an apparent 90–95% inhibition of chloride influx by high external osmotic pressure during aging. In addition, an effect of osmotic pressure on chloride influx must be differentiated from an effect on development.

The internal osmotic pressure in excised carrot tissue is approximately 500 kPa (5 bars). The hydrostatic pressure inside the cells in the intact tissue is unknown, but must be between 0 and 500 kPa above atmospheric, depending mainly on the osmotic pressure in the extracellular spaces.

When tissue is excised in a solution of osmoticum, the hydrostatic pressure in the cells will remain constant in some concentration between 0 and 500 milliosmolar (0 and 1200 kPa).

TABLE 1

EFFECT OF RAISING THE OSMOTIC PRESSURE OF THE BATHING SOLUTION ON CHLORIDE INFLUX TO FRESHLY CUT CARROT DISKS

Influx expressed in terms of the fresh weight of 20 disks (one replicate) after being in 5 mM KCl alone (after being in 500 mM sucrose 20 disks weighed 85% of those in no sucrose). Influx shown as mean ± standard error of the mean of three replicates and was measured from 5 mM $K^{36}Cl$ plus various concentrations of sucrose. Temperature 20°C

Sucrose concn. (mM):	0	50	100	200	500
Chloride influx (μmole g^{-1} hr^{-1}):	0·11 ±0·002	0·10 ±0·01	0·09 ±0·01	0·09 ±0·004	0·10 ±0·01

Carrot disks were cut in air and within a few seconds transferred in sequence to sucrose solutions of the concentrations shown in Table 1. After washing for 90 min in these solutions, chloride influx was measured over 1 hr from 5 mM $K^{36}Cl$ plus the same concentration of sucrose as in the washing solutions. The results are shown in Table 1. When allowance is made for the slight shrinkage in the sucrose solutions, there is no significant difference between the chloride influx in the different treatments. There is therefore no rapid change on excision induced by the sudden change in the hydrostatic pressure inside the cells, nor is the influx in fresh tissue influenced by the hydrostatic pressure inside the cells, unless there is a complicated interaction between the effect of external osmoticum on the changes during excision and directly on chloride influx.

In the second experiment (Table 2) the disks were cut under 200 mM sucrose to avoid the possibility of a very rapid response when the tissue was cut in air, and the possibility of a direct effect of 200 mM sucrose on chloride influx was checked. The amount of chloride accumulated from 1 mM KCl over the first 17 hr after excision was measured in tissue which was

(1) cut and washed in sucrose plus 1 mM KCl;
(2) cut in sucrose, gradually transferred to water by dilution of the sucrose with water over a period of 0·5 hr, and then washed in 1 mM KCl (to find the effect of a gradual as opposed to a sudden increase in internal pressure);
(3) cut under water and transferred after 15 min to sucrose plus 1 mM KCl; and
(4) cut under water and washed in 1 mM KCl.

Comparison of treatments 3 and 4 shows that there is little direct effect of the internal hydrostatic pressure of the cells on the influx of chloride in tissue excised in

TABLE 2

EFFECT OF RAISING THE OSMOTIC PRESSURE OF THE BATHING SOLUTION ON CHLORIDE ACCUMULATED BY FRESHLY CUT CARROT DISKS

The disks were cut under water or sucrose solution and aerated for 17 hr in 1 mM KCl plus 200 mM sucrose. Temperature 17°C. The initial chloride content of comparable disks was 10 μmoles g^{-1}. The table shows the contents of duplicate samples at the end of the 17-hr treatment

Treatment No.	Sucrose concn. (mM)		Final chloride content (μmoles g^{-1})*
	In slicing medium	In incubating medium	
1	200	200	11·5, 11·6
2	200	0	10·3, 11·7
3	0	200	13·1, 13·4
4	0	0	11·2, 10·9

* Final fresh weight.

water. Therefore, since there is no difference between tissue cut under water and cut under 200 mM sucrose (treatments 1 and 4, and Table 1), there is also no internal hydrostatic-pressure-dependent change on excision. This is also shown directly by the similarity between treatments 1 and 3 in Table 2. Further, there is no influence of cellular hydrostatic pressure on development during the first 17 hr after excision.

Although there is no hydrostatic-pressure-dependent change immediately after cutting, it still remains possible that the sudden change in hydrostatic pressure in the cells on cutting initiates the long-term changes, one of which is the development of the capacity to accumulate salt. This occurs about 3 days after excision in carrot tissue (Cram 1967). There is no direct effect of external osmolarity on influx of chloride in tissue washed in water for 4 days (Briggs 1971; Cram, unpublished data), so any differences between chloride accumulation by tissue in water and in osmoticum would be due to effects on development.

Carrot tissue was therefore cut and washed in 200 mM mannitol plus 0·5 mM $CaCl_2$, or 0·5 mM $CaCl_2$ alone, for 4 days. By this time water-washed tissue would have developed salt-accumulating capacity and have absorbed some chloride. As shown in Table 3, the amount of chloride absorbed and the chloride influx from 1 mM KCl at 96 hr after excision are nearly the same in the presence and absence of 200 mM mannitol. Allowing for the shrinkage in 200 mM mannitol (10% of the volume in water) the chloride contents per gram fresh weight are the same, and the chloride influxes are only just significantly different ($P > 0{\cdot}02$).

TABLE 3

EFFECT OF RAISING THE OSMOTIC PRESSURE OF THE WASHING SOLUTION ON THE DEVELOPMENT OF CHLORIDE-ACCUMULATING CAPACITY IN EXCISED CARROT TISSUE

Blocks of tissue were cut under 200 mM mannitol. Half of each block was cut into slices under mannitol and remained in mannitol for the rest of the experiment, and half was cut in water. Slices were then washed several times in mannitol or water and left in aerated solutions of 200 mM mannitol plus 0·5 mM $CaCl_2$ or 0·5 mM $CaCl_2$ for 96 hr. Chloride influx from 1 mM $K^{36}Cl$ ± 200 mM mannitol and final chloride content were then measured. Values are shown as mean ± standard error of the mean of four replicates

Mannitol concn. (mM)	Chloride influx (μmole g^{-1} hr^{-1})	Chloride content (μmoles g^{-1})
0	0·47±0·02	44±1
200	0·62±0·03	49±1

Conclusions

Briggs (1971) has examined the effects of sucrose on the development of chloride-accumulating capacity in excised carrot tissue. Concentrations of sucrose up to 200 mM had no effect on net chloride influx in aged tissue, but aging in sucrose depressed net chloride influx by 20–25%. However, aging in 20 mM sucrose (50 kPa) had the same effect as aging in 200 mM sucrose (500 kPa). Therefore, the relatively small effect of sucrose is not an osmotic effect.

Sucrose is not an ideal osmoticum as it may penetrate the tissue, whereas not more than a small amount of external mannitol penetrates potato storage tissue disks in 3 days (Thimann, Loos, and Samuel 1960).

Tables 1–3 show that a rapid uptake of water following excision and a concomitant increase in internal hydrostatic pressure cannot be the primary factor determining the changes occurring during or after excision, at least for chloride accumulation in carrot tissue. The primary effect of excision, therefore, is probably related to the movement of a substance out of the tissue. This hypothetical substance would have to be volatile, as developmental changes in potato tissue take place in moist air (Laties 1962). The only known volatile-regulating substance is ethylene. Recent work (Pratt and Goeschl 1969) does not seem to rule out the possibility that ethylene is involved in initiating the changes that take place in excised tissues.

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