SUGAR AND ION TRANSPORT IN ISOLATED ONION EPIDERMIS

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Summary

The apparent unidirectional transport of sugars and ions in isolated onion epidermis was shown to be due to the presence of a cuticle on the outer surface of the epidermis. The cuticle probably is partially permeable for ions and impermeable for hexoses. Sugar transport across the inner surface of isolated epidermis had a temperature coefficient ($k_{20°C}/k_{10°C}$) of 2·4, the corresponding activation energy was 14·5 kcal/mole, and the permeability coefficient was approximately 10^{-8} cm/sec. Sugar transport was not inhibited by carbonyl cyanide *m*-chlorophenylhydrazone, potassium cyanide, or sodium azide.

I. Introduction

Jackson and Brown (1963) reported unidirectional transport of glucose through isolated onion epidermis and undirectional active secretion of glucose from this tissue. Since these properties would make the onion epidermis an excellent model for studies of active sugar transport in plants, an attempt was made to investigate the phenomenon in detail.

It turned out that the polarity of sugar transport, which is probably mediated by activated diffusion, is a structural and not a physiological property of the tissue.

II. Material and Methods

Onions (*Allium cepa* L.) were supplied from the local food stores. The outer leaves or scales were discarded. From the inner scales, the upper (adaxial) epidermis was readily stripped. In many cases the epidermis separates from the scales by itself when the scales are taken apart. Protoplasmic streaming and staining of the vacuoles with neutral red demonstrated that the epidermal cells were intact after stripping. Disks were cut from this tissue with a cork-borer and screwed into place between two neoprene rings at the end of the diffusion tube described earlier (Lüttge 1964).

The inner diameter of the tube was 1 cm and the free area of the disks was therefore 0·785 cm^2. For each treatment 6 or 12 replicates were run. 1·5 ml of 10^{-4}M $CaSO_4$ were pipetted into the tube, which was then placed vertically in small Petri dishes containing 20 ml 10^{-4}M $CaSO_4$ with sugars or KCl added at varied molarity. The level of the solution within the tube was 5 mm above that in the Petri dishes so that leaky tubes could easily be recognized and discarded. No volume change of the solutions in the tubes was observed within the experimental periods. Unless otherwise stated experiments were carried out at room temperature (25°C). At the end of the experiments the solution in the tubes was tested for sugars and ions.

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With the arrangement described, transport from the Petri dishes across the epidermis disks into the tubes was investigated. To test polarity of the transport, disks were placed in the tubes in two inverted positions. To describe the direction of transport the following terms will be used throughout: the upper surface of the epidermis, i.e. the adaxial surface or the surface closer to the centre of the onion, will be referred to as the outer surface or, in respect to transport across the disks, as the outside; the opposite side, i.e. the surface more remote from the centre of the onion or the abaxial surface, will be called the inner surface or the inside.

Sugars were analysed colorimetrically using the test of Somogyi and Nelson for reducing sugars and that of Roe for keto-sugars (cf. Kakáč and Vejdělek 1966). Chloride was measured with an Aminco-Cotlove electric chloride titrator. Potassium was determined by flame-photometry.

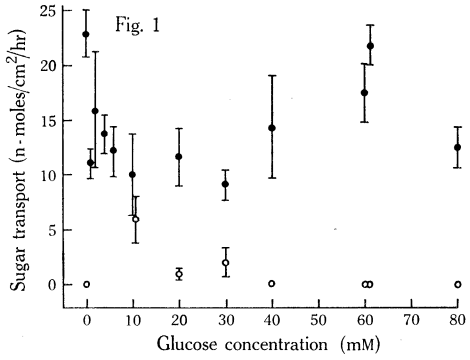
For paper chromatography solutions were desalted on ion exchange columns of Amerlite R, MB-3. Separation was achieved by descending chromatography in butanol–pyridine–water (6:4:3 v/v) in about 48 hr at room temperature. The spots were developed with silver nitrate as described by Trevelyan, Proctor, and Harrison (1950).

Standard deviations of mean values are shown on the figures and are given where appropriate in the text.

III. Results and Discussion

(*a*) *Sugar Transport*

In Figure 1 the rates of glucose transport into the solution facing the inner and the outer surface of onion epidermis disks are shown. Transport through the outer surface is very small or undetectable, while transport through the inner surface is prominent. The polarity described by Jackson and Brown (1963) is clearly reproduced in our experiments. The data of Figure 1 indicate, however, that polar sugar transport is unaffected by the sugar concentration in the opposite compartment.



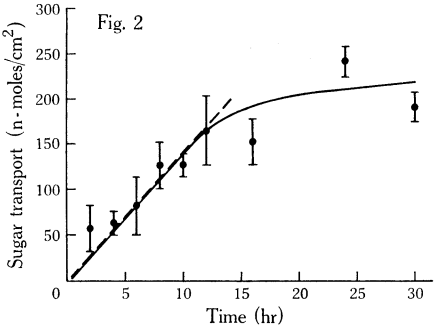


Fig. 1.—Transport of reducing sugar to the inside of isolated onion epidermis for various glucose concentrations at the outside (●) and to the outside for various glucose concentrations at the inside (○). Experimental period 12 hr.

Fig. 2.—Time course of the transport of reducing sugar to the inside of isolated onion epidermis. Glucose concentration at the outside 1 mM.

Furthermore, the amount of reducing and keto-sugars appearing in the solution facing the inner surface of the epidermis disks is unaffected by the kind of sugar applied on the opposite side. For example, when 60 mM glucose, galactose, mannose, or fructose is applied on the outside of the epidermis for 12 hr, the transport of sugar to the inside is, respectively, 70 ± 13, 60 ± 14, 67 ± 10, and 70 ± 10 n-moles/cm²/hr.

In another experiment, mixtures of glucose+fructose, glucose+galactose, and fructose+galactose (60 mM each, total sugar concentration 120 mM) were in the solution facing the outer surface of the epidermis. After 12 hr the solution bathing the inner surface was tested chromatographically. With any combination of sugars at the outside all three sugars were found at the inside.

From these results one has to conclude that there is no sugar transport across isolated onion epidermis. The sugar appearing at the inside must come from the epidermal cells themselves.

The time course of the transport of reducing sugar into the solution bathing the inner surface of the epidermis is linear over the first 12 hr and then rapidly levels off (Fig. 2). Most of the experiments described were therefore carried out over a period of 12 hr. The rapid decline in the rate of sugar secretion after 12 hr may be due to decrease of internal sugar concentration.

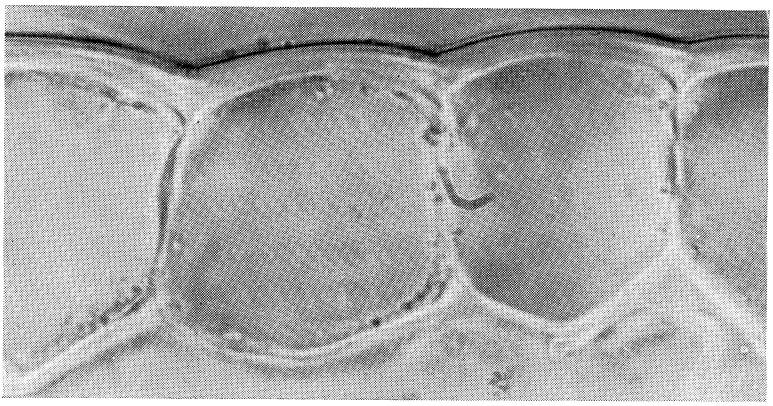


Fig. 3.—Cross-section of an isolated onion epidermis. The outside and the Sudan III-stained cuticle are towards the top. Magnification $\times 1000$.

This is further illustrated by the comparison of fresh and aged disks of isolated epidermis. In the first 12 hr after isolation, epidermis disks secrete about 4–5 times as much sugar into the solution facing the inner epidermal surface than disks washed for 48 hr in aerated 10^{-4}M $CaSO_4$ solution prior to the 12 hr secretion period. (The results of two experiments were: reducing hexoses secreted by fresh disks 382 ± 13 and 268 ± 57 n-moles/cm^2 per 12 hr; by washed disks 102 ± 32 and 46 ± 4 n-moles/cm^2 per 12 hr. A 60 mM glucose solution was applied on the outside.)

The decline of internal sugar concentration was also demonstrated by semi-quantitative paper chromatography. Disks of epidermis were extracted in boiling 80% ethanol after 0, 6, 12, and 24 hr of secretion when glucose concentration in the solution bathing the outer epidermis surface was 60 mM. The extracts were brought to a constant volume and aliquots were chromatographed. The intensity of the developed spots decreased considerably from 0 to 6 hr and then slowly between 6 and 24 hr.

Since these results indicate that no sugar can move into the epidermis cells through the outer surface, and that all sugar appearing on the inside is secreted from within the cells, the nature of the barrier at the epidermal surface had to be

investigated. The onion epidermis was cross-sectioned by hand with a sharp razor-blade, the sections were stained overnight in Sudan III solution, and then washed briefly with water. Microscopic inspection revealed a cuticle on the outer surface of the epidermis which stained heavily with Sudan III (Fig. 3) and so provided a simple explanation for the polarity of sugar transport.

Although the polarity is therefore not based on physiological properties of the onion epidermis, the mechanism of sugar transport from the cells across the inner surface was interesting enough to be investigated further.

Metabolic inhibitors did not decrease the rate of sugar secretion (Fig. 4). Potassium cyanide was ineffective. Increasing concentrations of carbonyl cyanide *m*-

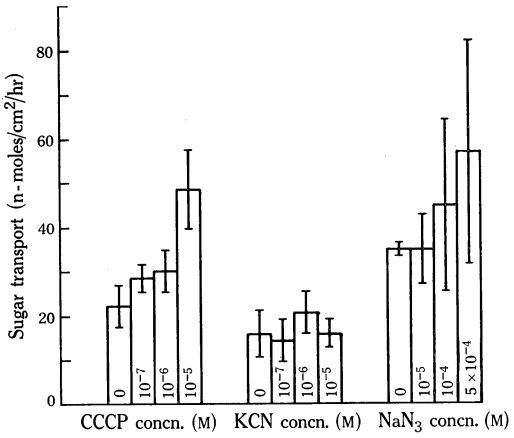


Fig. 4.—Transport of reducing sugar to the inside of isolated onion epidermis with different inhibitors added to both sides. Experimental period 12 hr.

chlorophenylhydrazone (CCCP) and sodium azide obviously enhanced transport but this effect, however, was not highly significant. Temperature, on the other hand, considerably affected the rate of sugar transport from the tissue in the first 12 hr after isolation (Fig. 5). From this graph the temperature coefficient of sugar transport

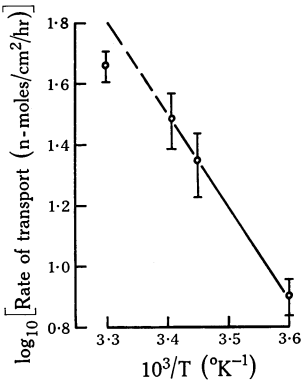


Fig. 5.—Effect of temperature on transport of reducing sugar to the inside of isolated onion epidermis. Experimental period 12 hr.

(k_{t+10}/k_t) can be calculated, where k_t is the specific rate at t°C and k_{t+10} at 10 degC higher. For $t = 10$°C and $t+10 = 20$°C the temperature coefficient is 2·4. The corresponding activation energy is 14·5 kcal/mole. The levelling off at higher tem-

peratures (30°C, $10^3/T = 3{\cdot}3°K^{-1}$) may be due to a beginning of heat inactivation of the transport mechanism.

(*b*) *Ion Transport*

Jackson and Brown (1963) reported that potassium and sodium were transported in both directions across isolated onion epidermis. Fluxes in the two directions were different in magnitude. However, the higher cation flow was opposite to the direction of sugar transport. This indicated a reversed polarity of ion transport compared with the polarity of sugar transport.

Figure 6(*a*) shows that the results obtained with our material differed from these findings. Potassium and chloride were excreted from the tissue only across the inner surface. When the solution bathing the outer surface contained KCl, the

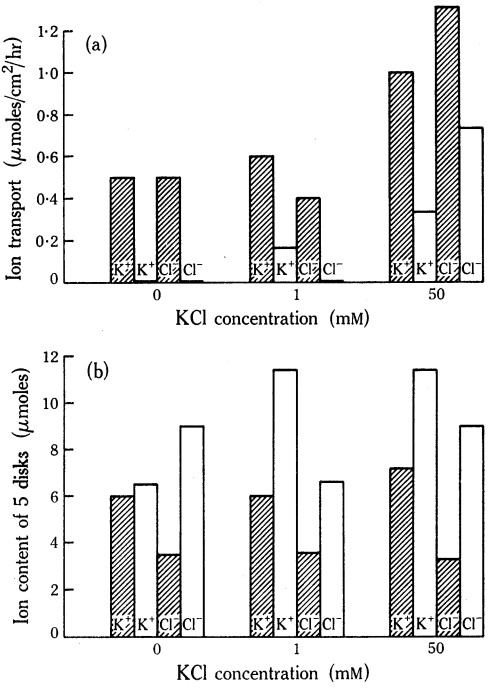


Fig. 6.—(*a*) Transport of K^+ and Cl^- to the inside (shaded columns) and to the outside (unshaded columns) of isolated onion epidermis. KCl concentration was varied in the opposite compartment. Experimental period 12 hr. (*b*) K^+ and Cl^- content of the disks used in the experiment of Figure 6(*a*).

amount of potassium and chloride appearing on the other side was increased. When KCl was given in the solution facing the inner surface of the disks K^+ and Cl^- transport from this side to the outside was observed. The rate of this transport, however, was much lower than that of the movement in the opposite direction. In contrast to the results of Jackson and Brown our experiments show that the polarity of ion transport has the same direction as that of sugar transport. Ion transport polarity is only less pronounced. This means that not only potassium and chloride present in the tissue are secreted, but that KCl can also penetrate through the cuticle. The cuticle diminishes K^+ and Cl^- transport but does not entirely block it as it does sugar transport.

Figure 6(*b*) shows the K^+ and Cl^- contents of the disks used in the experiment of Figure 6(*a*) at the end of the experiment. KCl content of the tissue was high when the inner surface was bathed with the KCl solution. In this situation KCl uptake was high and transport was low. In the inversed situation when KCl transport was high, salt uptake and hence concentration in the tissue were low.

IV. Concluding Remarks

The experimental results indicating a polar sugar and ion transport in isolated onion epidermis can be explained by the presence of the cuticle on the outer surface. The cuticle obviously is impermeable for sugars but partially permeable for ions. The fact that ions can be transported from either side of the onion epidermis to the opposite side indicates that unlike hexose molecules ions can move across the cuticle. This passage may occur in small holes in the cuticle which are accessible for the ions but not for the sugars or along possible exchange pathways along charged sites fixed in the cuticle, or both may occur.

The rates of transport of sugar to the inside vary between 20 and 70 n-moles per square centimetre of epidermal surface per hour. Efflux into the free space and then into the solution facing the inner surface of the epidermis occurs probably across the plasmalemma at all faces of each individual cell except at the outer surface which is covered by the cuticle. The true efflux is therefore somewhat smaller than the data based on disk surface indicate. A consideration of the geometry of individual epidermis cells leads to the assumption that this overestimate is about three- to four-fold. Therefore, a better average estimate of sugar efflux (ϕ) is a value of 10 n-moles/cm^2/hr. The concentration of reducing sugar in the tissue was measured to about 230 μmoles per gram of tissue water or about 0·23 moles/l. The permeability coefficient for hexoses (P) can be calculated from $\phi = P(C_i - C_o)$, where C_i is the intracellular and C_o the external sugar concentration. Since C_o is zero at the beginning of the experiments and still negligible after the 12-hr period through which efflux is measured, $P \simeq 10^{-8}$ cm/sec.

For an evaluation of the mechanism of glucose transport in the onion epidermis it is also remarkable that the flow of sugar from the epidermal cells is not inhibited or even slightly increased by metabolic inhibitors and yet has a high temperature coefficient and activation energy. This shows that sugar transport involves passages across an energy barrier which is probably constituted by a lipid membrane, but that the required activation energy not necessarily derives from metabolic sources.

It is unlikely that the high activation energy indicates an enzymatic polysaccharide breakdown as a source of the hexoses diffusing out of the onion epidermis cells, because onion is known to be a glucose-storing plant, and it does not store starch.

V. Acknowledgments

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VI. References

JACKSON, R. T., and BROWN, H. D. (1963).—*J. Cell. Comp. Physiol.* **61**, 215.

KAKÁČ, B., and VEJDĚLEK, J. (1966).—"Handbuch der Kolorimetrie." Vol. III. Section 1. pp. 287 and 352. (Gustav Fischer: Jena.)

LÜTTGE, U. (1964).—*Ber. dt. bot. Ges.* **77**, 181.

TREVELYAN, W. E., PROCTER, D. P., and HARRISON, G. S. (1950).—*Nature, Lond.* **166**, 44.