

NECTAR PRODUCTION IN *ABUTILON*

IV.* WATER AND SOLUTE RELATIONS

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Abstract

Nectar secreted by isolated nectaries floated on sucrose solution was of a higher total molar concentration than the medium. However, under steady-state conditions, the *sucrose* concentration was lower than that of the medium, the nectar containing glucose and fructose in addition to sucrose. When mannitol was added to the medium the osmotic pressure of the nectar exceeded that of the medium although the total molar sugar concentration of the nectar could be somewhat less.

The osmotic pressure of phloem exudate (13 atm) was found to be less than that of nectar produced *in situ* (19 atm).

Various models to account for nectar production are examined.

I. INTRODUCTION

Two important questions concerning nectar production have not been satisfactorily resolved. Firstly, is the sugar moved against a concentration gradient? Secondly, what controls water flow—does it fit an osmotic model?

It has been assumed that in the nectary sugar is moved against a concentration gradient (Lüttge 1966). However, the only thorough investigation is that of Shuel (1956) who found that the nectar produced by isolated snapdragon flowers was of the same sugar concentration as the medium on which they were floating. Interpretation of the concentration relationships is often complicated by the fact that isolated nectaries transform sugars (Zimmermann 1953; Frey-Wyssling, Zimmermann, and Maurizio 1954; Shuel 1956).

In this paper the relationship of the sugar concentration of nectar secreted by *Abutilon* nectaries to the concentration of sugar supplied both *in situ* and in isolated nectaries floating on sugar solutions is investigated. The effect of mannitol on sugar and water relations in isolated nectaries is also described.

Various models of nectar production and water flow in *Abutilon* nectaries are considered.

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II. MATERIALS AND METHODS

The forms of *Abutilon* used have been described previously (Findlay and Mercer 1971a).

(a) Culture of Isolated Nectary Cups

The nectary cups were prepared by removing the corolla and stamens and cutting off the sepals a little beyond the level of the nectary. The five nectaries of each flower were not separated from each other to form nectary segments as in previous experiments (Findlay, Reed, and Mercer 1971). A coverslip was sealed with Vaseline over the cup-shaped basal portion of the calyx containing the nectaries to prevent changes in the concentration of the nectar through evaporation. Preliminary experiments showed that nectary cups cut off through the pedicel at the base of the calyx and floated on solution produced nectar at a low rate. If, however, the base of the calyx was trimmed off close below the nectary tissue, exposing a larger cut surface to the medium, the rate of nectar secretion was comparable to that observed in isolated nectary segments (Findlay, Reed, and Mercer 1971). The base of the calyx, therefore, was removed in all experiments.

The details of culture of the nectaries were the same as those described previously (Findlay, Reed, and Mercer 1971). The baths in which the nectary cups were kept were larger than those used for the nectary segments.

(b) Sugar Content of Nectar

The nectar was collected and analysed for both total and reducing sugar as described previously (Findlay, Reed, and Mercer 1971). The molar concentration of sugar was calculated from the total and reducing sugar in the sample and its volume. As glucose, fructose, and sucrose are the major sugars present in the nectar, the reducing sugar was assumed to be hexose and the non-reducing sugar sucrose.

(c) Nectar Water Volume

Water content of the nectar was determined by weighing. The nectar was absorbed in filter paper which was weighed immediately in small air-tight tubes. The paper was oven-dried and cooled in a desiccator until constant weight was reached.

Alternatively, when the sample was wanted for other analyses, the nectar was collected in capillary tubes and weighed. The weight was corrected for sugar content estimated from the refractive index of the sample.

(d) Osmotic Pressure of the Nectar

Samples remaining from determinations of water content were kept by sealing the capillary with Parafilm and storing frozen. Standard solutions given similar treatment showed no change in osmotic pressure.

The osmotic pressure of nectar was measured using a thermoelectric osmometer similar to that of Baldes (1934). A drop of nectar was suspended from a loop in a thermocouple. The technique of Stover (1960) was used to butt weld the 0.002 in. diameter constantan and manganin wire of the thermocouple. The drop was sealed in a moist chamber held in a temperature-controlled water-bath. Condensation raises the temperature of the drop, and by measuring the voltage induced by the thermocouple (thermal e.m.f. $38\mu\text{V}/1^\circ\text{C}$) a measure of the vapour pressure difference between the drop and the moist atmosphere is obtained. Osmotic pressure differences of 30 atm gave an e.m.f. of $10\mu\text{V}$. With such small voltages to be detected, parasitic e.m.f.'s must be controlled, and the water temperature retained within less than $\pm 0.04^\circ\text{C}$. Precautions against evaporation of the small drops are necessary. The level of control used in the present measurements limited accuracy to ± 0.5 atm.

(e) Chromatography of Sugars

Appropriate samples were spotted on No. 1 or 3 MM Whatman paper and run in ethyl acetate-n-propanol-water (57 : 32 : 13 by vol.) (Wager, unpublished data, quoted by Turner, Turner, and Lee 1957). The papers were treated by the silver nitrate method of Trevelyan, Proctor, and Harrison (1950) to make the sugar spots visible. Mannitol could be detected by this method.

(f) Collection of Phloem Sap

The technique of Kennedy and Mittler (1953) was used to obtain an exudate of phloem sap from *Abutilon* as wounding techniques were unsuccessful. The potato aphid *Microsiphum euphorbiae* was anaesthetized with ether whilst feeding on intact petioles on the bush. The aphid stylet was cut with a sliver of razor blade, leaving the labial sheath to prevent the separation of the stylet components. A few such stylets exuded at c. $1 \mu\text{l}$ per hour. Exudate was collected in a capillary tube sheathing the labial sheath in an effort to reduce evaporation. Sections showed the aphid stylet to have penetrated the phloem. The osmotic pressure of the exudate was determined with a thermoelectric osmometer as above. If evaporation occurred values of the osmotic pressure would be overestimated.

III. RESULTS

(a) Isolated Nectaries

The time course of nectar sugar secretion by the nectary cups floating on media of different sucrose concentrations was similar to that reported by Findlay, Reed, and Mercer (1971) for nectary segments. However, the rates of sugar secretion varied considerably from one nectary cup to another with the same treatment.

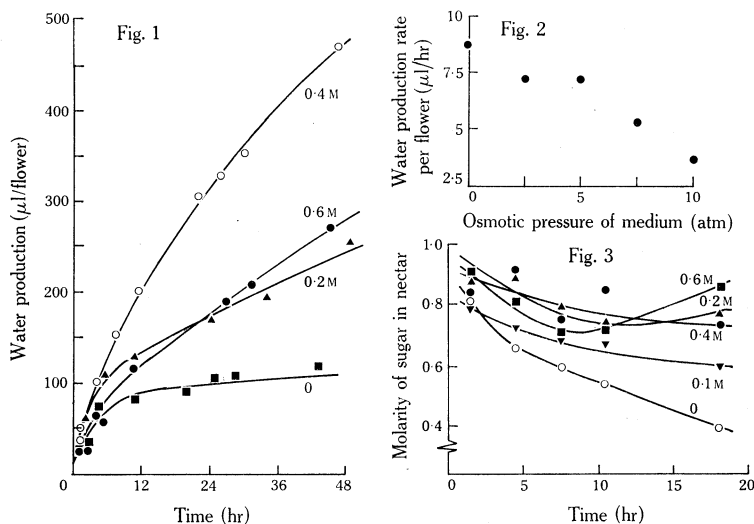


Fig. 1.—Time course of water production by nectaries on sucrose media of the different molarities indicated. Each point is the mean of 1–4 nectary cups.

Fig. 2.—Effect of osmotic pressure of media containing mannitol only on the rate of water production in phase I.

Fig. 3.—Changes with time in the total molarity of sugar in nectar secreted by nectaries on sucrose media of the different molarities indicated. The points are plotted at the midpoint of each collection period. Each point is the mean of two nectary cups.

The pattern of the time course of nectar water production is similar to that for sucrose secretion and occurs in two phases (Fig. 1). In phase I the rate is independent, in phase II it is dependent on the sucrose concentration of the medium. On media containing concentrations of sucrose greater than 0.4 M, the rate of water production is decreased during phase I as well as phase II by increase in medium concentration. This has been found to apply also to sugar secretion.

The initial rate of water production is decreased by increasing the osmotic pressure of the media with mannitol (Fig. 2).

Figure 3 shows the concentration of total sugar in the nectar collected at different times from nectary cups on media of different sucrose concentration. The total sugar concentration of the sample of nectar collected after the first 3 hr was approximately the same ($0.8\text{--}1.0\text{M}$) for all concentrations of the medium from water to 0.6M sucrose. Thereafter the concentration of the nectar gradually changed, the nectar from nectaries on the lowest media concentrations showing the greatest decrease.

The total sugar concentration of the nectar was always higher than the medium sucrose concentration. However, the sucrose (in contrast to total sugar, mainly glucose, fructose, and sucrose) concentration of the nectar was always lower than the sucrose concentration of the medium except on water or during the initial period on 0.1M sucrose.

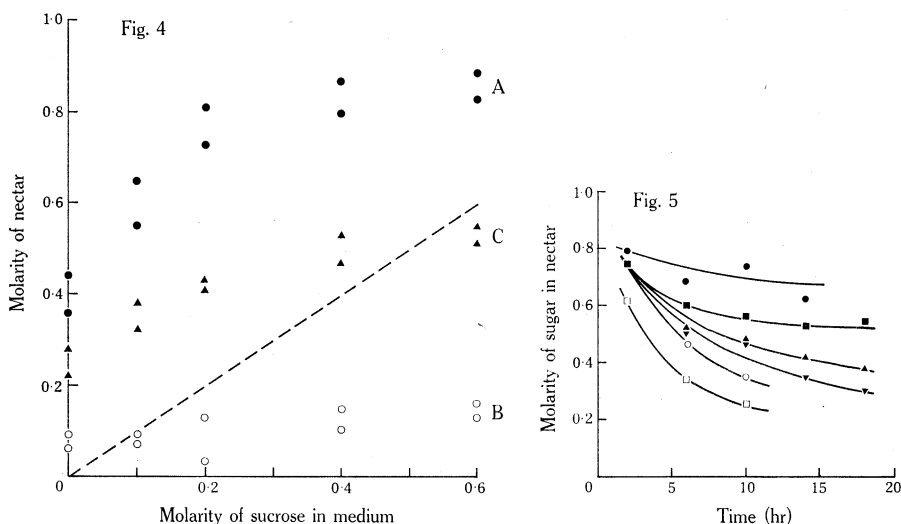


Fig. 4.—Molar concentration of nectar secreted during phase II (12–24 hr) by nectaries on media of different sucrose concentrations. *A*, total sugar concentration; *B*, sucrose concentration; *C*, total sugar concentration expressed as molarity of sucrose. Dotted line indicates equality of molarity of medium and nectar.

Fig. 5.—Changes with time in the total molarity of sugar in nectar secreted by nectaries on zero sucrose (\circ), 0.1M sucrose (\blacktriangledown), 0.2M sucrose (\blacktriangle), 0.3M sucrose (\blacksquare)—all with mannitol to give a total osmotic pressure of 10.8 atm ; 0.4M sucrose (\bullet); and water only (\square). Points are plotted at the midpoint of each collection period. Each point is the mean of three nectary cups.

In phase II the concentration of nectar depends on the sucrose concentration of the medium (Fig. 4). Since the total sugar concentration expressed as molarity of sucrose (graph *C*) is not equal to the medium sucrose concentration it is clear the higher molar concentration of the nectar is not merely due to hydrolysis of some of the sucrose in its passage through the nectary and in the nectar.

The total sugar concentration of nectar secreted by nectary cups floated on media containing $0\text{--}0.4\text{M}$ sucrose and with the total osmotic pressure adjusted to the

same value (10.8 atm, equivalent to 0.4M sucrose) by the addition of mannitol, showed similar changes (Fig. 5). The magnitude of the decrease depended on the sucrose concentration of the medium as in the absence of mannitol. The sugar concentration of nectar was also influenced by the presence of mannitol, the decrease being less rapid on 0.4M mannitol than on water (Fig. 5). In a similar experiment with the osmotic pressure of the medium adjusted to 10.8 atm the osmotic pressure of the nectar produced during phase II as measured with the thermoelectric osmometer was always higher than that of the medium and was dependent on the sucrose concentration of the medium (Fig. 6).

The osmotic pressure of nectar secreted in phase II by nectary cups floating on media of constant sucrose concentration (0.1M), but of differing osmotic pressure obtained by the addition of various concentrations of mannitol, varied with the osmotic pressure of the media and always exceeded the osmotic pressure of the medium (Fig. 7). The rate of water production under these circumstances is shown in Figure 8.

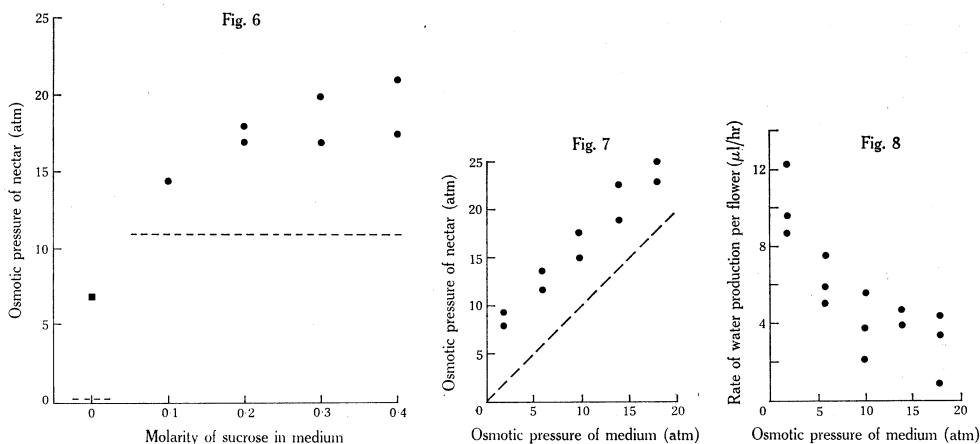


Fig. 6.—Osmotic pressure of the nectar secreted during phase II (25–31 hr) by nectaries floating on sucrose media of different molarities but adjusted to an osmotic pressure of 10.8 atm with mannitol. ■ Nectaries on water only. Dotted lines show osmotic pressure of medium.

Fig. 7.—Osmotic pressure of nectar secreted during phase II on media containing 0.1M sucrose with mannitol to raise the osmotic pressure. The dotted line shows equality of medium and nectar osmotic pressure.

Fig. 8.—Rate of water production during phase II; media with 0.1M sucrose, and mannitol to adjust the osmotic pressure.

Chromatograms of nectar produced during phase II by nectaries which had been floated on 0.4M mannitol plus 0.1M sucrose showed faint spots at the mannitol position. However, no mannitol was detected in nectar from nectaries which had floated on 0.2M mannitol for 24 hr.

The nectar produced by nectaries floating on sucrose solutions was shown by chromatography to contain very approximately equal concentrations of sucrose, glucose, and fructose by weight. In the experiment of Figure 3, chemical analysis showed that non-reducing sugar made up $20.4 \pm 1.1\%$ by weight of the total sugar (44 observations). No large consistent changes in the ratios of the three sugars during the course of the experiment were evident from chromatograms or chemical analysis.

Extracts of nectary tissue contained a higher proportion of sucrose to glucose and fructose than the nectar. This was found not only in nectaries which had been floating on sucrose solution but also in the nectaries of freshly picked flowers and in nectaries which had been floating on water, thus excluding the possibility that the high sucrose content might have been due entirely to sucrose which had diffused into the free space of the nectary tissue from the medium. The chromatograms also showed two faint spots having a lower R_F than sucrose. These may have been trisaccharides or other oligosaccharides such as have been reported for some other species (Zimmerman 1953).

(b) *Concentration of Sugar in Phloem Exudate, Nectar, and Nectaries on the Plant*

Only one measurement of phloem exudate from the petioles of *Abutilon* was completed successfully. This sample had an osmotic pressure of 13 atm which is equivalent to that of a 0.47M sucrose solution.

A nectary cup *in situ* sealed with a coverslip in a similar manner to the isolated nectary cups produced nectar with an osmotic pressure of 19 atm which is equivalent to that of a 0.6M sucrose solution.

TABLE 1
SUGAR CONTENTS OF THE PARTS OF NECTARY SEGMENTS

Reducing sugar (R) and total sugar (T) in the various parts expressed as milligrams per gram fresh weight of tissue

Expt. No.	Sepal*			Nectary Tissue*			Hairs*			Nectar: R/T (%)
	R	T	R/T (%)	R	T	R/T (%)	R	T	R/T (%)	
1	5	8	63	10	22	43	25			
2	8	16	50	20	53	38		91		62
3	5			14			32			
4	6	13	46	16	69	23		108		57
Mean	6	12	53	15	48	35	28	100	28	60

* Mean sugar content per flower of sepals, nectary tissue, and hairs 4.8, 4.0, and 1.2 mg respectively.

The distribution of sugar in nectaries of mature flowers is shown in Table 1. The nectary hairs comprise only a small fraction of the nectary and only a small part of the total sugar is contained in them. However, the average concentration of sugar is highest in the nectary hairs.

IV. DISCUSSION

The results may be summarized as follows:

- (1) The osmotic pressure of the nectar exceeds the osmotic pressure of the medium.
- (2) Where sucrose only is supplied in the medium, the *total molar sugar* concentration of the nectar exceeds that of the medium.
- (3) The *sucrose* concentration of the nectar, under steady-state conditions, is less than that of the medium.

On the plant it appears that the nectar sugar concentration is higher than the concentration of sugar in the phloem (0.47M). The single value obtained for the phloem sap concentration is similar to that reported for the phloem sap of a number of other woody species (Zimmermann 1960). It is interesting that the maximum rate of nectar secretion by isolated nectaries occurs in nectaries on about 0.4M sucrose (about the concentration in the phloem) and this is also the medium concentration when there is a transition from a two-phase to a one-phase nectar secretion pattern. Possibly, the physiological state of the isolated nectaries floating on 0.4M sucrose may correspond to the normal physiological state of nectaries on the plant. Analysis of the distribution of sugar in different parts of the nectary shows that the average content of sugar is higher in the nectary hairs than in the nectary tissue. Since some of the nectary tissue cells may be low in sugar and the sugar in the nectary tissue may be largely in the phloem strands (which terminate about three cells from the base of the hairs) this does not necessarily demonstrate an increasing total sugar concentration from the phloem endings to the hair tips but it is likely that this is the case.

Several models of nectar production may be considered:

- (1) The transformation of sugars by the nectary maintaining a gradient both for the further diffusion of sugars and a passive movement of water through the nectary and into the nectar.
- (2) The active transport of the constituents of nectar as solution.
- (3) The active transport and concentration of sugar accompanied by passive water movement.

(a) *Model 1: Transformation of Sugars*

The nectar produced by most nectaries, including those of *Abutilon*, contains glucose and fructose as well as sucrose when sucrose is supplied in the phloem or in the medium. The diffusion or facilitated diffusion of sucrose into the nectary tissue followed by its transformation to glucose and fructose could maintain concentration gradients favouring the further diffusion of sucrose into the nectary and nectar and simultaneously maintaining a water potential gradient between the medium and the prenectar. For nectaries floating on glucose or fructose a synthesis of sucrose could allow the further diffusion of the hexose into the nectary and so maintain a water potential gradient. The formation of trisaccharides (Zimmermann 1952, 1953, 1954) could also assist in maintaining gradients. Many of the enzymes required for sugar transformation have been demonstrated to be very active in nectaries of *Deutzia* and *Convolvulus* (Fekete, Ziegler, and Wolf 1967).

The situation in *Abutilon* is consistent with this model in so far as the sucrose concentration of the nectar secreted by isolated nectaries was always found to be lower than that of the medium during phase II and, for both isolated nectaries and nectaries on the plant, the proportion of reducing sugar to sucrose was higher in the nectar than in the nectary. However, no gradient in the proportion of sugars was found within the nectaries of freshly picked flowers.

A difficulty with the model, however, is that the diffusion into the medium of the sugars formed in isolated nectaries will be large unless there is some factor preventing this diffusion. Also, some means of transport of nectar out of the cells and

into the subcuticular space is still required. Hydrolysis of sucrose could occur in the subcuticular space, as invertase is known to occur in nectar (Zimmermann 1953, 1954), thus maintaining movement of sucrose into the subcuticular space in nectaries on sucrose media or on the plant. However, this does not apply to nectaries on glucose or fructose, which sugars also support nectar production in *Abutilon* (Matile 1956).

Thus sugar transformations which maintain diffusion gradients may be involved in sugar transport in nectaries, but some active process is also necessary for this transport.

(b) *Model 2: Active Transport of Sugar Solution*

Nectar could be transported as solution through the nectary if a process such as exocytosis or pinocytosis occurs. Ziegler (1965) has considered such a model. There is some evidence from electron micrographs (Findlay and Mercer 1971b) for a process of this type in *Abutilon*.

If all the movement of water and solute occurred by solution transport the steady state concentration (measured as total weight per unit volume) of the nectar would be equal to that of the medium, and this is not the case (graph C, Fig. 4). However, there appears to be at least some additional passive water movement which would result in a change in concentration of the nectar, but there are insufficient data to predict the expected direction or magnitude of such a passive water flow.

Although there is no clear evidence against this model, the various physiological phenomena observed in *Abutilon* are more easily explained by other models.

(c) *Model 3: Active Transport of Sugar, Accompanied by Passive Water Movement*

The transport of sugar in nectaries appears to differ in characteristics from active sugar accumulation, such as occurs into the vacuoles of storage tissues, in its energy requirements and in the external concentrations involved (Bialeski 1962; Grant and Beever 1964; Findlay, Reed, and Mercer 1971). This is not surprising since most of the sugar transport in nectaries is likely to be through the cytoplasm and not into the vacuoles.

Of the several possibilities for the location of the active transport two will be considered.

(i) *Active Step of Sugar Transport Located within the Nectary*

A similar model has been proposed by Arisz *et al.* (1955) for salt glands and has been considered for nectaries by Ziegler (1965).

In *Abutilon*, the stalk cell at the base of the hairs could be the site for this active transport as here movement of substances appears to be restricted to a route through the cell protoplast because the lateral walls of these cells are modified (Findlay and Mercer 1971b). As the sugar concentration in the hair increases, the water potential decreases and water will flow into the hair. The resultant increase in hydrostatic pressure in the hair then causes a leakage or squeezing of solution from the hair cells and through the cuticle. This model would require the active sugar transport into the hair to occur across a cell relatively impermeable to the diffusion of sugar whereas the other cells of the hair, or at least some part of them, would be relatively freely permeable to sugar.

If it is assumed that there is mixing of the solution within the nectary hair it would be expected that the rate of secretion of sugar by nectaries would decline if transport of sugar into the hair ceased and the sugar within the hair became depleted hence decreasing the water potential gradient. This does not happen immediately with nectaries floated on water as they continue secretion at a steady rate for about 7 hr (Findlay, Reed, and Mercer 1971). Continued uptake of sugar from the nectary tissue into the hairs and hydrolysis of sucrose to glucose and fructose within the hair could be sufficient to maintain secretion for this time.

The effects of addition of a non-permeating solute such as mannitol on sugar secretion can be interpreted in terms of this model. The presence of mannitol in the medium should have a rapid effect on the rate of sugar secretion. The mannitol in the medium would reduce the water potential gradient thus decreasing the rate of water flow into the nectary hairs and hence the rate at which solution and consequently sugar will leak out of the nectary. The higher the mannitol concentration the lower the initial rate of sugar secretion. This agrees with the experimental findings (see Fig. 8 of Findlay, Reed, and Mercer 1971). On media with mannitol but no sucrose, the secretion of sugar would cease when the water potential gradient between medium and hair reached zero, i.e. the higher the medium mannitol concentration the less sugar secreted before the nectaries stopped secretion. This again agrees with the experimental findings [Fig. 7(a) of Findlay, Reed, and Mercer 1971]. On media containing sucrose as well as mannitol, the concentration of sugar in the hair will increase as compared with nectaries on the same sucrose concentration but without mannitol. Eventually the rate of sugar secretion will be the same as that without mannitol, but the molar concentration of the nectar will be higher the higher the medium mannitol concentration. This has been found to be the case during phase II (Fig. 7).

The pattern of secretion by immature nectaries floated on sugar solutions is consistent with this model (Findlay, Reed, and Mercer 1971).

Also the continued secretion by hairs in sections, in sections treated with inhibitors, by hairs with spontaneously plasmolysed cells, and in isolated hairs (Findlay and Mercer 1971a) are consistent with this model.

(ii) *Active Step of Sugar Transport Located at the Tip of the Hair*

Ziegler (1965) has considered the general evidence for this model.

In *Abutilon* the effect of mannitol in the medium on the initial rate of sugar secretion by isolated nectaries is difficult to explain by this model. A rapid effect would be expected if mannitol acted by changing the sugar secretion rate as a result of changing the osmotic pressure at the sugar transport site but this interpretation has been dismissed for other reasons (Findlay, Reed, and Mercer 1971). On the other hand, mannitol may act by changing the rate of water flow. This would not be expected to affect the initial rate of sugar secretion unless the water flow affected the concentration of sugar at the transport site similarly to the polarization of solute in transcellular osmosis (Kamiya and Kuroda 1956). The possible magnitude of such an effect cannot be predicted at present.

The reduction of the total amount of endogenous sugar secreted when nectaries are floated on media with mannitol but no sucrose (Findlay, Reed, and Mercer 1971) cannot be readily explained by this model.

The observations on exudation by sections of nectary (Findlay and Mercer 1971*a*) are compatible with this model with the possible exception of the continuation of exudation by spontaneously plasmolysed hairs.

However, neither of the above proposed sites for an active transport step gains any support from the electron-microscope studies (Findlay and Mercer 1971*b*) since there is no particular accumulation of mitochondria or other organelles either at the hair tips or at the base of the hairs, but the mitochondria and endoplasmic reticulum are abundant throughout the hair cells. Nevertheless, it appears that although the active transport actually may be widespread in the hair its physiological effect (perhaps because of the construction of the hair) can be predicted from model 3(i).

(iii) *Water Flow*

In *Abutilon* nectaries the nectar passes through the cuticle covering the nectary hairs via valve-like pores (Findlay and Mercer 1971*a*) and there will be little exchange of water with the nectary cells once the nectar is exuded through the cuticle. Any change in water content of the nectar will be by exchange with the external environment, including other parts of the flower. On the other hand, the water potential of the prenectar in the subcuticular space could be of importance in influencing water flow through the nectary. The hydrostatic pressure (P_n) in the subcuticular space will be greater than the outside pressure if exudation through the cuticle is to take place. This pressure changes cyclically, rising slowly as prenectar collects in the subcuticular space and then falling sharply as prenectar is exuded through the pores. However, the mean value of P_n will normally remain constant over long periods of time assuming that the mechanical properties of the cuticle and the pores in it remain the same.

A major part of the water flow is passive because the initial rate of water movement into the nectar is decreased by lowering the water potential of the medium by the addition of mannitol (Fig. 2). The osmotic effect of sucrose is less. This is to be expected because of the greater movement of sucrose into the nectary and nectar, i.e. the reflection coefficient, σ , for sucrose is less than 1. The rate of water flow in phase I was decreased by high concentrations (above 0.4M) of sucrose, but these high concentrations appear to have some effect in addition to altering the water potential since the rate was also decreased in phase II, unlike the results obtained with mannitol.

The factors controlling water flow depend on which model is appropriate for nectar production. In model 3(i) the water flow would be similar to that in the model described by Curran and MacIntosh (1962). The water flow would depend on the water potential gradient between the medium (corresponding to compartment A of Curran and MacIntosh) and the compartment into which sugar is actively transported (corresponding to compartment B). Water flow would be independent of water potential in the prenectar or nectar (corresponding to compartment C). Thus, for water flow to occur,

$$\sigma\Pi_m < \sigma\Pi_c - P_c,$$

where Π_c and Π_m are the osmotic pressures in the stalk cell (or hair cells or whatever cells into which the active transport of sugar occurs) and the medium respectively,

P_c the hydrostatic pressure in the stalk cells, and σ the reflection coefficient for the solute. The solution from these cells leaks into the subcuticular space and is then exuded through the cuticle. Thus in a steady state the concentration of nectar formed should equal the concentration in the sugar transporting cells except for changes due to sugar transformations.

In models 2 or 3(ii) the force producing water flow would be the water potential difference between the medium and the prenectar in the subcuticular space. For water movement to take place,

$$\sigma\Pi_m < \sigma\Pi_n - P_n,$$

where Π_m and Π_n are the osmotic pressure of the medium and prenectar respectively, P_n the hydrostatic pressure in the subcuticular space, and σ the reflection coefficient for the solute.

In the present experiments Π_n exceeded Π_m . In the experiment of Figure 7 the concentration of the nectar exceeded that of the medium by 6.6 ± 0.3 atm and similar or larger differences were found in other experiments. However, P_n , P_c , and σ for both sugars and mannitol are unknown, leaving in doubt whether water movement can be explained by classical osmosis. Certainly in some circumstances such as shown in Figure 8 the rates of water production were not readily explicable in terms of classical osmosis.

At the present time it appears that a model involving the active transport of sugars within the nectary, possibly at the stalk cell, resulting in an uptake of water into the hair and hence leaking of sugar solution from the permeable hair cells, combined with transformation of sugars increasing the water potential gradient, is the most likely explanation of nectar production in *Abutilon* nectaries.

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