NECTAR PRODUCTION IN *ABUTILON*

III.* SUGAR SECRETION

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Abstract

Analysis of *Abutilon* nectaries shows that the sugar content of the nectary rises sharply as nectar secretion begins. Nectar secretion on the plant occurs at a steady rate of 2–5 mg total sugar per flower.hour for 36–48 hr. A total of about 100 mg sugar per flower is secreted, and this is about seven times the maximum sugar content of the nectary at any time. Secretion of sugar in nectar by isolated nectaries floating on 0–0·4M sucrose solution is in two phases. During phase I the rate is independent of concentration of sucrose in the external medium, but the rate is decreased by increasing the osmotic pressure of the medium with mannitol. In phase II the rate of secretion of sugar depends on the concentration of sucrose in the external medium and is independent of increase in osmotic pressure with mannitol. The rate is zero on water and reaches a maximum on about 0·4M sucrose. Secretion is reduced in both phases on media of sucrose concentration greater than 0·4M.

By floating immature nectaries on sucrose solutions, secretion of nectar is induced earlier than would occur from nectaries on the plant.

Respiratory inhibitors and anaerobic conditions reduce nectar secretion in both phases. Temperature has a marked effect on the rate of secretion, with a temperature coefficient ($k_{20°C}/k_{10°C}$) of 2·3.

The total respiration of the nectaries was sufficient to supply only about one $\sim$P per sugar molecule transported through an actively secreting nectary.

The movement of the sugar component of nectar appears to be an active process (though influenced by water movement), but the mechanism is not understood.

I. INTRODUCTION

Isolated nectaries of several species of plants, floating on water, continue to secrete nectar and this secretion is often enhanced by the addition of various sugars to the medium (Radtke 1926; Agthe 1951; Matile 1956). However, little is known about the quantitative details of nectar secretion under these conditions.

This paper deals with physiological aspects of nectar production by *Abutilon* nectaries either when on the plant or when isolated.

II. Materials and Methods

Nectaries of two forms of *Abutilon* (Findlay and Mercer 1971) were used and behaved similarly in the present study. If quantitative differences did exist between nectaries of the two forms they were masked by the variability within each form.

Unless otherwise indicated the nectaries were taken from flowers which were just opening and in which nectar secretion was just beginning. These flowers are termed "mature".

(a) *Preparation of Isolated Nectary Segments*

The nectaries from four to six flowers were isolated and each split into five segments corresponding to the individual sepals. Only that part of the sepal extending beyond the nectary was cut off. One segment from each flower was assigned to each treatment.

(b) *Culture of Isolated Nectaries*

Fig. 1

The nectary segments (N) were kept in Perspex baths floating on the medium which was continuously circulated and aerated by compressed air passed through a hypodermic needle (H) into Nylex tubing (Fig. 1). The medium was aerated before use and was changed frequently so that the concentration of the medium did not alter significantly during an experiment. Experiments were done at room temperature, which remained constant within about 1°C during an experiment.

For experiments under anaerobic conditions the isolated nectary segments were floated on a shallow layer of solution in a wide-mouthed bottle through which nitrogen gas was passed. The bottles were kept in a nitrogen-filled glove-box in which the nectar could be collected without disturbing the anaerobic conditions. Control nectaries were treated similarly except that air was used in place of nitrogen. The nectaries were kept in the dark except during nectar collection.
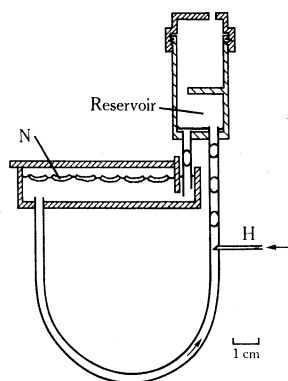
In the experiments with inhibitors, M/30 phosphate buffer, pH 6·0, was included in the medium. In the other experiments the medium was not buffered. No detectable pH changes occurred in the media prior to their renewal.

Mannitol was used to adjust the osmotic pressure of the medium which was taken as the sum of the osmotic pressures attributable to sucrose and mannitol and was expressed as the equivalent sucrose concentration having the same osmotic pressure as the total osmotic pressure of the medium.

(c) *Nectar Collection*

The nectaries were removed from the solutions one at a time and the nectar collected with a calibrated pipette. The nectar samples and the rinsings from the pipette were transferred to small glass specimen tubes and stored frozen until the nectar was analysed.

(d) *Tissue Extraction*

Tissue samples were extracted twice with 80% ethanol at 80°C for a total of 30 min. A total of 7·5 ml of ethanol was used for tissue samples which contained up to 1 mg total sugar. The extract was evaporated to dryness on a water-bath.

(e) *Sugar Analysis*

Sugar was determined by the method of Somogyi (1945) modified by McIlroy (1950). Small samples of nectar were each used whole for a single determination. Larger samples were diluted and suitable aliquots containing up to 4 mg sugar taken. In some experiments total and reducing sugar were determined, but in most only total sugar was determined.

Dried extracts of nectary tissues were dissolved in a suitable volume of water and treated in the same way as the nectar samples.

For hydrolysis a volume of $0 \cdot 1$N HCl equal to half the sample volume was added and boiled for 10 min and then cooled and neutralized with NaOH using phenol red indicator.

The total sugar values are given as the number of milligrams of hexose present after hydrolysis. The nectar contains 20–30% by weight of sucrose, the remainder being mainly glucose and fructose in approximately equal amounts. Since sucrose has a molecular weight 5% less than two hexose molecules the total sugar values given are 1–2% too high but this is not significant in the present experiments.

(f) Experiments with [14C]Sucrose

The media contained $0 \cdot 5$ μCi/ml of uniformly labelled [14C]sucrose. To determine the 14C content of the tissues, segments were rinsed for 20 sec in unlabelled solution then the nectary tissue was cut from the underlying sepal tissue. Nectary and sepal tissue were extracted separately [see Section II(e)] except that it was done overnight at room temperature. Nectar or concentrated extracts from the tissue were counted after drying on aluminium planchets.

(g) Respiration

The respiration rate was determined using standard Warburg technique. The experimental solution was 3 ml M/30 phosphate buffer, pH $6 \cdot 0$, containing $0–0 \cdot 2$M sucrose depending on the experiment. During the experiment inhibitors were added in appropriate amounts from a side-arm. The experiments were done at 25°C in the dark.

III. RESULTS

(a) Nectaries on the Plant

Nectar secretion was measured in flowers which were enclosed in gauze bags to exclude birds and insects. Nectar secretion begins at about the time the corolla unfolds and continues at a steady rate for 36–48 hr, ceasing when the flower wilts.
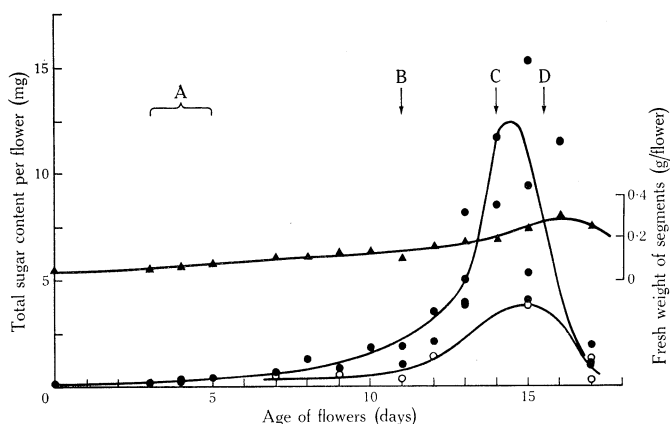

Fig. 2.—Total sugar content of nectary segments (●) and underlying sepal only (○) in freshly picked flowers of different ages. ▲ Fresh weight of segments. The times are shown when nectary hairs form (A), sepals begin to unfold (B), corolla unfolds (C), and wilting begins (D).

Diurnal variations in the amount of total sugar secreted were not detected. Rates varied from 2–5 mg total sugar per flower.hour. The total amount of sugar secreted averaged about 100 mg per flower, which is seven times the maximum amount of sugar present in a nectary at any one time.

The changes in sugar content of the nectary as the flower develops were followed using nectaries of different ages collected from a bush on one day shortly after sunrise. No starch was present in either the nectaries or the sepals. The sugar
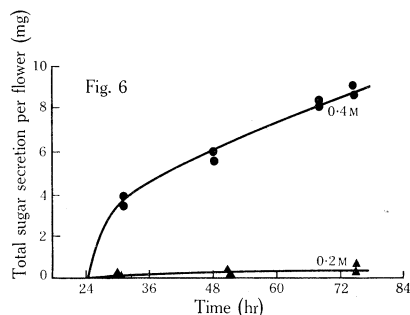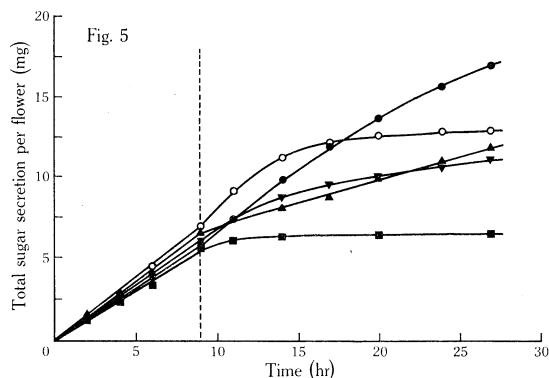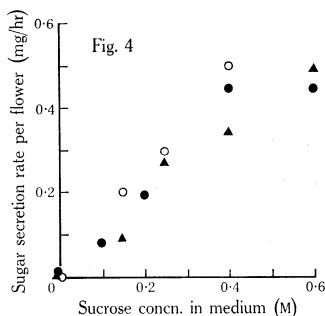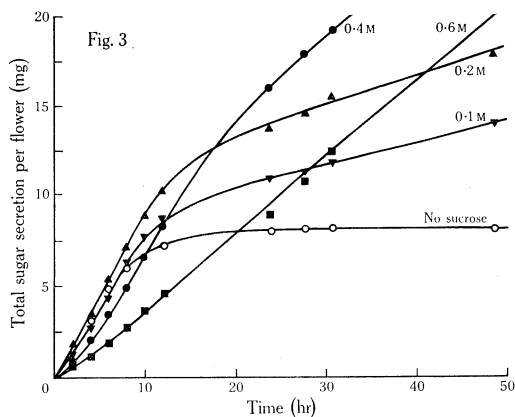

Fig. 3.—Time course of total sugar secretion by isolated nectaries on media of different sucrose molarities as shown.

Fig. 4.—Rate of total sugar secretion by isolated nectaries during phase II at different sucrose molarities. The results are from three experiments. ● No mannitol. ○ Total molarity adjusted to 0·4M with mannitol. ▲ Total molarity adjusted to 0·6M with mannitol.

Fig. 5.—Time course of total sugar secretion by nectaries floated on 0·4M sucrose and transferred (dotted line) to: water (○), 0·4M sucrose + 10^{-4}M KCN (▼), 0·4M sucrose + 10^{-4}M dinitrophenol (▲), 0·4M sucrose + 10^{-3}M NaN$_3$ (■), 0·4M sucrose (●). All solutions contained M/30 phosphate buffer, pH 6·0.

Fig. 6.—Time course of total sugar secretion on 0·4M and 0·2M sucrose by flowers picked about 3 days before nectar secretion in control flowers on the plant began. No secretion occurred in nectaries floated on water. Nectary cups (Reed, Findlay, and Mercer 1971) were used in this experiment.

content of the nectary increased rapidly prior to the beginning of nectar secretion and decreased as secretion ceased (Fig. 2).

The fresh weight of isolated whole segments (nectary plus adjoining tissue) increased during the 11 days preceding the onset of nectar secretion (Fig. 2). Since the area and thickness of the nectary remained almost constant over this time most of the increase in weight of the segment occurred in the sepal tissue and not the nectary tissue suggesting that the changes in concentration of sugar in the nectary tissue are proportional to the changes in total sugar content. The sugar content of the underlying sepal tissue increases much less than that of the nectary tissue.

The total sugar content of secreting nectaries (including underlying sepal tissue) analysed at various times of the year was variable and could be as high as 15 mg per flower.

Although no starch is present in the early morning, nectaries collected later in the day contained starch granules in the nectary tissue though not in the nectary hairs.

(b) *Isolated Nectaries*

(i) *Effect of Concentration of Sucrose in the External Medium*

Figure 3 shows the time course of nectar sugar secretion on media containing different concentrations of sucrose only. On solutions of zero up to $0 \cdot 4$M sucrose concentration nectar sugar secretion occurs in two phases. In phase I, over the first 7 hr, the rate of secretion at about $0 \cdot 5$–$1 \cdot 0$ mg sugar per flower.hour is independent of the concentration of the external medium. After about 7 hr the rate begins to change until after 10–15 hr it reaches a new steady state, phase II. In phase II the rate is proportional to the concentration of sucrose in the external medium and in the absence of external sucrose no nectar is produced (Fig. 4). On media of about $0 \cdot 4$M sucrose concentration or higher the two phases are not apparent, the rate of sugar secretion being virtually constant throughout. This rate decreases with increase in the sucrose concentration of the medium above $0 \cdot 4$M. The rate of secretion usually declined after about 30 or 40 hr on all solutions when fungal contamination often became obvious.

On $0 \cdot 4$M sucrose the rate of sugar secretion (*c.* $0 \cdot 5$–$1 \cdot 0$ mg per flower.hour) was somewhat less than the rate for nectaries on the plant (about 5 mg per flower.hour).

Nectaries which have been floating on $0 \cdot 4$M sucrose for some time show an immediate small increase in the rate of sugar secretion when transferred to water but the rate decreases to zero over about 7 hr (Fig. 5).

In nectary segments on $0 \cdot 2$M sucrose medium containing [U-^{14}C]sucrose, the label appears in the nectar within about 3 hr.

(ii) *Initiation of Nectar Secretion in Nectaries from Buds*

Abutilon buds were picked (at *c.* day 11, Fig. 2) when the sepals were beginning to separate, and before the sugar content of the nectaries increases. When these nectaries were floated on sugar solution, nectar secretion began 3 days before that in control nectaries in flowers left on the plant. Secretion was greater on $0 \cdot 4$M sucrose than on $0 \cdot 2$M sucrose and secretion did not occur on water (Fig. 6). Though the two-phase pattern of nectar secretion was similar to that of the flowers picked later, the rate during the first phase was not independent of the medium concentration.

(iii) *Effect of Osmotic Pressure of the Medium*

The effect on sugar secretion of increasing the osmotic pressure of media without sucrose by the addition of mannitol is shown in Figure 7(a). The initial rate of sugar secretion in the first phase decreased as the mannitol concentration
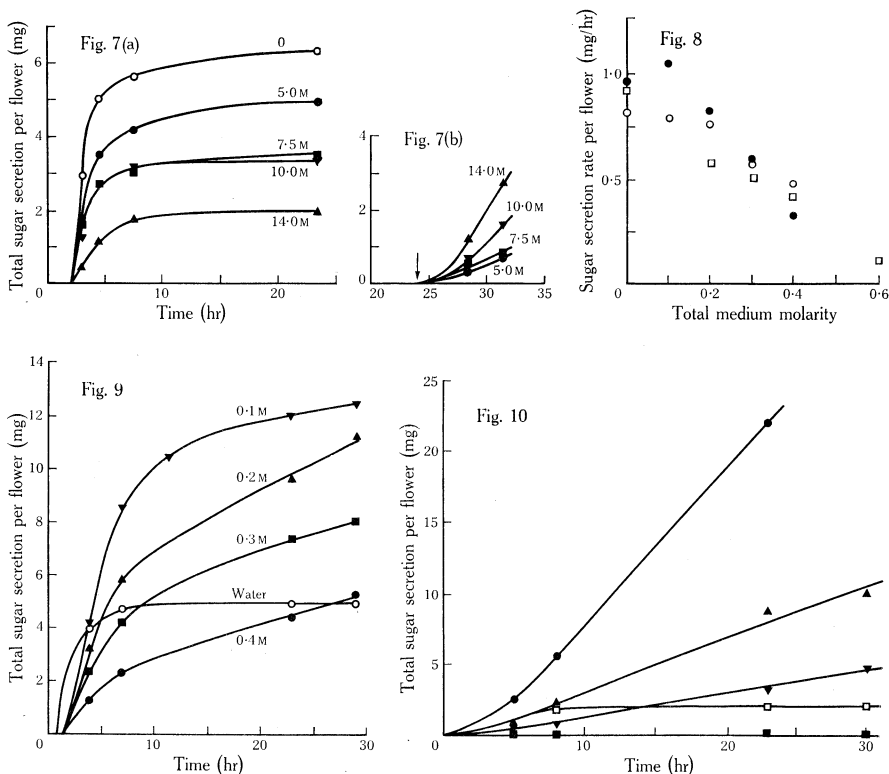
Fig. 7.—(a) Time course of total sugar secretion on media of different mannitol molarities as indicated for each curve. No sucrose was present in the media. (b) Time course of total sugar secretion by the above nectaries after transfer (arrow) to sucrose solutions of equivalent molar concentration.

Fig. 8.—Initial rate of total sugar secretion on media of different total concentrations containing mannitol with no sucrose ($\bigcirc$, $\square$) (two experiments) or $0 \cdot 1$M sucrose ($\bullet$).

Fig. 9.—Time course of total sugar secretion on media containing $0 \cdot 1$M sucrose plus mannitol to give the total molarities shown on the curves. Time course with water only as medium is also shown ($\bigcirc$). The flowers were slightly younger than normal.

Fig. 10.—Time course of total sugar secretion on media containing: no sucrose ($\blacksquare$); $0 \cdot 15$M sucrose ($\blacktriangledown$); $0 \cdot 25$M sucrose ($\blacktriangle$), these three with mannitol added to make a total molarity of $0 \cdot 4$; $0 \cdot 4$M sucrose ($\bullet$); water only ($\square$).

of the medium was increased (Fig. 8). When nectaries which had ceased nectar secretion were transferred to sucrose solutions of equivalent osmotic concentration, secretion resumed [Fig. 7(b)], the rate being dependent upon the sucrose concentration as for a normal phase II.

Various concentrations of mannitol in the presence of 0·1M sucrose in the medium had no detectable effect on the rate of sugar secretion during phase II (Fig. 9).

The effect on nectar sugar secretion of media of the same total osmotic pressure but different concentrations of sucrose is shown in Figure 10. The rate of sugar secretion in phase I is reduced on low sucrose concentrations and concomitant high mannitol concentrations, and it may be reduced to zero in the absence of sucrose. However, the magnitude of this reduction in rate varies in different experiments (the experiment of Figure 10 showed an abnormally small amount of sugar secreted in the first phase even on water alone). In phase II the rate of sugar secretion is dependent on the sucrose concentration of the medium as is the case in the absence of mannitol (Fig. 4).

Nectaries on mannitol solutions secrete only small amounts of mannitol (Reed, Findlay, and Mercer 1971).

(iv) *Effect of Inhibitors, Anaerobic Conditions, and Temperature*

Respiratory inhibitors were found to decrease nectar sugar secretion in isolated nectary segments of *Abutilon* (Fig. 5), confirming observations of Matile (1956) and Ziegler (1956). The degree of inhibition was variable but occasionally approached 100% with sodium azide (10^{-3}M).

Potassium cyanide (10^{-4}M) decreased the rate of sugar secretion during both the first phase (Fig. 11) and the second phase (Fig. 5). When nectaries were floated on a medium lacking sucrose, the total amount of sugar secreted by the end of phase I was also reduced by potassium cyanide (Fig. 11). The inhibition was reversible (Fig. 11). 2,4-Dinitrophenol (10^{-4}M) also inhibited both phases of secretion with or without sucrose in the medium.
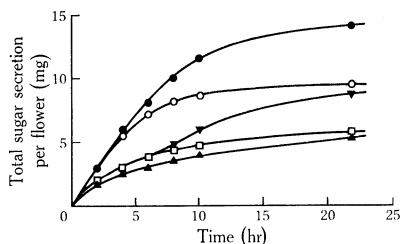

Fig. 11.—Time course of total sugar secretion on media containing: 0·1M sucrose (●); no sucrose (○); 2×10^{-4}M KCN, 0·1M sucrose (▲); 2×10^{-4}M KCN, 0·1M sucrose for the first 6 hr and then 0·1M sucrose only (▼); 2×10^{-4}M KCN, no sucrose (□). All media contained M/30 phosphate buffer, pH 6·0.

Sodium fluoride (10^{-2}M) brought about a partial inhibition of nectar secretion, but phloridzin (10^{-5}–10^{-3}M) and mercuric chloride (10^{-5}M) were without effect.

Under anaerobic conditions both the amount of sugar and the volume of nectar secreted were decreased (Fig. 12). The rate of volume increase of nectar varied from 0 to 90% of that in air. The inhibition was nearly complete in flowers picked just before they opened. The effect was reversible [Fig. 12(*b*)]. Under nitrogen, the amount of label appearing in nectaries floated on uniformly labelled [^{14}C]sucrose was less than in those in air (Fig. 13). The difference was greater when both the content of the nectaries and the nectar produced by them was taken into account.

Temperature has a marked effect on the rate of sugar secretion (Fig. 14), with a temperature coefficient of 2·3 between 10 and 20°C.

(v) *Respiration of Nectaries*

Nectary segments (minus sepal tissue) from mature flowers were found to have a respiration rate of $62 \cdot 0 \pm 2 \cdot 5$ μl O_2 per flower . hour. The tissues of the nectary had a respiration rate of 820 ± 60 μl O_2 per gram fresh weight . hour as compared with 227 ± 27 μl O_2 per gram fresh weight . hour for the underlying sepal tissue.

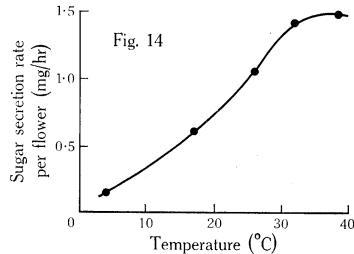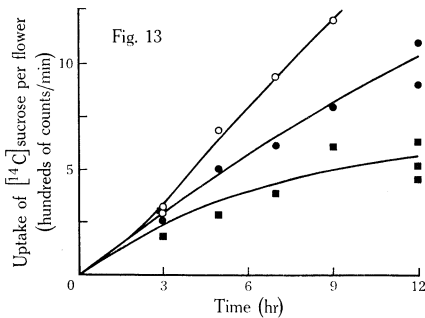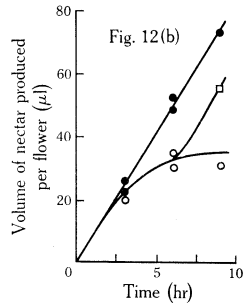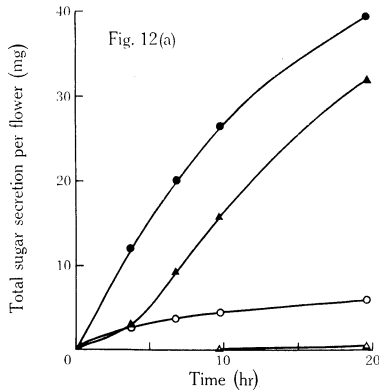Fig. 12.—(*a*) Time course of total sugar secretion by mature nectaries in air ($\bullet$), mature nectaries in nitrogen ($\circ$), young (buds about to open, no nectar) nectaries in air ($\blacktriangle$), young nectaries in nitrogen ($\triangle$). All nectaries were on $0 \cdot 4$M sucrose. Each point is the mean of three nectary cups (Reed, Findlay, and Mercer 1971). (*b*) Time course of volume of nectar produced in air ($\bullet$), in nitrogen ($\circ$), after transfer to air from nitrogen at 6 hr ($\square$). Medium $0 \cdot 4$M sucrose. Each point represents nectar from one nectary segment.

Fig. 13.—Amount of ^{14}C label appearing in nectaries in nitrogen (these produced only a trace of nectar) ($\blacksquare$), nectaries in air ($\bullet$), nectaries in air and the nectar produced by them ($\circ$). All nectaries on $0 \cdot 2$M sucrose solution containing [U-^{14}C]sucrose. The sepal tissue adjoining the nectaries was removed before assay.

Fig. 14.—Effect of temperature on the mean rate of total sugar secretion by nectaries on $0 \cdot 4$M sucrose.

The addition of $0 \cdot 1$ or $0 \cdot 2$M sucrose did not increase the rate of respiration above that without added sucrose. Ziegler (1956), on the other hand, found that addition of glucose and fructose did increase the rate of respiration of *Abutilon* nectaries. Of the inhibitors which were found to depress nectar secretion, potassium cyanide (10^{-4}M) and sodium azide (10^{-3}M) decreased respiration by about 50% whereas 2,4-dinitrophenol (10^{-4}M) increased it about 10% (Fig. 15).

IV. Discussion

(a) Sugar Secretion by the Nectaries on the Plant

The onset of nectar secretion on the plant coincides with a build-up of sugar in the nectary. Since the total amount of sugar secreted by a nectary can be at least seven times that present in the nectary at the onset of nectar secretion, sugar must continue to be supplied to the nectary during nectar secretion.

A comparison of the behaviour of isolated nectaries and nectaries on the plant suggests that nectar production is similar in the two systems. It seems likely that the steady-state condition observed in isolated nectaries floating on 0·4M sucrose approximates to the steady-state situation in nectaries on the plant in which sugar is supplied from the phloem. Further evidence for this will be discussed in Part IV of this series (Reed, Findlay, and Mercer 1971).
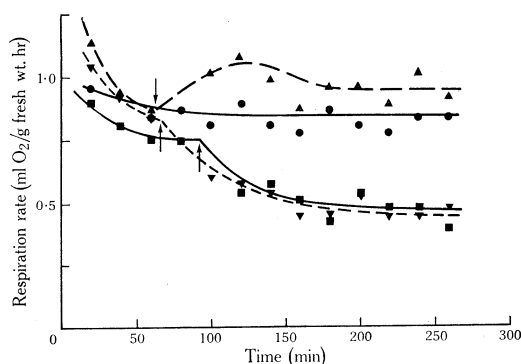

Fig. 15.—Effect on the rate of respiration of nectary segments of 10^{-4}M dinitrophenol (▲), 10^{-3}M NaN_3 (▼), 10^{-4}M KCN (■), added at the arrows. ● Control. All media contained 0·2M sucrose and M/30 phosphate buffer, pH 6·0.

(b) Sugar Secretion by Isolated Nectaries

The two-phase nature of the sugar secretion suggests that somewhere in the nectary, possibly near the base of the hair (Reed, Findlay, and Mercer 1971), there is a sugar transport process, saturated at high internal sugar concentrations. In such a model, the rate of secretion during phase I is independent of the sucrose concentration in the medium because the sugar concentration at the base of the hair is sufficient to saturate the transport process. The absence of a change in secretion rate between phase I and phase II when the concentration of sucrose in the medium .exceeds about 0·4M suggests that this concentration is sufficient to maintain the internal sugar concentration at saturation level. When the secreting nectary is placed on media of sucrose concentration less than 0·4M the internal sugar concentration will eventually fall below the saturating level and the secretion rate then becomes a function of the sucrose concentration in the medium. This functional relationship appears as the distinct phase II of the sugar secretion.

There is the possibility that the two-phase pattern of secretion in isolated nectaries is due to some change in physiological state (e.g. enzyme activity) induced by isolating the nectaries. However, the initial delay preceding the two-phase pattern of secretion in young nectaries and the two-phase pattern induced by transferring nectaries from a medium with a high sucrose concentration to water (Fig. 5) seem to rule out his interpretation.

The decrease in the rate of sugar transport during phase I, caused by the presence in the medium of mannitol or sucrose at concentrations higher than $0 \cdot 4$M, could be the result of either an increase of osmotic pressure at the transport site or a decrease of water flow through the nectary. The first alternative appears unlikely since the rate of sugar secretion in phase II is independent of the mannitol concentration. Furthermore, the rapid effect of the addition of mannitol suggests that the effect is not due to the competition of mannitol with sugars at the transport site.

The pattern of secretion in young nectaries cut before they had accumulated any substantial amount of sugar may be interpreted as follows. The initial delay is due to the necessity for some sugar to be accumulated in the nectary before secretion can begin. Presumably on the plant virtually no sugar is being supplied through the phloem at this stage. That there is a higher rate of secretion on $0 \cdot 4$M than on $0 \cdot 2$M sucrose during phase I and that secretion starts about the same time suggests that sugar uptake is less on $0 \cdot 2$M than on $0 \cdot 4$M sucrose and the nectary sugar concentration needed to start secretion is not an absolute amount but some value above that of the medium.

(c) Active Processes in Sugar Secretion

The following evidence indicates that nectar production is an energy-requiring process:

(1) Nectaries can begin nectar secretion when isolated and can continue to take up and secrete both sugar and water.

(2) Nectar secretion is decreased or ceases under anaerobic conditions or on the application of inhibitors of respiration such as cyanide or azide or an uncoupler of oxidative phosphorylation, dinitrophenol. The metabolically linked process is involved not only in the uptake of sugar into the nectary since secretion by nectaries on water can be inhibited by the above inhibitors.

(3) The high value of $2 \cdot 3$ for the temperature coefficient ($k_{20°C}/k_{10°C}$) is consistent with the process being active but cannot stand as evidence on its own because Steinbrecher and Lüttge (1969) found a similar value for the transport of sugar from onion epidermis, a process which was not inhibited by metabolic inhibitors and hence not active.

It has often been suggested that a carrier system involving phosphorylation and dephosphorylation is responsible for the active transport of sugar in nectaries (Ziegler 1965; Lüttge 1966). An estimate of the total energy (as ATP or $\sim$P) available from the respiration of isolated nectaries may be made and compared with the sugar transported by the nectaries. The maximum high energy phosphate ($\sim$P) available from the mean respiration rate of nectaries of 60 μl O_2 per flower.hour would be $0 \cdot 015$ mmoles $\sim$P per flower.hour as the respiration using 1 mole O_2 produces a maximum of 6 moles $\sim$P. Since, on the plant, up to 5 mg sugar (c. one-third sucrose, two-thirds hexose by weight) is secreted by the nectaries of one flower per hour, barely one $\sim$P is available per sugar molecule secreted. Isolated nectaries usually secrete about 1 mg per flower.hour giving, in this case, about five $\sim$P available per sugar molecule secreted. At least some of the energy available is consumed by the cell for processes other than the transport and secretion of sugar.

In the accumulation of sucrose by sugar-cane tissue nine $\sim$P per sucrose molecule accumulated are available from the sugar-induced respiration alone (Bieleski 1960).

This suggests that in the nectaries a process more efficient in the utilization of energy than a carrier involving phosphorylation and dephosphorylation is responsible for the transport of sugar. Certainly each sugar molecule, on the average, need be transported once only in its passage through the nectary by the suggested phosphorylating carrier, at least on the plant.

Models to explain the overall nectar production process are discussed in Part IV (Reed, Findlay, and Mercer 1971).

V. REFERENCES

AGTHE, C. (1951).—Über die physiologische Herkunft des Pflanzennektars. *Ber. schweiz. bot. Ges.* **61**, 240.

BIELESKI, R. L. (1960).—The physiology of sugar-cane. III. Characteristics of sugar uptake in slices of mature and immature storage tissue. *Aust. J. biol. Sci.* **13**, 203.

FINDLAY, N., and MERCER, F. V. (1971).—Nectar production in *Abutilon*. I. Movement of nectar through the cuticle. *Aust. J. biol. Sci.* **24**, 647.

LÜTTGE, U. (1966).—Funktion und Struktur pflanzlicher Drüsen. *Naturwissenschaften* **53**, 96.

MATILE, P. (1956).—Über den Stoffwechsel und die Auxinabhängigkeit der Nektarsekretion. *Ber. schweiz. bot. Ges.* **66**, 237.

MCILROY, R. J. (1950).—"The Plant Glycosides." (Edward Arnold: London.)

RADTKE, F. (1926).—Anatomisch-physiologische Untersuchungen von Blütennektarien. *Planta* **1**, 379.

REED, M. L., FINDLAY, N., and MERCER, F. V. (1971).—Nectar production in *Abutilon*. IV. Water and solute relations. *Aust. J. biol. Sci.* **24**, 677.

SOMOGYI, M. (1945).—The new reagent for the determination of sugars. *J. biol. Chem.* **160**, 61.

STEINBRECHER, W., and LÜTTGE, U. (1969).—Sugar and ion transport in isolated onion epidermis. *Aust. J. biol. Sci.* **22**, 1137.

ZIEGLER, H. (1956).—Untersuchungen über die Leitung und Sekretion der Assimilate. *Planta* **47**, 447.

ZIEGLER, H. (1965).—Die Physiologie pflanzlicher Drüsen. *Ber. dt. bot. Ges.* **78**, 446.