NECTAR PRODUCTION IN *ABUTILON*

I. MOVEMENT OF NECTAR THROUGH THE CUTICLE

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Abstract

Observations by light microscopy of the process of exudation of nectar from the nectary hairs of *Abutilon* show that the nectar passes through the relatively impermeable cuticle in discrete droplets at regular intervals. From the observations it is concluded that there are pores through the cuticle at the hair tips which act as valves to produce these discrete drops. Numerous small pores at the expected positions are visible in electron micrographs.

I. INTRODUCTION

Nectaries in a variety of plants have been found to be covered with a cuticle through which the nectar must pass. In nectaries without hairs the nectar passes through the cuticle either by rupture of the cuticle, through stomates, or by diffusion through a thin or modified cuticle (Fahn 1952; Helder 1958). Nieuwenhuis van Ůxküll-Güldenband (1914) found that in some nectaries lines passing through the cuticle are visible after staining. However, it was not ascertained that these are pores allowing passage of nectar.

Feldhofen (1933) has studied the multicellular hairs of *Abutilon* nectaries. He observed changes in the nectary hairs which he interpreted as being directly involved with the nectar exudation process, but apparently did not observe the actual passage of nectar through the cuticle.

This paper deals with the final step of nectar production in *Abutilon*: the exudation of nectar through the cuticle covering the nectary, and the structure of this cuticle. The present observations are in conflict with many of the observations and the interpretation of nectar exudation by Feldhofen. Subsequent papers in this series will deal with the fine structure of the nectaries and with other aspects of sugar and water movement.

II. MATERIAL AND METHODS

The nectaries of two horticultural forms of *Abutilon* were used. Voucher specimens of these have been placed in the University of Sydney Herbarium. One form appears close to *A. venosum* Lem. but differs slightly from published descriptions of that species (e.g. Hooker 1849; Chittenden 1951). The other is referred to as *A. hydridum* Sieb. et Voss (a form close to *A. darwinii* J. Hook). Bushes of both forms were grown in the grounds of the Botany Building, Sydney University.

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The nectary is situated on the adaxial proximal surface of the calyx and occupies an area of about 1 cm^2. The nectaries proved suitable for observing exudation under the microscope since sectioning does not damage the cuticle close to the point of exudation.

For light microscope studies radial sections of nectary were cut with a moistened razor-blade. The most usual method for observing exudation was to mount the section on a water-saturated strip of filter paper with the hair tips projecting into a drop of paraffin oil (Fig. 1). Observations were made with a Zeiss Lumipan microscope. An oil-immersion objective and a light blue filter were used for all detailed observations.

Except where otherwise stated, nectaries were taken from freshly picked flowers which had opened but whose anthers were not or were only partly dehisced. These nectaries had formed a small amount of nectar and were rapidly secreting.

For electron microscope studies of the cuticle, nectaries were either fixed in 2% potassium permanganate and embedded in Araldite or were fixed in 2% osmium tetroxide and embedded in methacrylate. Sections were cut with a glass or a diamond knife on a Porter–Blum or LKB microtome and examined with a Siemens Elmiskop I electron microscope.

III. Results

The structure of the nectary of *Abutilon* has been described previously by Feldhofen (1933), Agthe (1951), and Frei (1955) and is shown in Figure 2. The size and shape of the hairs are fairly uniform over the surface of the nectary except at the edges. The cells of the nectary, particularly those of the hairs, are rich in cytoplasm.

(*a*) *Nectar Exudation from the Hair Tip*

The process of nectar exudation from the hair tip has been described briefly elsewhere (Mercer and Rathgeber 1962). If sections from secreting nectaries are mounted as described (Fig. 1), droplets of nectar rapidly appear at the tips of the hairs and increase in size (Figs. 5–7). Only rarely do small drops appear on other parts of the hair.

Detailed observation of nectary hairs mounted with the tips in paraffin oil showed that the exudation of nectar takes place as illustrated in Figure 3. The cuticle surrounding the cell at the tip of the hair becomes separated from the cell and stretched as solution (or "prenectar") collects between the cell wall and the cuticle. When the cuticle is sufficiently stretched a small droplet of prenectar is suddenly released through the cuticle. The cuticle returns to a position closer to, though usually not touching, the cell wall and the process is repeated. The droplets of nectar attached to the tip of the hair can be observed to increase in volume in sudden steps or "pops" at more or less regular intervals. Measurement with a camera lucida or photographically of droplet size between pops show that about 100 μm^3 of prenectar is released at each pop. The nectar usually appears to be released instantaneously but a few hairs have been observed where the release is somewhat slower. The process of exudation is similar in hairs mounted with the tips in air instead of the paraffin oil. If the sections are mounted completely in water, the released nectar is of course not visible but the movement of the cuticle can be readily observed.

During nectar exudation the thin cell wall remains attached to the protoplast and does not expand with the cuticle, nor does it "split" as was claimed by Feldhofen (1933). The location of the cell wall was confirmed by observing exuding tip cells continuously during plasmolysis with hypertonic sucrose solution. The protoplasts shrank away from the wall leaving it clearly visible.

Successive nectar droplets usually appear from the same spot on the cuticle. This behaviour is apparent if the newly formed droplets coalesce with another nearby nectar drop and leave the surface of the hair dry. Occasionally, however, droplets appear at one spot for some time and then suddenly start to appear at another place instead.

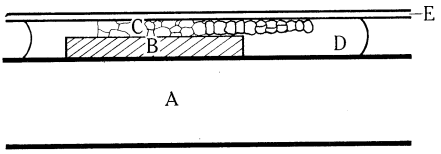


Fig. 1

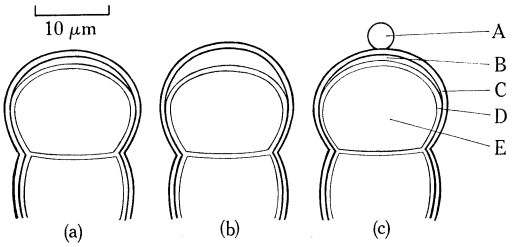


Fig. 3

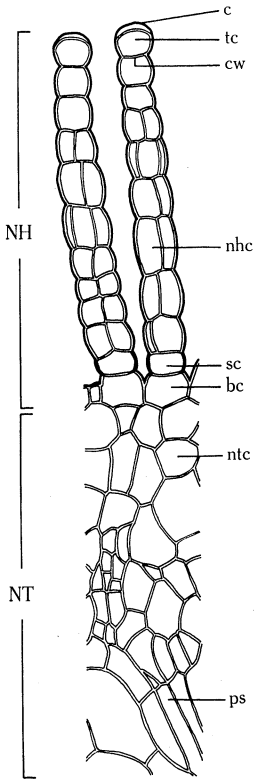


Fig. 2

Fig. 1.—Method of mounting a section of nectary for observing exudation of nectar. *A*, microscope slide; *B*, moist filter paper; *C*, section of nectary with hairs projecting into a drop of paraffin oil (*D*); *E*, coverslip. Not to scale.

Fig. 2.—Diagram of a section of nectary. The nectary tissue (*NT*) consists of nectary tissue cells (*ntc*) and is penetrated by phloem strands. A cell of a phloem strand is shown (*ps*). A nectary hair (*NH*) is made up of a basal cell (*bc*), a stalk cell (*sc*) with a cutinized lateral wall, nectary hair cells (*nhc*), and a tip cell (*tc*). At the widest part of the hair there may be about six cells per tier. Occasionally there may be two or more stalk cells adjacent to one another in the longitudinal direction of the hair. Sometimes there may also be two tip cells instead of one. *c*, cuticle; *cw*, cell wall.

Fig. 3.—The process of nectar exudation. Diagrams (*a*) and (*b*) show how prenectar (*B*) collects between the cuticle (*C*) and cell wall (*D*) until [diagram (*c*)] a droplet of nectar (*A*) is suddenly released. The process is repeated, the droplet of nectar increasing in size in discrete steps. *E*, tip cell protoplast.

The rate of exudation of nectar, even from the hairs of one section, is variable and changes slowly with time. Figure 4(*a*) shows two typical examples of the time course of the rate of exudation from individual hairs. Many examples were observed where a rate of above one pop per 10 sec was maintained for about 2 hr. Rates of about one pop per sec were occasionally observed soon after cutting. In many hairs the rate of exudation declined rapidly from an initial high rate. The rate of exudation appeared to depend to a large extent on the section thickness and the distance of the

base of the hair from the section surface, and was not a guide as to the variability of the rate of exudation in different hairs in the intact nectary.

Several instances have been observed of the cells of exuding hairs becoming plasmolysed some minutes after the sections are mounted in water. The plasmolysis was associated with an increase in the rate of exudation. After some time the cells deplasmolysed with a decrease in the rate of exudation. Occasionally successive protoplasts along the hair separated from one another except for very fine protoplasmic strands. These protoplasts moved when nectar was released through the cuticle. For instance, the boundary of a protoplast which had become detached from the proximal wall rapidly moved a short distance towards the tip of the hair when the nectar was released and then more slowly returned to its original position.

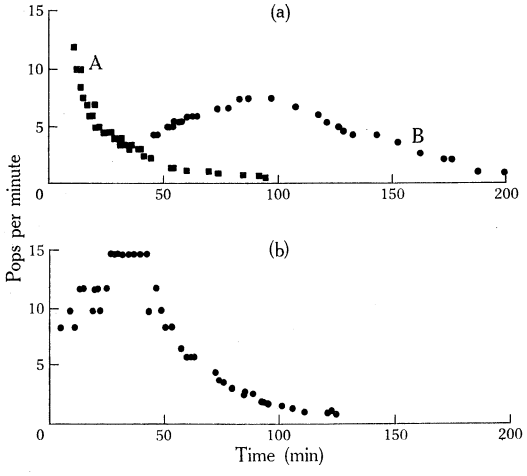


Fig. 4.—(*a*) Time course of popping of the cuticle in two hairs from sections mounted in water (■ and ●) and (*b*) in a hair from a section in 10^{-5}M 2,4-dinitrophenol.

Nectary hairs which have been cut off from the nectary tissue and mounted in water may show movement of the cuticle for some time. Hairs with stalk cells attached have been observed to exude for over 30 min, while hairs without a stalk cell rarely showed any exudation and even then for no longer than 5 min.

Exudation of nectar was observed to occur in the same way in *Hibiscus* and *Tilia* nectaries. In *Abutilon* exudation takes place in the same way, but more slowly, from glandular hairs which are similar to the nectary hairs but occur scattered on other parts of the plant.

(*b*) *Effect of Mounting Medium on Exudation*

When sections were mounted in 10^{-5}M 2,4-dinitrophenol (in phosphate buffer, pH 6·0) or in 5×10^{-3}M sodium azide, hairs could be found which exuded as well as and as long as hairs from similar sections mounted in water [Fig. 4(*b*)].

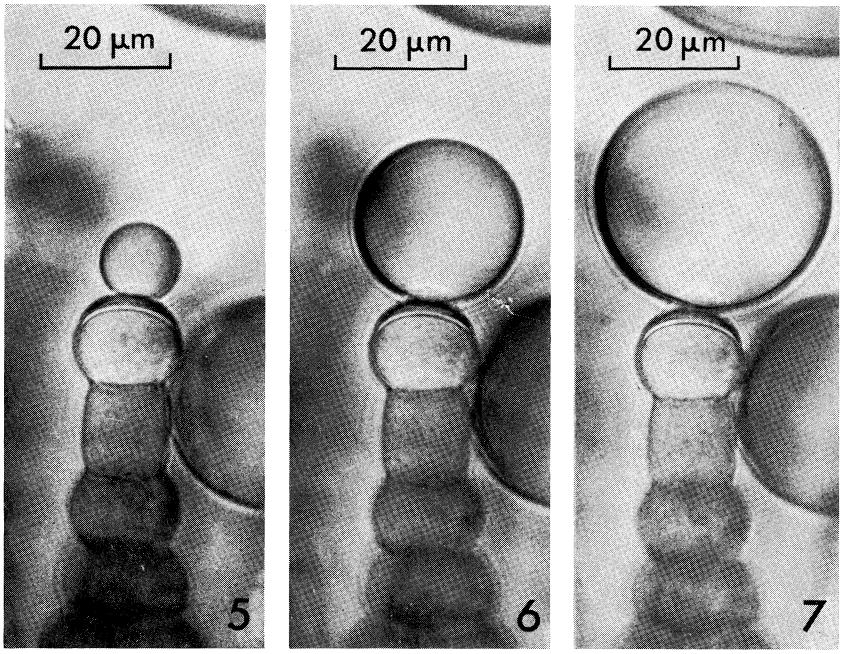
Hairs from sections mounted in sucrose solutions up to 0·4M could exude as well as those mounted in water.

(*c*) *Exudation Rates in Sections and in Excized and Intact Nectaries*

In sections mounted in water a rate of exudation of about 1000 μm^3 per hair . minute is quite common, with popping every 5–10 sec.

Isolated nectaries floated on water exude at a rate of about 5–30 μl per flower . hour (corresponding to a secretion of sugar of 0·5–4·0 mg per flower . hour for some hours after they are isolated. Since the nectaries of one flower contain about 2×10^5 hairs, this gives a rate of exudation of $0{\cdot}4 \times 10^3 - 2{\cdot}5 \times 10^3$ μm^3 per hair . minute which is comparable to that in sections.

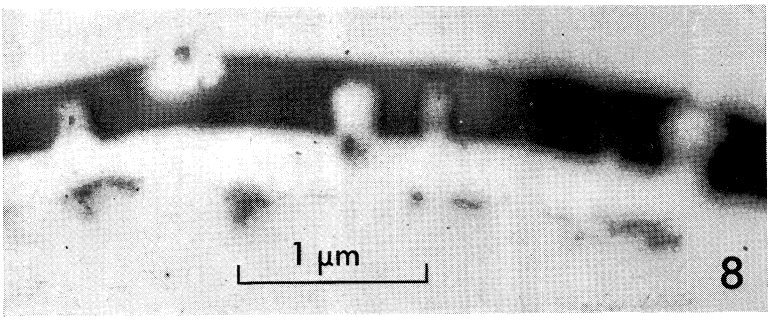
On the plant, exudation of sugar occurs at a rate of up to about 5 mg per flower . hour. The volume of nectar exuded is uncertain as changes in water content may occur after exudation. Various data (Reed, Findlay, and Mercer 1971) suggest a concentration of about 0·6M with approximately one-third by weight each of sucrose, glucose, and fructose. This would give a rate of exudation on the plant of 35 μl per flower . hour or 3×10^3 μm^3 per hair . minute which is comparable with that in sections or isolated nectaries.

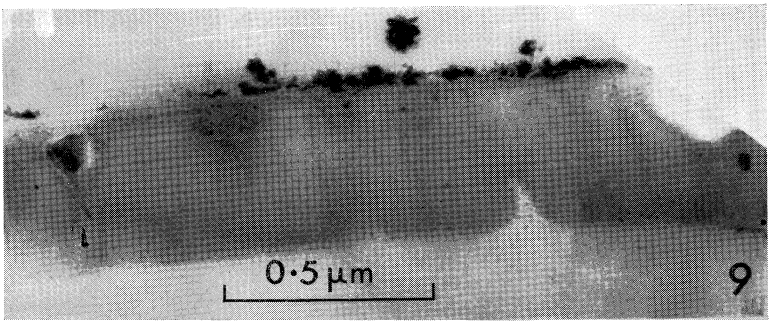


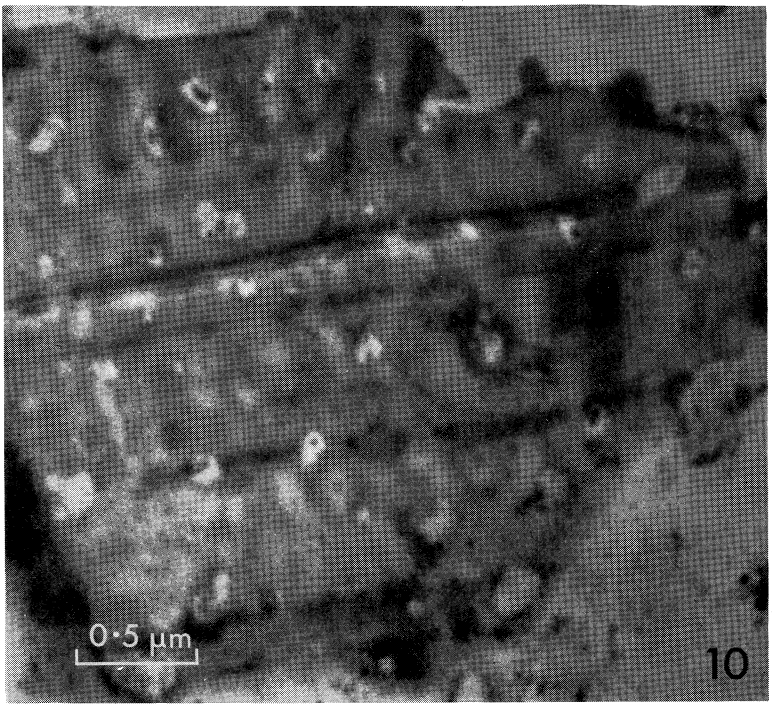
Figs. 5–7.—Nectar exudation from a tip cell: *5*, initial photograph; *6*, 5 min later; *7*, 15 min later. The droplet has grown by 6×10^3 μm^3 between Figures 5 and 6 and 14×10^3 μm^3 between Figures 6 and 7. The cuticle was popping every 4 sec.

(*d*) *Submicroscopic Structure of the Cuticle*

In longitudinal sections of the hair tips (Figs. 8 and 9) pores of diameters up to 0·4 μm were found in the cuticle. These pores occurred in large numbers at the hair tips but were not found elsewhere. Many of the pores do not appear to be continuous through the cuticle, probably as a result of the section being cut at an angle to the pores although it may be that some pores do not completely penetrate the cuticle. There is no indication of a separate cuticle and cuticular layer making up what has here been termed cuticle. In many instances the pores are partially







Figs. 8–9.—Electron micrographs of longitudinal sections through the cuticle showing pores at the tips of exuding hairs. *8*, osmium tetroxide fixation; *9*, permanganate fixation.

Fig. 10.—Electron micrograph of tangential section through cuticle from the tip of an exuding hair. Osmium tetroxide fixation.

filled with an electron-dense material indicating that they are not merely cutting artefacts. A tangential section through the cuticle at a hair tip (Fig. 10) showed about four pores per square micron.

(*e*) *Penetration of Dyes*

When sections of nectary were mounted in various aqueous dye solutions, such as 0·05% neutral red, 0·1–0·5% methylene blue, or 2% potassium permanganate, entry of the dye into the nectary hairs was much slower than into the nectary tissue. The dyes penetrated the nectary hairs through the stalk cells and, occasionally, to a lesser extent through the tip cells. For instance, in sections mounted in neutral red for 3 hr the nectary tissue was densely stained but the nectary hairs were stained in only a few proximal cells. In methylene blue the lateral walls of the stalk cells were faintly stained compared with those of the adjoining hair cells, presumably because of their modfied structure (Findlay and Mercer 1971). Nectary hairs cut from the nectary stained rapidly and intensely in the above solutions.

Dyes placed on the surface of a nectary floating on water entered the nectary hairs very slowly except when the hairs were damaged or their tips had been cut off. When a drop of 0·05% neutral red was placed on a rapidly secreting nectary only the tip cells and sometimes the adjoining cells showed any stain after 4 hr. The tip cells then had faint pink vacuoles containing a few red granules undergoing brownian motion. If the tips of the hairs were cut before the application of the dye, the cut hairs stained rapidly and intensely. There was practically no entry of dye into hairs of nectaries which had nearly stopped secreting. When methylene blue was applied to secreting nectaries there was virtually no staining except in a few hairs, presumably damaged ones, which stained intensely.

(*f*) *Plasmolysis of Hair Cells*

The nectary hair cells can be plasmolysed by salt solutions. As found by Agthe (1951) they can also be plasmolysed by sucrose solution. The concentration required to plasmolyse more than half of the tip cells varied considerably from flower to flower. Sometimes 0·5M sucrose was sufficient but at other times even 1·0M sucrose resulted in very little plasmolysis.

There are two forms of plasmolysis. In one form the protoplasts contracted away from the cell wall, in the other the cell wall and cuticle collapsed together with the protoplast.

The stalk cells, as observed in other species (Schrödter 1925), usually remain attached to the lateral walls on plasmolysis.

Plasmolysis occurs first in either the cells at the base of the hair or the tip cell. Some of the intermediate cells may take more than half an hour to plasmolyse. Several cells were observed to deplasmolyse 0·5–2 hr after plasmolysis in sucrose solution.

IV. Discussion

The rate of exudation of nectar as observed under the microscope is about the same as that calculated for intact nectaries. While some aspects of nectar production may be modified by the artificial conditions it seems unlikely that the process by which nectar passes through the cuticle will be substantially altered. Under optimal conditions droplets of nectar are exuded at 2- or 3-sec intervals and a volume of nectar equivalent to the volume of the tip cell may be produced in about 10 min.

The accumulation of secretion between the cell wall and the cuticle occurs at the tips of a wide variety of glandular hairs (e.g. Schnepf 1969). In many of these glands the secretion either remains in the subcuticular space, except for any volatile components, or the cuticle is torn (e.g. Kisser 1958). Pores have been found in the cuticle of the glandular hairs of *Drosophyllum* (Schnepf 1963), the oil glands of *Mentha piperita* (Amelunxen 1964), the mucilage secreting hairs of *Rumex* and *Rheum* (Schnepf 1968), the trichome hydathodes of *Cicer arietinum* (Schnepf 1965), and they also occur in salt glands (e.g. Ziegler and Lüttge 1966). However, they have apparently not been recorded in nectaries.

The pores in *Cicer arietinum* are similar to those in *Abutilon* in that they allow the secretion to escape in discrete drops. However, their structure is somewhat more complicated as the pores in the cuticular layer and the cuticle are not aligned and the secretion, after passing through the pore in the cuticular layer, usually has to move some distance between the two layers before it can escape through the cuticle. The pores appear to be closed in electron micrographs. In nectar exudation in *Abutilon* there appears to be no delay during the passage of nectar through the cuticle, nor is there any indication of a non-alignment of pores in two layers in the cuticle.

The behaviour of the nectary hairs during exudation, on plasmolysis, and with dye solutions shows that most of the cuticle covering the nectary hairs is relatively impermeable to solutes and water. At the hair tips, however, there are a large number of pores in the cuticle which can allow solute and water movement. The periodic movement of nectar through the cuticle is caused by a building up of hydrostatic pressure within the hairs until prenectar flows through the pores. This flow results in a sudden decrease of the hydrostatic pressure and flow of prenectar ceases. The movement of plasmolysed hair cells during the "popping" cycle provides some direct evidence that periodic hydrostatic pressure changes do occur in the hairs during nectar exudation.

The intermittent flow could be due to a structural opening and closing of the pores, but this appears unlikely from the size of the pores as seen in electron micrographs and the relatively small expansion and contraction of the cuticle, or to a threshold pressure or size below which no flow through a structurally open pore occurs. In either case the popping cycle requires that there is a hysteresis whereby the hydrostatic pressure at which nectar flow commences is appreciably higher than the pressure at which it stops. The physical basis for such a hysteresis is not clear. The fact that exudation occurs at a small region in the cuticle and probably at a single pore can then be explained if the further assumption is made that the pores have different threshold pressures for the start of the flow. Nectar will flow through the pore with the lowest threshold pressure. If this pore becomes blocked, the resulting rise in pressure would cause the pore with the next lowest threshold to take over the nectar exudation. Such sudden changes in the position of nectar exudation have been observed.

It follows from the proposed model that movement of nectar through the cuticle is predominently a one-way process and that nectar, once exuded, is essentially separated from the nectary cells. The difference in the rate of penetration of dye into intact and cut hairs supports this conclusion. However, a small amount of penetration can occur as is shown by the slight entry of dyes into the hair tips, particularly in

exuding nectaries. This entry appears to be mainly through the pores and not through the cuticle. Lüttge (1962) has demonstrated the entry of various labelled substances inwards through the surface of the nectaries of *Abutilon*.

The nectary hair can be visualized as a sac which is impermeable, except for the cuticular pores (that allow exit of solution as a result of internal pressure) and the stalk cell. Since the stalk cell has modified lateral walls (Findlay and Mercer 1971) to which the protoplast remains attached even on plasmolysis, movement of solution between the nectary tissue and the hair is restricted to a pathway through the protoplast of this cell. Because of this structural arrangement exudation can continue in hairs with plasmolysed hair cells provided the hydrostatic pressure in the hair is maintained by a volume flow in through the stalk cell.

Models to account for nectar production and the build up of hydrostatic pressure in the nectary hairs will be considered in Part IV of this series (Reed, Findlay, and Mercer 1971).

Various interpretations can be placed on the fact that respiratory inhibitors have no marked effect on exudation in sections within 2 hr although they do in isolated nectaries after longer periods (Findlay, Reed, and Mercer 1971). The inhibitors may penetrate too slowly to have any marked effect. On the other hand, the sugar content of the nectary hairs and conversion of sucrose to hexoses may be sufficiently high to maintain a water potential gradient without further active transport of sugar and hence maintain the water flow into the hair. This water flow will allow leakage of sugar solution from the nectary hair cells if they are relatively permeable to sugar compared with the stalk cell.

The collection of prenectar in the tip of the nectary hairs has been observed by Feldhofen (1933), who believed that the prenectar collected between two layers of the cellulose wall of the tip cell. Our light microscope observations show that the collection of prenectar is actually between the cell wall and the cuticle. Further support for our conclusion comes from electron micrographs that show no separation of the cell wall into two layers (Findlay and Mercer 1971).

Feldhofen also described a progressive shrinking of the tip cell protoplast, accompanied by a depression inwards of the inner cell wall and finally also of the cuticle, as the nectary aged. He interpreted the shrinking of the protoplast as due to the expression of nectar from the cell, this formation of nectar either limiting the expansion of the cell or using up the substances of the cell. Towards the end of activity of the protoplast the concentrated secretion takes up water osmotically and can then permeate through the cuticle. Feldhofen appears not to have observed the nectar passing through the cuticle. The shrinking of the protoplast and collapse of the cell wall were not observed by the present authors in exuding cells. An exception was the very occasional transient spontaneous plasmolysis of the cells of exuding hairs. This plasmolysis is certainly not an essential part of the exudation process. Changes similar to those observed by Feldhofen were seen in damaged or plasmolysed cells or cells fixed and embedded for microscopy. Moreover, the mechanism suggested by Feldhofen would not be adequate either to explain the large amounts of nectar formed by these nectaries or the fact that nectar exudation on the plant begins at the time when the collection of prenectar between the cuticle and the cell wall of the tip cells is first observed.

V. Acknowledgments

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