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Development and transfer character of secretory glands in the broad bean (*Vicia faba* L.)

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Abstract

The development and transfer character of the secretory glands developing on the stipules (from node I to X) in *Vicia faba* L. was investigated. It was found that the state of developmental and functional maturity is reached by them in nodes IV-VI from the shoot apex. In mature glands the characteristic ingrowths from the cell wall to the interior of the protoplast develop to different extents (number, size and shape) in the particular gland cells. Their most intensive development up to the formation of labyrinths was observed in the outher walls of the head, and particularly in the cells of its upper tier. In all the other cells of the gland (up to the subepidermal one) less developed ingrowths occur on the transverse walls and even less along the long axis of the gland. This arrangement of the ingrowths shows the direction of flow of secretive substances.

INTRODUCTION

Transfer cells do not form a specific homogeneous type of tissue, various types of cells may belong here. They are localised at the sites of intensive transport of substances, at the sites of absorption and secretion to or from the internal or external habitant. It is considered that they play an essential role in effective and rapid transport of substances for short distances. So large surface of their plasmalemma is connected with their absorbing and secreting function (Gunning and Pate 1969). For instance in *Pisum arvense* the plasmalemma surface in the transfer cells is about 10 times larger than in other types of cells (Gunning et al. 1968).

A characteristic feature of transfer cells is the occurrence of simple or branched ingrowths. Their formation and development occur before

the start of intensive transport of substances, but as compared with the development of the cell as a whole the ingrowths of the cell wall develop rather late. They result from the specialisation of the cell and nonuniform deposition of a secondary cell wall at the inner surface of the primary wall (Pate and Gunning 1972).

In higher plants transfer cells are common component of conducting tissues: the phloem and xylem (Pate et al. 1970, Yeung and Peterson 1972, Letvenuk and Peterson 1976). The glandular secretive structures are also transfer cells (Thompson and Liu 1967, Schnepf 1964, 1969, Schnepf and Pross 1976).

Wrischer (1962) described the transfer type of some nectary cells on the stipules of *Vicia faba*. These nectaries are formed of numerous secretory glands each consists of a basal cell (at the epidermis level), the cell of the stalk and eight cells of the head arranged in two tiers (four cells in each tier). This author was mainly interested in the submicroscopic structure of the cells forming the gland, he did not study their development: either of the single gland or of the relation between the degree of gland development and its distance from the shoot apex. He claimed that ingrowths to the inside of the protoplast characteristic for transfer cells would occur only in the outer cell walls of the upper cells of the head. The walls separating the head cells of the same tier or of two different tiers are deprived of ingrowths. In some cases Wrischer (l.c.) noted ingrowths also in the basal and subepidermal cells.

The aim of the present study was to follow on the same material the development of glands and to find the site and time of ingrowth formation in all the cells composing the gland.

MATERIAL AND METHODS

The object of the studies were nectaries of stipule of *Vicia faba* L. The culture was run at constant temperature 25° ±1°C and light intensity of 30 000 lux for 16 h daily until the plants reached a length of about 40 cm and had 10-11 nodes.

Fragments of the stipules were fixed with the whole nectary from all the successive nodes:

— for light microscopy (LM) in CrAF (0.5:1:20), and in absolute ethyl alcohol and glacial acetic acid (AA — 3:1)

— for electron microscopy (EM) in 2.5 per cent glutaraldehyde with postfixation in 2 per cent OsO_4 (Karnovsky 1965) or only in 2 per cent $KMnO_4$.

Parafin microtome sections (20 μm thick) were stained with 0.1 per cent alcian blue at pH 2 (phosphate-citrate buffer), or iron haemato-

xylin after Heidenhain or Sudan III and IV. The material fixed in AA was investigated as squash in acetocarmine. This metod was particularly convenient for observation of gland development. Semithin and ultrathin sections were examined in Tesla-BS 500 electron microscope.

RESULTS

LOCALISATION AND STRUCTURE OF SECRETORY GLANDS

The nectaries outside the flowers of the broad bean are surface formations occurring in each node on the side of the stipules facing the axis. The nectary is a grouping of secretory glands (Figs. 1 and 2) which shape and colour vary due to on the distance of the stipules from the shoot apex, depending on the stage of development of the glands and their activity. On the surface of very young stipules (I-III node from growth apex), in their central part there is a small lighter dot. On the surface of older stipules (IV-VI node) nectaries outside the flower appear in the form of brown spots covered with a drop of the secretum. In nodes still more distant from the growth apex (VII-X node) dry nectaries appear as an alongated dark line.

The outer walls of each gland and the cells of the epidermis are covered with a continuous layer of cuticule but much thicker on the head. The cell walls of the stalk are incrusted with cutine.

FORMATION AND DEVELOPMENT OF SECRETORY GLANDS

The secretory glands of *Vicia faba* are of epidermal origin. Each of them arises as the result of divisions and growth of the epidermal cell. The glands are very numerous within the nectary and they lie close to one another, but not every epidermal cell produces a gland (Figs. 2 and 3).

The first sign of gland formation is anticlinal division of the epidermal cell (Fig. 19a). One or both daugther cells higher than the other epidermal cells (Fig. 19b) divide then periclinally (Fig. 19c). From the two overlying sister cells the lower one reaches a height similar to that of the neighbouring epidermal cells and becomes the basal cell (B). The upper cell after the next periclinal division produces the stalk cell (T) and the mother cell of the head (MG) (Fig. 19d) from which as the result of further divisions the 8-cell head of the gland arises (Fig. 19e-g). The observed successive divisions of the mother cell of the head are illustrated by Figs. 5-10. In addition moreover, Fig. 4 presents different but similar stages of gland development within one nectary from node III. The first division in mother cell of the head

Fig. 1. Tangential section through mature nectary from node IV, heads of secretory glands are visible (in cross section). Iron haematoxylin (Heidenhain), 140 ×

Fig. 2. Secretory glands constituting nectary, cross section through stipule of node IV. Alcian blue, 200 ×

Fig. 3. Mature secretory gland: B — basal cell, T — stalk cell, G — head. Alcian blue, 1200 ×

Fig. 4. Various stages of secretory gland development in nectary on stipule of node III. Squash, acetocarmin, 250 ×

always occurs in the plane transverse to the long axis of the developing gland (Fig. 19e), whereas the next divisions may occur in various order. The first to divide is the upper (Fig. 5) or the lower cell of the head (Fig. 6) or the divisions in both these cells are synchronised (Fig. 7). This leads to the formation of four cells of the head, two at each tier (Figs. 8 and 19f). Further divisions of the four-cell head may occur in various order, but usually the first to divide are the cells of the upper tier (Fig. 9).

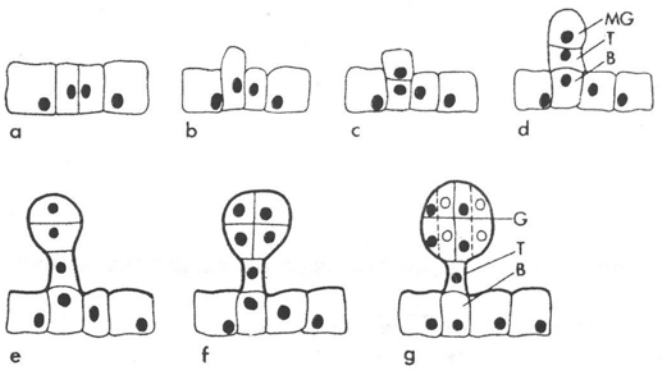
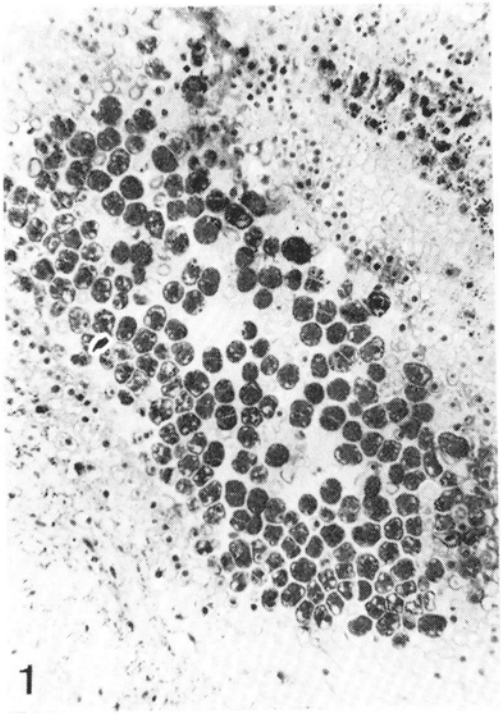


Fig. 19. Development of gland from the epidermal cell
B — basal cell, G — head cells, MG — mother cell of the head, T — stalk cell

As the result of the above described divisions, no matter to their order, a mature secretory gland forms (Figs. 3, 9 and 19g). It consists of one basal cell one elongated cell of the stalk and eight cells of the head arranged in two tiers, four in each.

The development and secretory activity of the glands can be investigated in the successive nodes of the plant beginning from the apex. On the youngest stipules, in nodes I-III, the first steps of gland development can be observed from the elongated epidermal cell (after its anticlinal division) to the four or less frequently, six-cell stage (basal cell, stalk and two or four cells of the head). In nodes IV to VI further development of the head takes place: the number of its cells increases to 8, that is the gland reaches morphological maturity and secretory

Plate I



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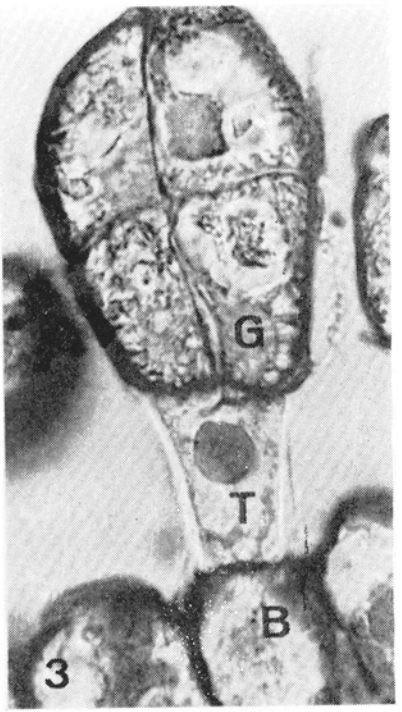

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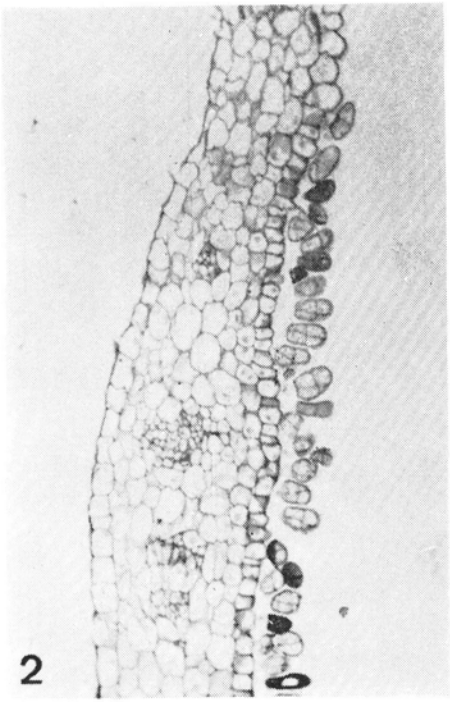

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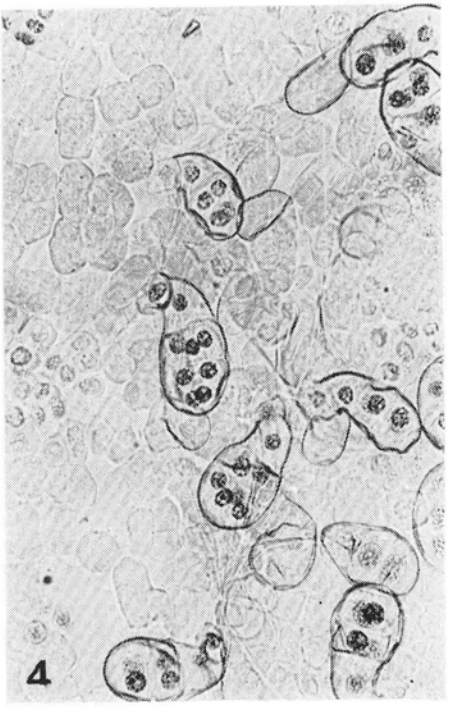

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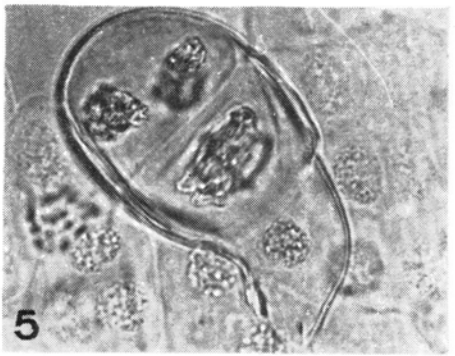
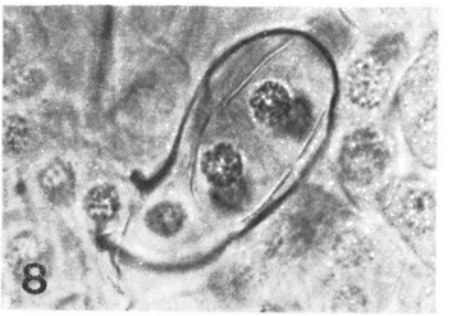
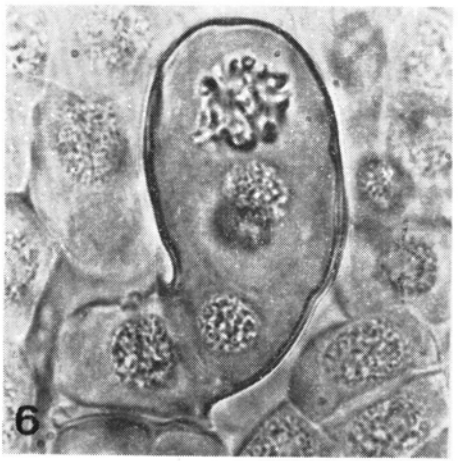
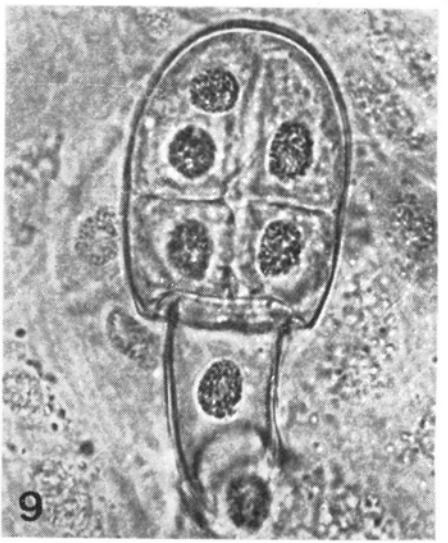
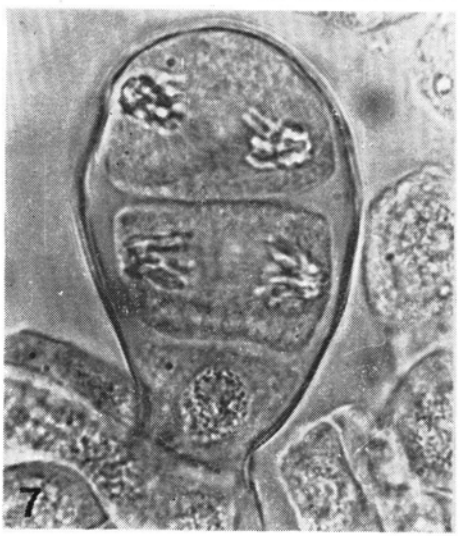
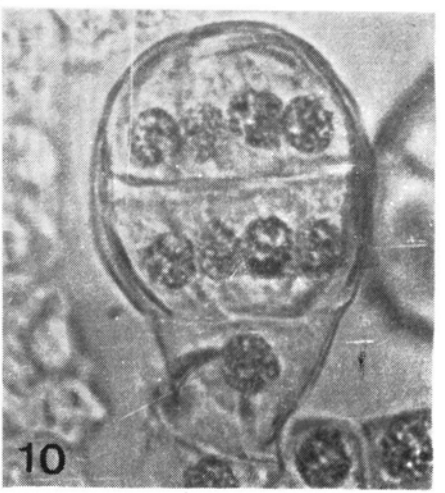












Development of head of secretory gland. Squashes, acetocarmin
Fig. 5. The upper cell of the head divides first, 580 ×;
Fig. 6. The lower cell of the head divides first, 640 ×;
Fig. 7. Simultaneous divisions in both cells of the head, 640 ×;
Fig. 8. 4-cell head, two in each tier, 400 ×;
Fig. 9. Upper tier of 3-cell, lower tier 2-cell, 640 ×;
Fig. 10. Mature gland with completely formed head, four cells both in upper and lower tier, 640 ×

activity what is indicated by the drop of secretum covering the nectary. In nodes VII-X, the oldest ones examined, the mature glands are no more active and have the appearance of a brown dry line not covered with secretum.

INGROWTHS OF CELL WALLS IN SECRETORY GLANDS

Presence of ingrowths into the protoplast of secretory glands are easy observed in the light microscope (LM) after staining with iron haematoxylin or with alcian blue (Fig. 3) but more detailed studies of their spread, shape and lengh are possible only in electron microscope (EM).

Studies in the EM demonstrated that the ingrowths in the cell walls appear in glands with 2- or 4-cell head (Figs. 11 and 18, arrows). During further development of the gland associated with deposition of thicker and thicker layers of a secondary wall, the number and variability of the shapes of ingrowths increase.

Completely developed ingrowths were observed in mature glands but the degree of their development in the particular glandular cells varied even in the walls of the same cell. Most numerous and longest ingrowths are formed on all the outer walls of the head cells, they are most developed in the cells of its upper tier where they often form "labyrinths" penetrating into the cytoplasm (Figs. 12-14). The inner cell walls of the head cells have less numerous and less branched ingrowths. It is noteworthy that the degree of development of ingrowths of the same anticlinal wall of the upper tier cells depends on its distance from the outer wall. The ingrowths closest to the outer wall are more developed (above all more numerous) than those lying deeper from this wall (Fig. 12, arrows). Poorest developed are the ingrowths of the walls separating the two tiers — they are short unbranched bulges (Fig. 13, double arrows, slanting section). Short ingrowths develop also on the walls of the stalk cell bordering the head and the basal cell (Figs. 15 and 16, arrows). No ingrowths were found, however, on the outer walls of the stalk. Similarly distributed were the ingrowths in the basal cell, they occur on the walls between the stalk and the subepidermal cell but not on the walls neighbouring the epidermal cells. In the subepidermal cells the ingrowths are formed on the walls bordering the basal cell of the gland (Fig. 17, slanting section) and also on the opposite wall bordering the deeper cortex parenchyma layers.

The wall ingrowths of the stalk cell, basal cell and subepidermal cells are straight not large, and they do not form labyrinths.

The above described distribution of ingrowths in the walls of the whole gland and also in the subepidermal cells seems to be important for the secretory function of the gland.

The distribution of ingrowths on the walls of the glandular cells, taking into account the degree of their development, is presented in Fig. 20.

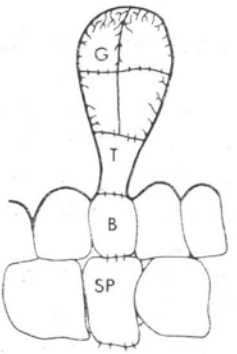
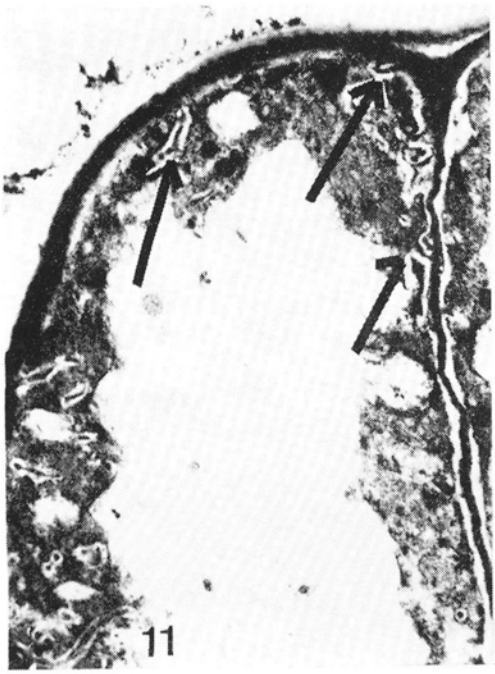


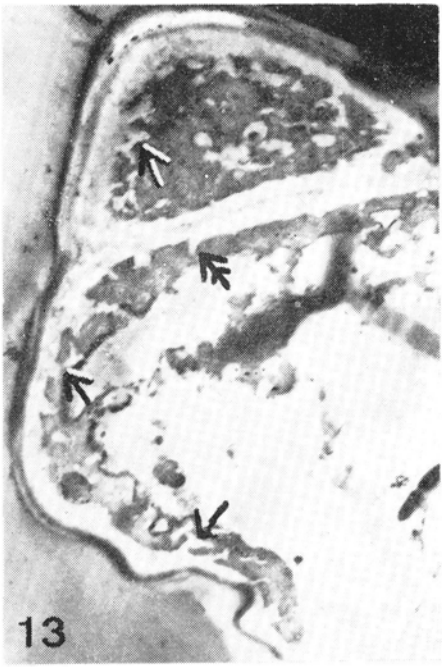
Fig. 20. The distribution of ingrowths on the walls of the glandular cells B — basal cell, G — head cells, SP — subepidermal cell, T — stalk cell

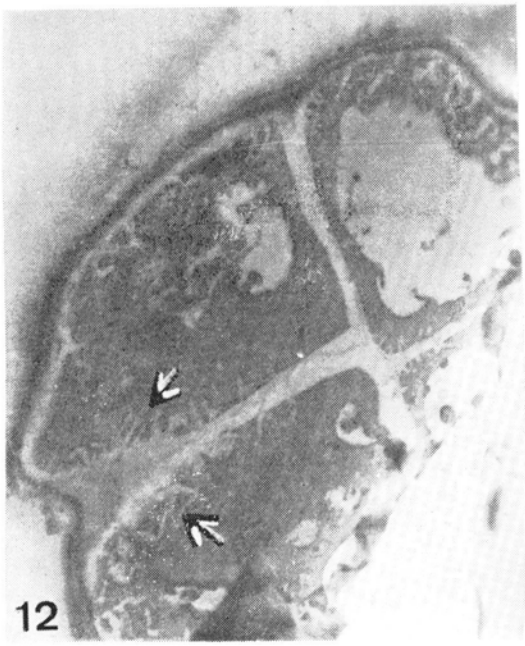
DISCUSSION AND CONCLUSIONS

The steps of gland differentiation in the successive nodes of the plant, can be observed, beginning with the youngest leaves most conveniently in squashes. A relation was found between the degree of development of the nectary as a whole and the distance of the stipule from the growth apex of the shoot on which it is developed. In the same node all glands constituting the nectary are usually in about the same stage of development. Nectaries lying closest to the apex (nodes I-III) are a set of still very young uncompletely formed glands. In nodes IV-VI they are mature and functionally active (covered with a drop of secretum). As the distance of the stipules from the growth apex increases the secretory function appears inhibited, although the glands do not degenerate.

The secretory gland cells of many plants are typical transfer cells: here belong among others the epidermal cells in the nectaries of *Gasteria* and *Aloë* (Schnepf and Pross 1976), some cells of the salt glands of *Tamarix aphylla* (Thompson and Liu 1967) and the already mentioned secretory glands of *Vicia faba* (Wrischer 1962). The latter author found that in *Vicia faba* glands the wall ingrowths occur only on the outer walls of the upper tier cells of the head and only in single cases in the stalk wall and subepidermal cells neighbouring the gland (the author does not mention on which walls of these cells). It is but probable that all the remaining cells of the head and the stalk walls would be completely deprived of ingrowths. If it were so, the presence







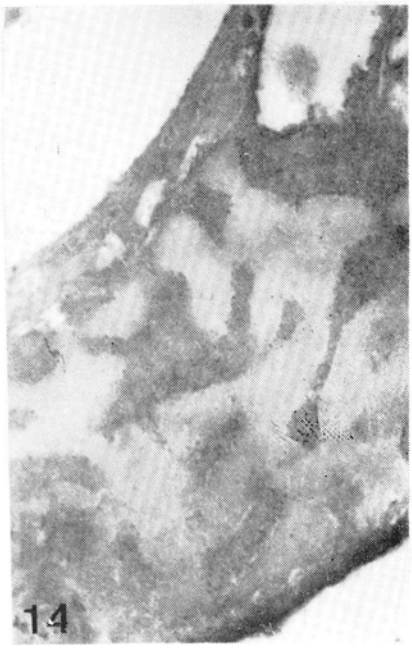
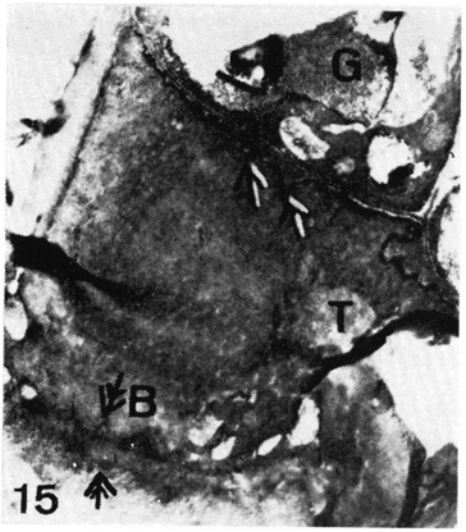
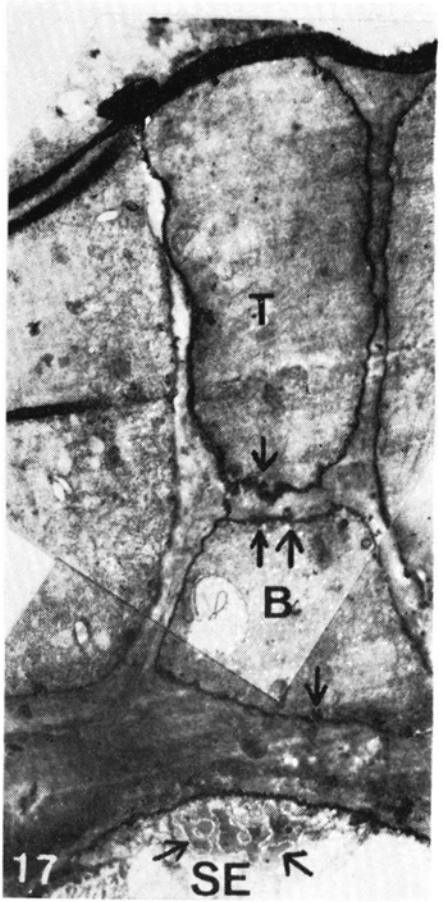
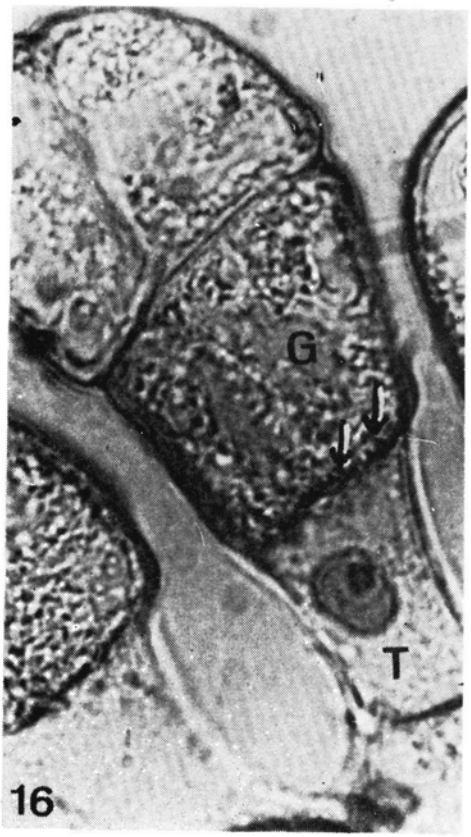


Fig. 11. Part of the outer wall of 4-cell gland head, with small ingrowths (arrows)
Fig. 12. 8-cell head, arrows indicate highly developed ingrowths in anticlinal wall close to outer walls of head
Fig. 13. Cells of the two tiers of mature head (slanting section). Large and branched ingrowths in outer walls (arrows with one head) and short not numerous ingrowths in wall separating the two tiers of the head (arrow with double head)
Fig. 14. Part of the outer wall of cell of upper tier of 8-cell head, wall ingrowths form "labyrinths"
Figs. 11-13 — 10 000 ×, Fig. 14 — 22 000 ×







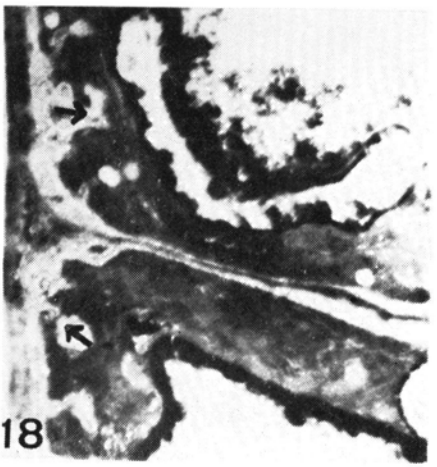


Fig. 15. Small unbranched ingrowths in stalk cell bordering head (arrows with single head) and basal cell (arrows with double head), 6400 ×

Fig. 16. Ingrowths of head cell bordering stalk wall (arrows), 1800 ×

Fig. 17. Ingrowths in subepidermal cells lying immediately under basal cell (arrows), 4000 ×

Fig. 18. Fragment of anticlinal wall of upper tier in 4-cell head, ingrowths (arrows), 10 000 ×

G — head cells, T — stalk cell, B — basal cell, SE — subepidermal cell

of ingrowths in the basal and subepidermal cell would not be understandable. It is possible that Wrischer (1962) being interested in fine structure of protoplast did not take care of proper sections of cell wall. Really it is actually very difficult to obtain good convincing cross sections (even semithin) through the glandular cells, besides, for instance the middle section through the stalk cell will not be the middle section through the head cells.

Shown in our work the fact that ingrowths in the glands of the broad bean are most developed on the outer walls of head cells, and that they are present on the transverse walls of all remain gland cells up to the subepidermal cell indicates the pathway of secretory substance along the gland long axis. This suggests that this flow is more intensive to the outside of gland head than among basal cell and surrounding epidermal cells.

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Rozwój i transferowy charakter gruczołów wydzielniczych bobu (Vicia faba L.)

Streszczenie

Badano rozwój i transferowy charakter gruczołów wydzielniczych rozwijających się na przylistkach (od węzła I do X) u *Vicia faba* L. Stwierdzono, że stan dojrzałości rozwojowej i funkcjonalnej osiągają one w węzłach IV—VI od wierzchołka pędu. W dojrzałych gruczołach charakterystyczne wrostki ścian komórkowych do wnętrza protoplastu rozwijają się w różnym stopniu (ilość, wielkość i kształty) w poszczególnych komórkach gruczołu. Najsilniejszy ich rozwój, aż do tworzenia labiryntów, stwierdzono w ścianach zewnętrznych główki, a szczególnie w komórkach jej górnego piętra. We wszystkich innych komórkach gruczołu (aż do komórki subepidermalnej), słabiej rozwinięte wrostki występują na ścianach poprzecznych, wzdłuż długiej osi gruczołu. Taki układ wrostków wskazuje na drogę przepływu substancji wydzielniczych.