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SUBCORTICAL FORMATION AND ABNORMAL DEVELOPMENT OF STOMATA IN ETIOLATED SHOOTS OF OPUNTIA BLAKEANA

J. G. BROWN

(WITH PLATES XXVII–XXX AND ONE FIGURE)

Introduction

Although experiments in the etiolation of plants date back to the activities of CHARLES BONNET, their contributions to morphological botany have largely been incidental to the prosecution of physiological studies, and therefore extensive only as regards the number of species subjected to investigation. Even up to the present century, literature contains little more than observations on gross structural changes induced or accompanying etiolation, such as the elongation of internodes and peduncles, the dwarfing of leaves, and the underdevelopment of aerating and conducting tissues. To this literature it is believed that the material discussed in this paper adds several new and important facts. Since other investigators (4) have given reviews of the literature on etiolation, the writer will pass directly to his own studies.

Material

Opuntia Blakeana is a common, low-spreading, prickly-pear cactus on the mesas of southern Arizona, having joints 10 cm. in length, 9 cm. in breadth, and 1–2 cm. in thickness (fig. 1). The joints exhibit purplish areoles. Each areole near the margin and in the middle region of a joint bears one or two brown spines 1–3 cm. long. On the basal portion of a joint and on the lowest joints of the plant, areoles are often without spines. In addition to the spines, areoles frequently bear glochids about half the length of the spines, which are especially numerous near the apex of the joints. Both spines and glochids are of the usual barbed type found in cacti. Many bristles about 4 mm. in length, consisting of a single row of cells, are inserted near the bases of the

spines. Small awl-shaped leaves about 15 cm. in length are also borne just below the spines in the spring, but are soon abscissed. The plant grows in the most exposed situations. It was first described as a new species by ROSE (5) in 1909, and the plant from which the type specimen was obtained furnished most of the

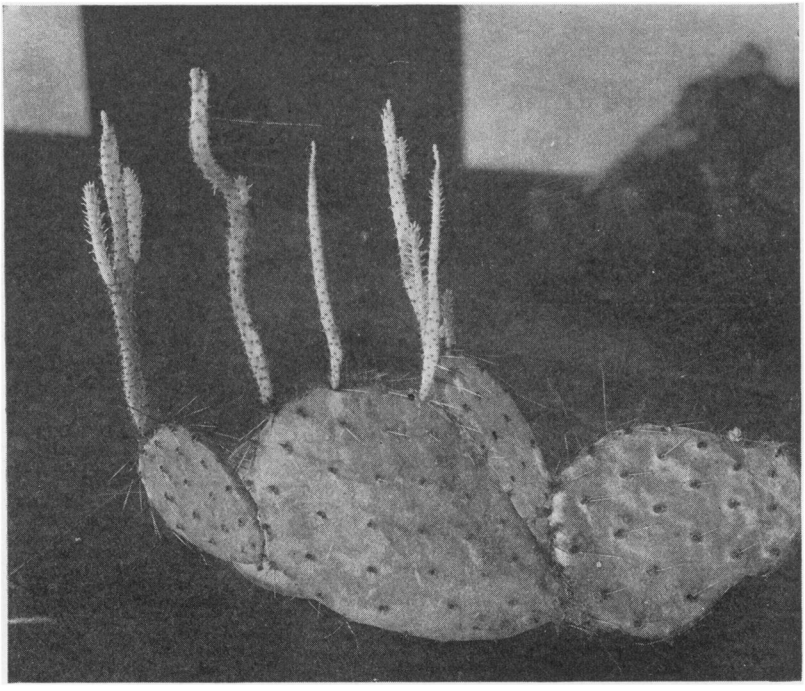

FIG. 1.—Etiolated shoots of *Opuntia Blakeana* sprouting from normal shoots which had been removed from open environment to dark chamber.—Photograph by D. T. MACDOUGAL.

normal shoots, and indirectly all of the etiolated shoots that were used in this study. The latter were grown from joints that had been removed to the dark constant temperature chamber of the Desert Laboratory.

Method

The chamber in which the etiolated shoots were grown has been fully described in publications of the Desert Laboratory,

but for convenience its main features will be given here. It is a small room situated under the floor of the main laboratory, from which light is absolutely excluded by means of an antechamber and two doors, the second door being a trapdoor in the ceiling of the dark chamber. The temperature remained between 56 and 75° F., with a maximum weekly fluctuation of 2° F. The humidity was 80–90 per cent. The room was absolutely dark at all times excepting for the candlelight used while collecting material.

Some of the etiolated shoots were killed in the dark chamber, others were removed to the light and allowed to grow for varying lengths of time before they were killed. The etiolated shoots removed to the light are referred to as etiolated-greened shoots. The killing agents used were chromo-acetic acid and Bensley's mercuric-formalin solution (1), and the material, both normal and etiolated, was then prepared for sectioning in the usual way. LAND'S (3) fixative proved useful in handling large sections. Several stains were used, including safranin-haematoxylon, orange G, and Haidenhain's iron-alum. Because of the mucilaginous nature of cactus material, it was found advantageous to continue the washing operation after the killing agent somewhat longer than is usually necessary with ordinary plant tissue.

Normal shoot

The gross structure of the normal shoot has already been given under the general description of the plant. Cross-sections (fig. 1) showed thick integumentary and palisade layers, an extensive spongy inner cortex, and a stele of elongated bundles. External to the phloem there appeared a mucilage mass which, with the bundle, presented a nail-shaped outline. The integumentary region consisted of cuticle, epidermis, and hypoderm (fig. 11). The cuticle averaged about 11 μ in thickness. Epidermal cells had straight anticlinal walls (fig. 4), and the longest cell measured was about 45 μ. One of the peculiarities of *Opuntia* and a few other cacti observed and mentioned by SCHLEIDEN (6) is the patchy decortication, in which the epidermal cells become active in spots, divide periclinally, and the inner of the two layers of cells thus formed continues to divide until a layer of tissue several

cells thick is present, whose outermost wall is the former external wall of the old epidermis. The tissue developed in this way is eventually entirely cut off, and a new outer epidermal wall is cutinized, so that the new epidermis resembles that which has just been lost.

The stomata were of the regular dicotyl type (fig. 4) and numbered 32–36 per sq. mm. of surface. Because of the thick cuticle they were sunken below the surface of the shoot (fig. 14). Below the epidermis extended the hypoderm, about six cells in thickness (figs. 11, 14). The first layer of the hypoderm consisted of a sheet one cell thick, with a large crystal of calcium oxalate in almost every cell. The remainder of the hypoderm was made up of about five layers of stone-cork cells. In places the crystal-containing cells almost closed the air chambers just under the stomata. The stone-cork cells were deeply pitted. They gave place abruptly to the palisade tissue with its long tubelike columns of cells, which was followed by the spongy cortical region reaching to the stele. The most external chloroplasts occurred in the palisade cells, where they were numerous on the lateral and end walls. Large air chambers extended from the stomata inward through the hypoderm into the palisade tissue, and the intercellular spaces were extensive in both palisade and spongy inner cortex. The structure of the stele will be described in a future paper.

Etiolated shoot

The appearance of the etiolated shoots presented a marked contrast with that of the normal ones (fig. 1). They were pinkish at first, but later changed to a very light green. They were longer than the normal shoots and more or less flattened in cross-section (fig. 3). Numerous sessile leaves like those of the normal plant in form were produced, which persisted for a short time. The longest measured was 7.5 mm. Spines, bristles, and glochids were grouped in a normal manner but were reduced in size, the spines averaging 3 mm. in length, the bristles about 1.5 mm., and the glochids intermediate between the two.

Unlike that of the normal plant, the epidermis of the etiolated shoot was without cuticle (fig. 13), although the walls showed

more or less cutinization. Individual cells varied in form and size from the base to the apex of the shoot. Those in the apical region were much elongated (fig. 6), some of them measuring 175 μ. Their lateral walls were straight, and their shorter end walls were wavy in outline. In the basal region the epidermal cells were not longer than 85 μ, and nearly all of the walls were slightly wavy when seen from the surface of the shoot (fig. 5).

However striking the changes in external form and gross structure due to etiolation may appear, the internal changes were even more interesting. When the surface of the etiolated shoot was examined under low magnification, numerous small elevations were observed which reflected the light. Closer investigation showed these structures to be minute papillae, each one bearing a stoma at the apex (figs. 13, 25, 27). Their nature was better seen in longitudinal and transverse sections of the shoot (figs. 25, 26), and they were usually found to be epidermal, the entire structure arising in most cases from a single cell; in others, chiefly from a single cell, but augmented to some extent by division of neighboring cells of the cortex. The papillary initial appeared to be analogous to a stoma mother cell. Evidently the stimulus to division had continued to act on the stoma mother cell and its progeny for some time and had met with a response, even after cutinization of the surface cell walls had occurred, for in one instance the resulting structure, unable to grow outward, had pushed inward among the undifferentiated cells of the cortex (fig. 28). The first division of the papillary initial was either a vertical one, as in the ordinary process of stoma formation (figs. 7–9), or else the cell became papillate and divided by an oblique wall (figs. 18, 19). The lower of the two cells thus formed then divided by a vertical wall, and the upper cell followed with a transverse division (fig. 20). After this the walls were chiefly oblique. Eventually two guard cells were cut off, which, seen from the surface, resembled the guard cells of a normal stoma (fig. 27), but which were more or less wedge-shaped in transverse sections of the shoot (figs. 21–25). The mature papilla might be compared to a hydathode in surface view, but in internal structure and in origin it was very different; the tissue was not glandular, no

vascular or conductive elements of any kind ended near it, and it usually originated from a single epidermal cell. Although large and solid papillate structures were most numerous, every transition was found between that type and the simplest form of stoma. Thus some papillae consisted of only a few cells in addition to the stomatal guard cells (fig. 26), and there was every gradation in size from this up to a papilla of dozens of cells. Some of the papillae were hollow also, and it appeared that the cavity had formed by the breaking down of the internal cells into a mass resembling mucilage. The simplest stomata consisted of guard cells with no differentiated auxiliary cells (figs. 32, 33), and often with neither intercellular space nor air chamber below. A few normal stomata had mucilage masses just underneath the stomatal opening.

Another peculiar situation was suggested by the occasional occurrence of a perfectly developed stoma lying almost under the margin of decorticating patches, and it was finally disclosed after a careful search. Three stomata were found developing under several layers of cork cells, one of which had guard cells just beginning to split apart (fig. 30). So far as can be discovered, the subcortical formation of stomata has never been reported. Perhaps it does not occur outside of the cacti.

In numbers the stomata of the etiolated shoot ran far below the normal plant, 12–16 being the maximal numbers found per sq. mm., even in the lower part of the shoot where they would be expected to be most numerous, in agreement with the nature of the epidermis which here most closely resembled that of the normal shoot. The stomata were mostly open.

The regions of a cross-section of the etiolated shoot also contrasted sharply with those of the normal stem (figs. 1, 3, 11, 13, 17). That the cuticle was absent has already been mentioned. The epidermis consisted of cells much broader than normal and much better supplied with protoplasm. Large budding chloroplasts were present which resembled chains of yeast cells in form, and varied in size from 30 μ to granules too small to be studied with the highest available power of the microscope (fig. 17). Below the epidermis there was neither a crystal-containing layer

nor stone-cork hypoderm, nor was a palisade tissue differentiated, but two general physiological regions could be recognized: an outer leucoplast-containing one 3–5 layers of cells thick, and an inner starch-containing one reaching to the stele (fig. 13). The leucoplasts of the outer cortical region were actively budding, like the chloroplasts of the epidermis, but were mostly smaller, and the smallest sizes increased in number in proportion to the distance from the surface of the shoot. Air spaces were much less extensive than in the normal cortex, and were often formed abnormally, as previously described.

Etiolated-greened shoot

Etiolated shoots placed in the laboratory windows and those transferred to the open presented similar changes in structure, but the changes were more rapid in the latter environment. Decortication removed the abnormal stomata with the papillae, and none reappeared (figs. 15, 29). Chloroplasts quickly disappeared from the epidermal cells. The whole shoot presented a shrunken appearance, due not only directly to the water loss from the almost unprotected tissues, but also to the actual death of many of the cells in the outer cortex (figs. 15, 16), a process by which air cavities were quickly enlarged. As cutinization progressed in the epidermis, and the turgidity of the cortical cells gradually became restored, the whole topography of the cross-section changed, for palisade tissue had appeared (fig. 12). Intracellular changes also occurred in the cortical cells. The chloroplasts were reduced in number as compared with the leucoplasts in the outer cortex of the etiolated shoot. They were regularly rounded in form and were present in cells as deep as the stelar region (fig. 12). They were necessarily confined to the peripheral region of the cell because of extensive vacuolization, and were found on end walls and lateral walls.

The new branches that appeared from the buds that had formed on the etiolated shoots before their removal to the light were larger, both in breadth and thickness, than the branches of the etiolated stems; and spines, glochids, and bristles were more like those of the normal plant in size and general appearance.

Discussion

The preceding description of the results obtained in the etiolation of *Opuntia Blakeana* suggest for discussion the factors concerned in the development of cuticle; the outline of epidermal cells; the number, origin, and development of stomata; the formation of palisade tissue; and the appearance of air spaces.

Cuticle formation, it has been suggested, approaches a maximum when transpiration is great in amount, and when it is high in proportion to absorption (**2**). It has also been stated that cuticle formation is favored by growth in concentrated nutrient media. Both factors were probably operating in these experiments. The etiolated shoots certainly transpired much less than they would have done had they been grown in the open, for they promptly wilted when transplanted to the latter environment. Gradual increase in the osmotic pressure of the sap in the joints from which the etiolated shoots obtained their nutrient supply must have occurred, but it was evidently insufficient to induce the development of cuticle. MACDOUGAL found no cuticle formation in any of the numerous plants with which he experimented.

The changes mentioned in connection with the outline of the epidermal cells have been observed by many investigators. Whether light is a factor in determining the shape of the cell walls in the epidermis could not be determined, for an attempt was not made to reduce transpiration when etiolated shoots were removed to an outdoor environment in order to separate the two factors. Mesophytic conditions in the dark chamber may be considered favorable to crenated walls in view of the results of other workers (**2**).

Decrease in the number of stomata per unit of surface area is in general harmony with the results of numerous investigators. Six or seven times as many epidermal cells per unit of surface appeared on the normal shoots, while the stomata were two to three times as numerous, compared with the etiolated ones. In his experiments with *Opuntia Opuntia*, MACDOUGAL found that the stomata on the etiolated shoots were reduced in size. Those measured in *Opuntia Blakeana* were not different in size from the stomata of the normal plant, excepting a few freakish forms of stomata.

Development of stomata under cortical tissue is a phenomenon that is difficult to explain. COWLES states that although the factors inducing the appearance of stomata are unknown, evidence is not lacking that light favors their development, and that stomata are abundant where transpiration is vigorous, and absent where it is reduced or wanting. Neither factor could have greatly influenced the development of subcortical stomata in a direct way in this case, for light was absolutely excluded and transpiration was much reduced, owing to the increased moisture content of the air in the dark chamber and the covering of cortical cells. In connection with the light factor, however, there is another interesting possibility. In MACDOUGAL's experiments, seedlings of *Aesculus*, whose basal internodes were briefly illuminated, developed laminar bodies in internodes formed some weeks after the stimulus had been given, which were entirely lacking in the absolutely etiolated seedlings. He states that "the stimulative effect of illumination . . . . may be received by one portion of the body and transmitted to another, and the impulses may even be communicated to organs not actually formed at the time the stimulating rays were received." The last resort appears to be to ascribe the phenomenon to some internal factor such as is included under the term heredity.

The papillary structures appear to be peculiar to the etiolated shoots of *Opuntia Blakeana*. That they result from the division of a cell analogous to a stoma initial seems to be a logical conclusion, since all stages are found, from the normally developed stoma with two guard cells and two adjacent cells originating from one initial, to the elevated structure consisting of many cells around and below the guard cells, all developed from one initial. The fact that the first division may be a periclinal one appears to be a matter of detail that does not exclude such an interpretation. Some stimulus, possibly previous illumination or some internal stimulus, starts the division process, which is favored by the increased moisture of the dark chamber and by an abundant food supply from the normal shoots at the base of the etiolated ones. Once started, the divisions continue until the cutinization of the epidermal walls offers sufficient resistance to check the growth

of the papilla. The appearance of the abnormal structure shown in fig. 28 seems to favor such a conclusion.

Among the theories advanced to explain palisade development are the light theory, the transpiration theory, and the lateral pressure theory (2). No attempt was made to separate the light and transpiration factors when the etiolated shoots were transplanted. Both factors were increased upon transferring the etiolated shoots to the light. Lateral pressure must have been reduced, for the cortical tissues had a minimum of intercellular air spaces in the dark chamber and a maximum in the etiolated-greened condition. Furthermore, the collapse and death of many cortical cells after exposure of the shoots to an outdoor environment must have further reduced lateral pressure. Another factor may have been operative. The partially cutinized epidermal walls resisted collapse, judging by the appearance of sections of fresh and killed tissues, and this cylinder of epidermis, as it gradually decreased its water loss by increased cutin secretion, must have set up an outwardly directed strain that could not fail to influence the shape of the cells attached to it.

The appearance of large intercellular spaces in the outer cortex is closely related to palisade development which has already been briefly discussed. Shrinking and subsequent turgidity and the death of cortical cells were important factors in increasing the intercellular spaces and enlarging air chambers. All of these factors are probably active in various plants growing in a natural environment in the southwest. It has already been shown for succulents that shrinkage and expansion are marked with the change from wet to dry seasons (7). An investigation of plants that survive continued dry winds would probably reveal intercellular changes in the cortex similar to those observed in these experiments in the etiolated-greened shoots.

Summary

1. This paper deals with a comparison of the appearance, form, and structure of normal, etiolated, and etiolated-greened shoots of *Opuntia Blakeana*, a prickly-pear cactus of the southwest.

2. The flat spiny joints of the normal plant, when carried into the dark chamber, produced roundish, light green, elongated, etiolated shoots which differed remarkably in form and structure from those of the normal plant, and which exhibited structural changes when transplanted in an outdoor environment that brought them to resemble the normal shoot.

3. The etiolated shoots lacked a cuticle, developed papillate structures and stomata abnormal in form and position, and lacked the cortical differentiation so characteristic of the normal shoot.

4. The etiolated-greened shoots lost water rapidly at first. Their air spaces increased rapidly by active intercellular splitting of walls and by the collapse and death of cells; then a cuticle appeared; cortical cells elongated, forming palisade tissue; and in other respects the shoots approached the normal ones in structure.

The study presented in part in this paper was made possible through the interest of Dr. D. T. MACDOUGAL, Director of the Desert Laboratory, who furnished the etiolated material used, and whose friendly criticism constituted a continual source of inspiration. Acknowledgments are also due Professors JOHN M. COULTER, CHARLES J. CHAMBERLAIN, and W. J. G. LAND, of the University of Chicago, for numerous helpful suggestions.

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EXPLANATION OF PLATES XXVII–XXX

PLATE XXVII

FIG. 1.—Cross-section of normal shoot showing topography: *a*, integumentary region; *b*, palisade region; *c*, mucilage mass at peripheral side of vascular bundle; *d*, stelar region; ×0.5.

FIG. 2.—Cross-section of etiolated-greened shoot showing topography: *a*, epidermis; *b*, cortex; *c*, leaf trace; *d*, vascular bundle; *e*, stele; ×12.

FIG. 3.—Cross-section of etiolated shoot showing topography; same ettering as fig. 2; ×12.

FIG. 4.—Epidermis from normal shoot after removal of cuticle; ×395.

FIG. 5.—Epidermis from basal region of etiolated shoot; ×395.

FIG. 6.—Epidermis from apical region of etiolated shoot; papillae not yet fully developed; ×395.

FIGS. 7–10.—Normal development of stoma in epidermis of etiolated shoot; fig. 8 is vertical section of stage shown in fig. 7; ×525.

PLATE XXVIII

FIG. 11.—Part of transverse section through outer region of normal shoot: *a*, epidermis with layer of cuticle above; *b*, hypoderm; *c*, palisade region; first layer of hypoderm is a crystal-containing sheet; 2 air chambers shown; ×85.

FIG. 12.—Part of transverse section through etiolated-greened shoot reaching from epidermis into stele; palisade tissue has appeared; round bodies represent chloroplasts; *a* and *c* as in fig. 11; *d*, inner spongy cortex; ×85.

FIG. 13.—Part of transverse section of etiolated shoot reaching from epidermis into stele; papilla shown in upper right-hand corner and part of mucilage mass in lower left-hand corner; black dots represent leucoplasts, and circles represent starch grains; ×85.

FIG. 14.—Detail of integumentary region of normal shoot; lettering as in fig. 11; ×525.

FIG. 15.—Part of transverse section of etiolated shoot shortly after removal to light; decortication of outer layers of shoot involving a stoma in progress; *a*, future surface wall of new epidermis; ×525.

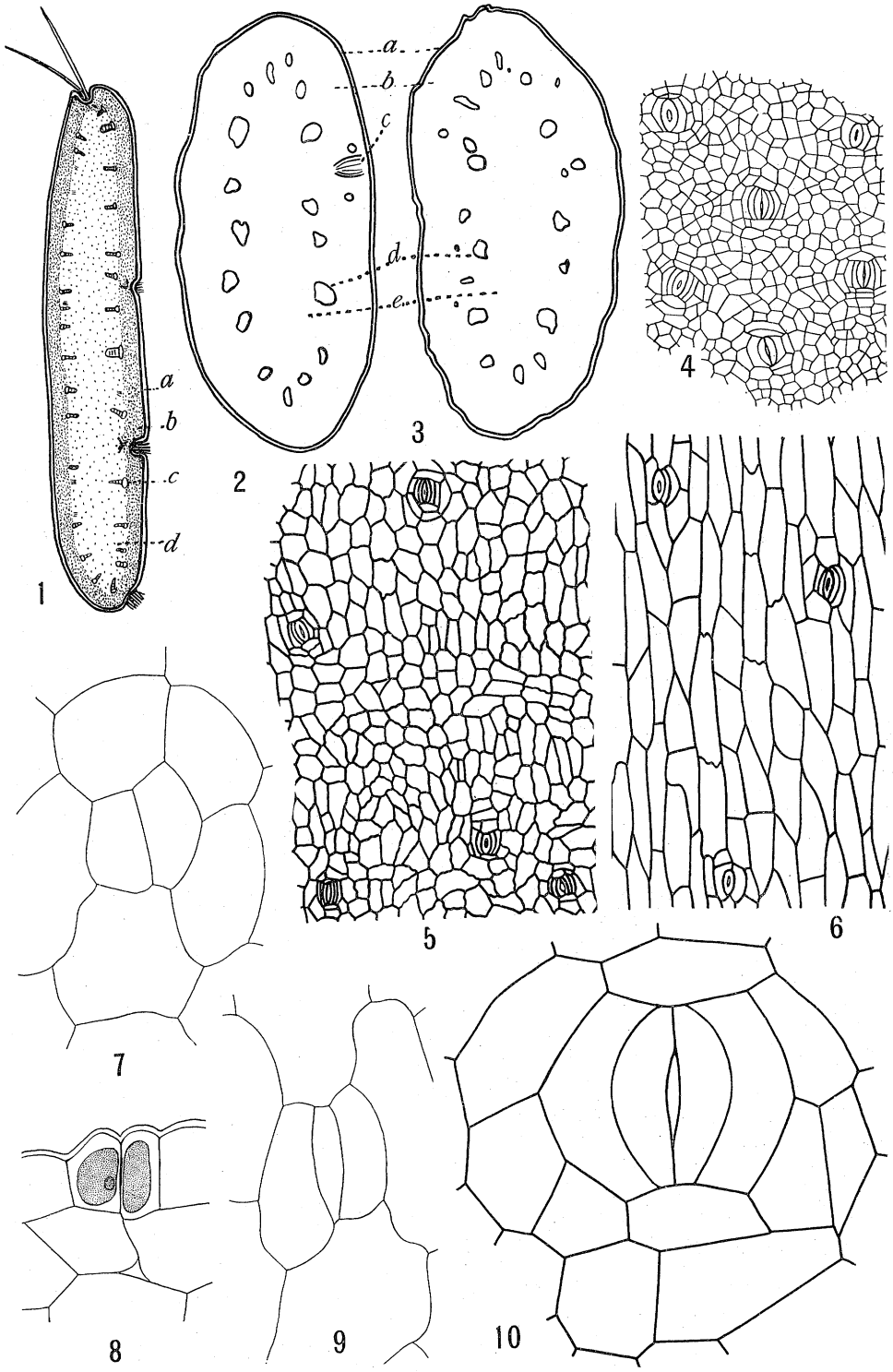
FIG. 16.—Detail of stage of adjustment of etiolated-greened shoot shown in fig. 12; remains of dead cell showing below, projecting into air space; ×525.

FIG. 17.—Detail of transverse section shown in fig. 13; reticulate bodies are nuclei; irregular bodies are chloroplasts and leucoplasts; large, round, lightly stippled bodies are grains of storage starch; ×525.

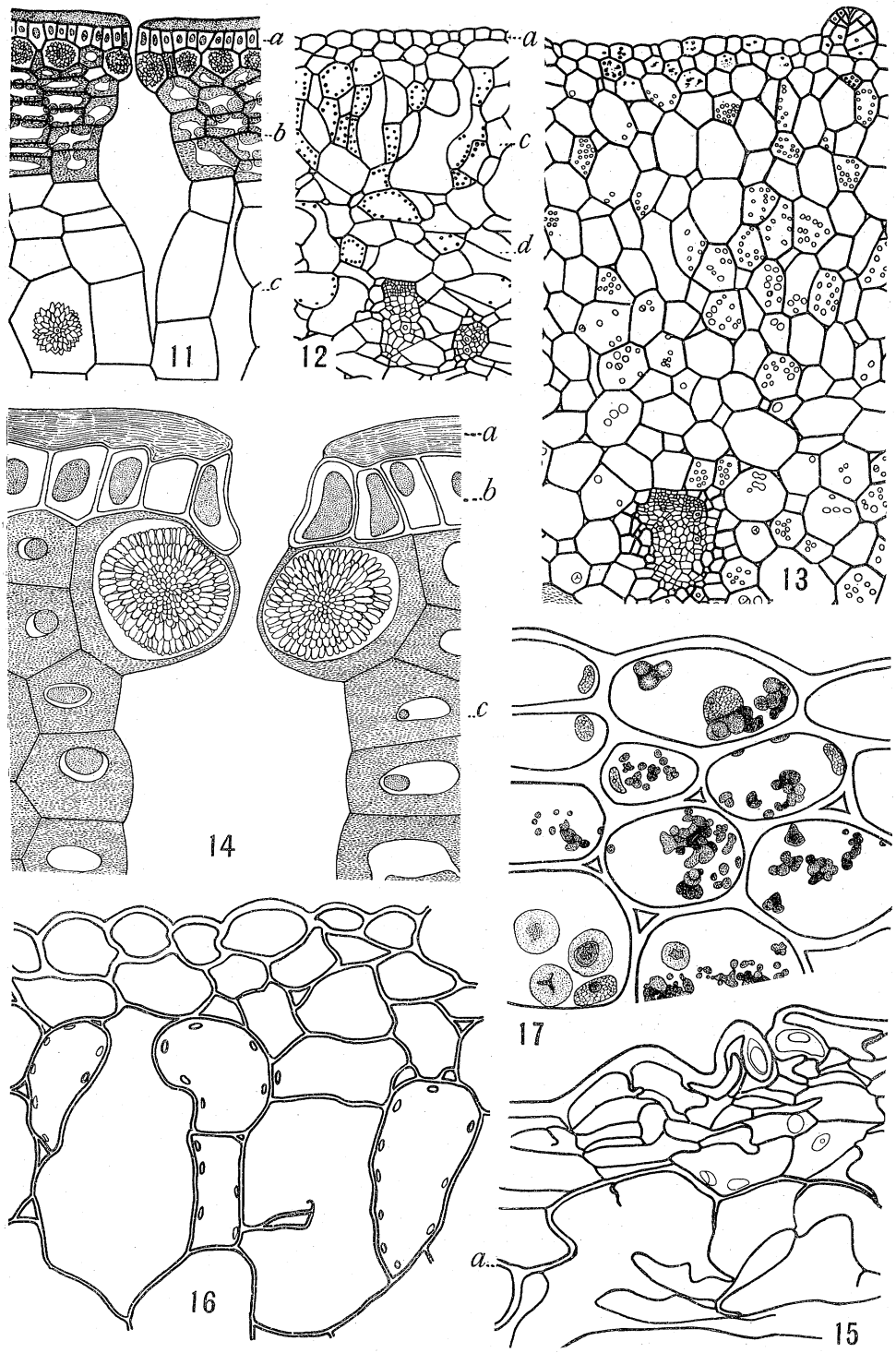
PLATE XXIX

FIGS. 18–25.—Development of papilla as seen in transverse sections of etiolated shoot: *a*, inner wall of initial cell; *b*, first transverse wall; *c*, stoma; fig. 19, ×390; figs. 20, 22, ×245; all others, ×525.

FIG. 26.—Vertical section of young papilla cut parallel with long axis of etiolated shoot, showing side view of guard cell; ×525.



BROWN on OPUNTIA



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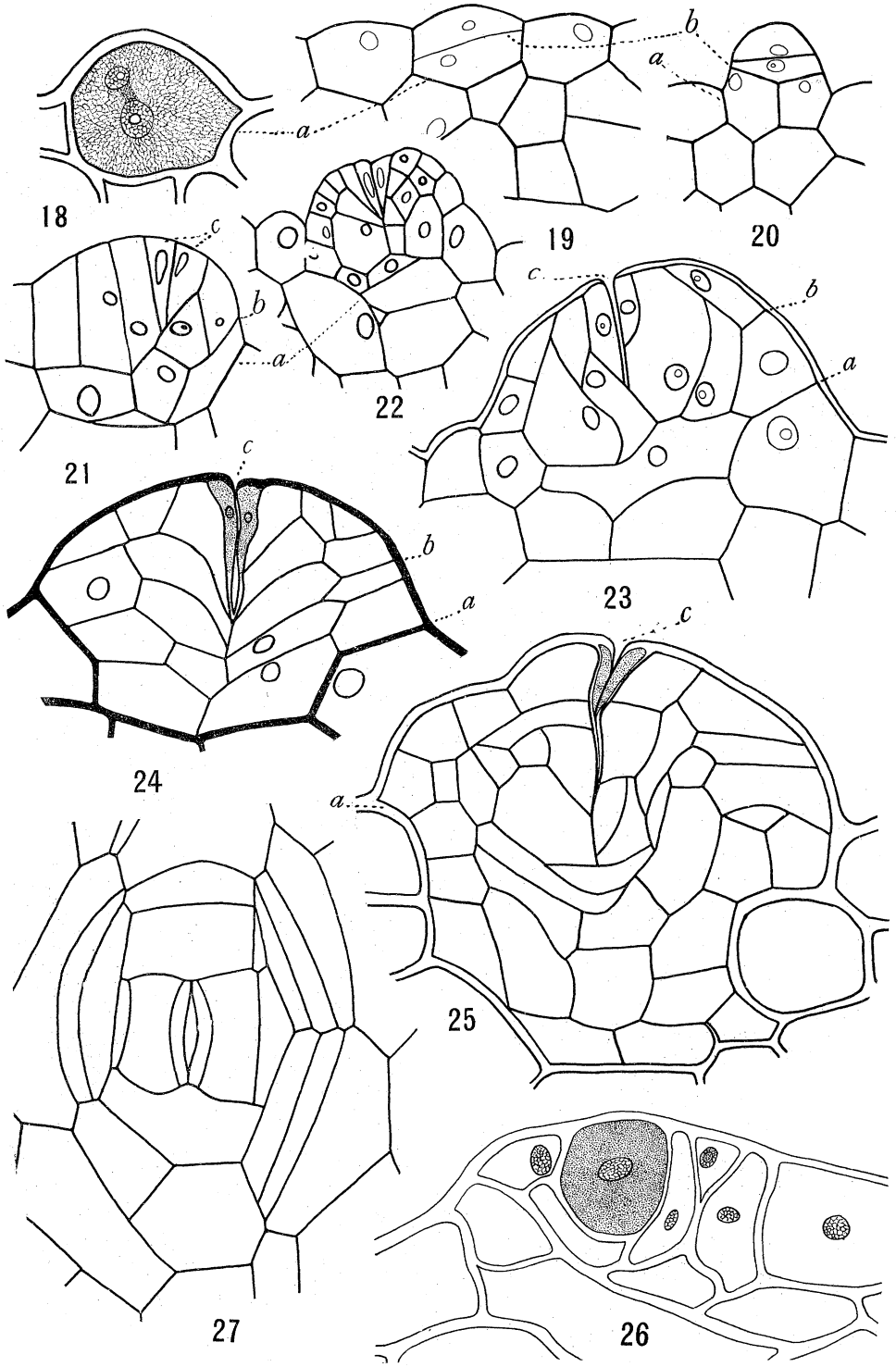

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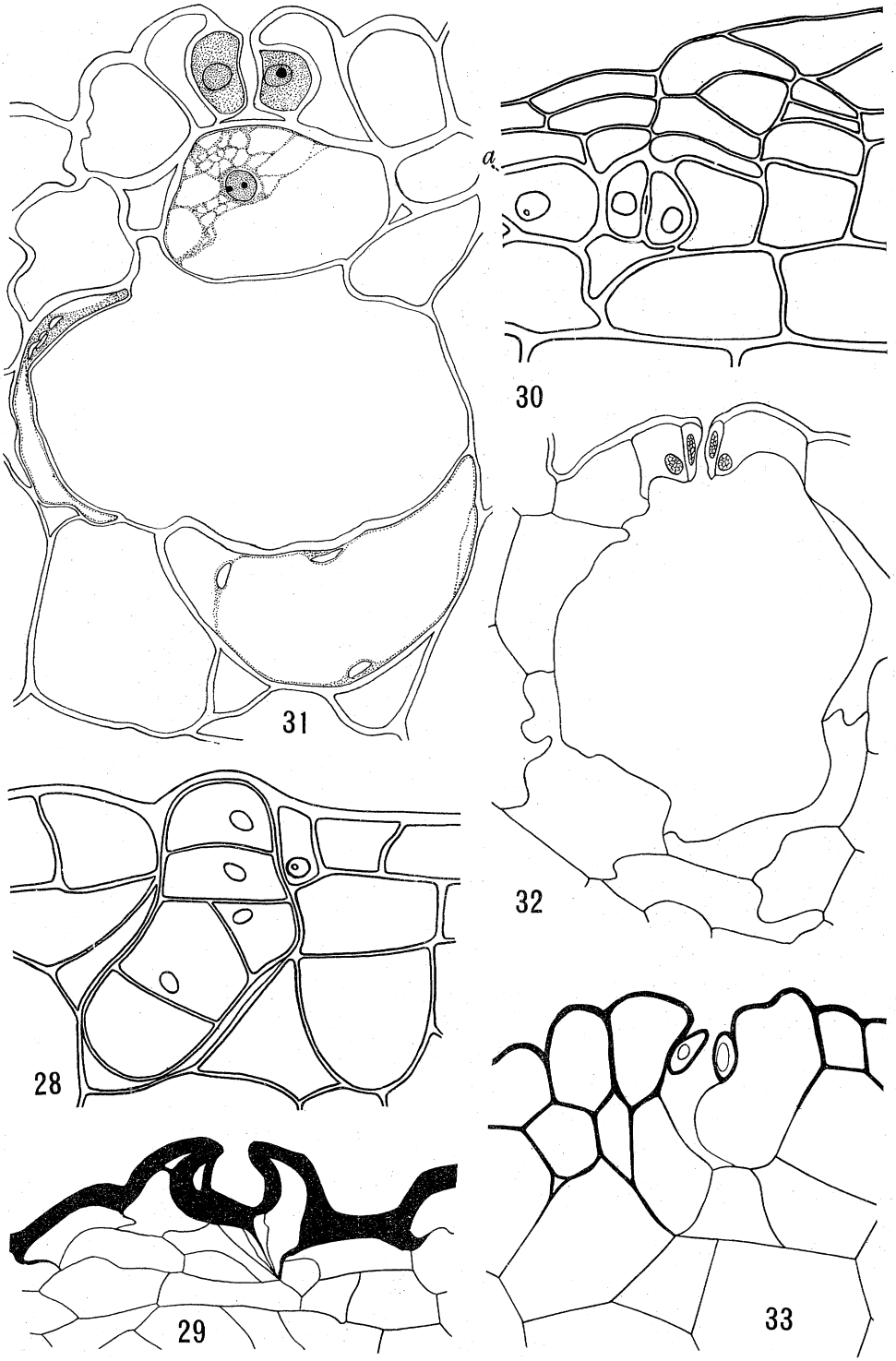

BROWN on OPUNTIA

PLATE XXX

FIG. 27.—Surface view of papilla with central stoma; ×525.

FIG. 28.—Structure found in cross-section of etiolated shoot arising like papilla, from epidermis, but growing inward; ×525.

FIG. 29.—Mass of decorticated tissue including papilla, drawn from transverse section of etiolated-greened shoot; ×525.

FIG. 30.—Subcortical development of stoma drawn from transverse section of etiolated shoot: *a*, future external epidermal wall; ×525.

FIG. 31.—Part of transverse section of etiolated-greened shoot showing stoma not yet connected with air chamber; collapsed cortical cell also appears to left; ×525.

FIG. 32.—Part of transverse section through etiolated-greened shoot: stoma lacked auxiliary cells and opened into air chamber of considerable size; similar simple stomata present in etiolated shoot; ×525.

FIG. 33.—Freakish stoma found in transverse section of etiolated shoot; ×395.