TYGE W. BÖCHER

STRUCTURE OF THE MULTINODAL PHOTOSYNTHETIC THORNS IN *PROSOPIS KUNTZEI* HARMS

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Synopsis

The structure of the photosynthetic, multinodal thorns in *Prosopis kuntzei* was studied anatomically. The stem anatomy in this xeromorphic subtropical tree deviates in many ways and exhibits several characteristics which raise questions of more general interest.

The external cell layer in the multiple epidermis is composed of elongate tapering cells terminating in brushes of delicate strands, which are believed to be microcanals and responsible for wax exudation. Wax occurs in the outer cuticular layer outside the brushes. Other delicate strands occur in or near the anticlinal cell walls and cuticular flanges and reach the cuticle and may penetrate it. Epicuticular wax covers seem largely to be exuded through the anticlinal system of microcanals. After heating and melting, wax is pushed out in the mounting medium, where, after cooling, it recrystallizes in various ways.

The stomatal complexes are initiated as multi-layered distinct cell groups. The guard cell precursors are first covered by several flat cells that degenerate, withdraw or remain as wall rests (pseudo-ledges) which frame an elliptic opening between the antechamber and the outer stomatal cavity. The latter frequently becomes filled with ± alveolar wax plugs, the wax probably being produced by the surrounding subsidiary cells. The stomatal complexes are compared with similar multi-layered complexes in *Prosopidastrum* and *Cercidium*, which both, however, possess a periderm-like outer covering of living cells.

The cortex has several palisade layers. A layer of crystal cells borders abaxially the primary fibre strands, while another endodermoid layer forms the transition from the green palisade layers to a distinct pericycle composed of 3–5 layers of thick-walled pitted cells. In young thorns the xylem is without vessels, or has few scattered solitary vessels. It is mainly composed of axial parenchyma. Later-formed wood has irregular tangential bands of fibres and parenchyma, while the latest wood contains clusters of wide vessels in bands of axial parenchyma. The rays are heterogeneous and often accompanied by radial rows of axial parenchyma.

Evolutionary trends in *Prosopis* and related genera are discussed. Comparisons are made between species with multiple and 1–2 layered epidermis. The structure of the stomatal complexes becomes elucidated by their origin as multiple structures. Other comparisons concern the different orientation of the stomatal apertures in green stems. Different degrees of xeromorphy are discussed in the light of diffusion resistance for water vapour due to differences in the length of the pathway for transfer of gases, and the number of narrowings along the pathway, as well as the various types of partial occlusions due to wax plugging in epistomatal cavities or formation of calcium oxalate sphaerites in the substomatal chambers.

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1. Introduction, Material, and Methods

The anatomy of a number of apophyllous shrubs was recently studied by BÖCHER & LYSHEDE (1968, 1972) and BÖCHER (1971,1972). The idea behind these studies was to attack the problems around the evolution of life-forms, or in other words to focus convergency as an evolutionary process. This attack may be launched from two sides, viz. by experiments elucidating the forces connected with the process and by comparison of the resulting entities. The papers mentioned above were contributions to a comparative anatomy of one life-form and have made possible a subdivision of this life-form according to anatomical structure.

The present paper may be considered a continuation of the same comparative line of research. However, the species being the subject of the study is not a shrub but a low tree and may be compared with other low trees, e.g. *Casuarina* or *Genista (G. aetnensis)*.

One weak point in comparative studies of this kind is the lack of access to living material that would make possible ecophysiological experiments and ontogenetic studies of tissue developments or studies on seedling and juvenile stages. Although seeds of wild plants are not available in Copenhagen in the present case, the results obtained by simple observations of dried and preserved material were in many ways so encouraging that it was thought worth-while to publish an account.

The investigation is classical, mainly descriptive, anatomical; but its purpose is intended to be wider in an attempt to understand structure from many points of view, ontogenetical, evolutionary background, structure in relation to adaptation, and function. Perhaps it would be appropriate to formulate a special branch of studies which could be called bio-morphology and which would not be merely descriptive but preferably dynamic, developmental, eco-physiological and -synthetical, though still founded on classical morphology and anatomy.

Material of *P. kuntzei* was collected by HAWKES, HJERTING & CRIBB 1971 (No. 4371) and determined by Dr. MARTIN CARDENAS. It originated from Departemento Santa Cruz, Prov. Florida, in Bolivia, one km from Matarel on the road to Valle Grande, 1400 m above sea level. A photograph of a 5–6 m tall specimen with a trunk of 40 cm in diameter, as well as herbarium and alcohol material, was brought to Denamrk by J. P. HJERTING and kindly given to me (see Fig. 1 a, b). Additional valuable herbarium material from the Botanical Museum in Copenhagen was also studied.

For sections three standard staining combinations were used: Safranin-fast green, Johansen's quadruple stain, and Sudan IV. Observations were made with the light microscope frequently combined with equipment for interference contrast or polarized light. The herbarium material was also used for SEM studies of the epistomatal cavities.

The author is indebted to Mrs Ella Buch, Miss Helle Feltrin, Messrs. Ole Lyshede, H. Elsted Jensen and Sv. Aa. Svendsen for excellent technical assistance, and to Mr J. P. Hjerting for the interesting material.

2. Taxonomy, Morphology, and Distribution

According to BURKART (1940: 83), *Prosopis kuntzei* Harms is closely related to *P. sericantha* Gill. Both have multinodal, large, green thorns. The small leaves are shed very early. The two species are distinguished by floral characters. However, a number of vegetative characters are clearly important also. *P. kuntzei* is a 5–10 m high tree, while *P. sericantha* is a 1–2 m high shrub. The year-shoots in *P. kuntzei* are 8–40 cm long and (1.5) 2–5 mm i diameter. In *P. sericantha* they reach 6–15 cm in length and are 0.8–2.5 mm thick.

P. sericantha was treated anatomically by BÖCHER & LYSHEDE (1972), as was a third apophyllous species, *Prosopis globosa*. The latter was transferred by BURKART (1964) to a separate genus *Prosopidastrum* Burkart. Its habit appears from the drawings in ROIG 1970, plate 50, Fig. 4–8. BURKART describes it as a subspinose, about 1 m tall shrub with year-shoots (dried), between 1–2 and 5 mm in diameter. The small stipules are persistent, recurved and spinous.

To the characters distinguishing *Prosopis* and *Prosopidastrum* the different orientation of the stomata and the very different developments of the epidermis should be added. In the two species of *Prosopis* the stomatal apertures are orientated across the longitudinal axis of the branch, while in *Prosopidastrum* the apertures are parallel to the axis. The epidermis is multiple and phellem-like in *Prosopidastrum* and multiple or two layered in the two *Prosopis* species, the external layer being developed as a palisade-epidermis.

Acccording to BURKART (1940), *P. kuntzei* has a relatively northern, sub-tropical distribution including Northern Argentina, Paraguay and Bolivia. *P. sericantha* is restricted to Northern Argentina, whereas *Prosopidastrum globosum* has a wider area in Argentina. *P. kuntzei* and *sericantha* are sympatric in Northern Argentina, where they are characteristic elements in the dry sub-tropical forest (Chako). *P. sericantha*, though, also enters the northernmost part of the sub-tropical shrub steppe (Monte), while *Prosopidastrum globosum* is connected with the regions dominated by Monte and the northern parts of the Patagonian province and the Pampa province.

The plants from Bolivia of *Prosopis kuntzei* to be dealt with in the present paper grew together with columnar *Cactaceae* in open, dry vegetation near the bottom af a valley (see Fig. 1).

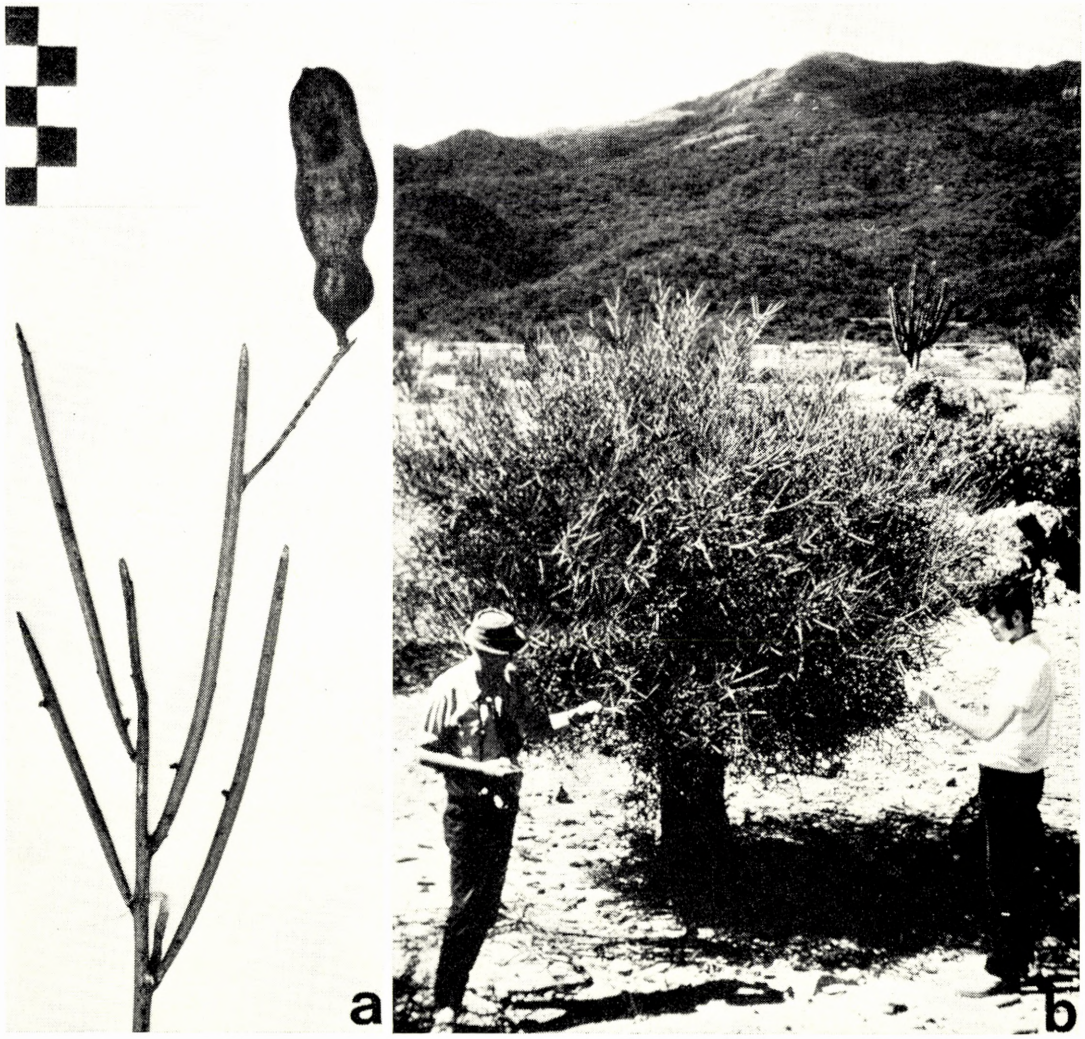

Fig. 1. *Prosopis kuntzei* from Santa Cruz, Prov. Florida in Bolivia. a. Fruiting branch (black and white squares 1 cm²). b. The 5–6 m tall tree (Hawkes, Hjerting & Cribb 1971 No. 4371). J. P. Hjerting phot.

The structure of the shoots in the apophyllous members of *Prosopis* (and *Prosopidastrum*) is interesting. In *Prosopidastrum globosum* three branches often issue apparently at the same node, one being a branch of first order, whereas the two others, which are opposite, are generally branches from the base of the first order branch. In *Proposis sericantha* a similar arrangement of three branches seemingly issuing from the same node may be found in several cases, but as a rule two branches are placed together and may be equally strong or one stronger than the other. Otherwise the structure is sympodial, composed of a series of relatively strong axes arranged in a zigzag (e.g. Krapovickas & Cristobal No. 14504 (C), A. Brizuela No. 576 (C) and Böcher,

Hjerting & Rahn No. 2460 (C)). The leaves which support branches are alternate, but in a number of cases two nodes tend to close up and the branches issuing from the nodes approach an opposite position. The morphology of *P. sericantha* was shortly mentioned by Böcher and Lyshede (1972 p. 77 and 80). "Scale leaves" in this account should be adjusted to rudimentary, persisting, scaly leaf bases. It is evident that a suppression of some of the branches takes place. In the proximal part of each shoot the branches are elongate, unbranched thorns and often occur singly. Stronger branches and the occurrence of two (or three) branches at the same node increase in the distal part, the result being an acrotonous main structure. However, in *P. sericantha* as well as in *P. kuntzei* the strongest lateral branches, which are members of the sympodial main axis system, issue at some distance from the top of each main axis.

The morphology of *P. kuntzei* appears from Fig. 1 and was further studied in herbarium material collected in Argentina and Paraguay by T. Myndel Pedersen. The plants from Paraguay (Myndel Pedersen Nos. 4172 and 4105, 1956 (C)) are similar and have more slender, long and flexible branches (thorns). According to Burkart (1940: 86) they belong to a separate taxon which corresponds to *P. casadensis* Penzig. Yet, the shoot structure may not deviate essentially from that found in the main type of *P. kuntzei* represented by the collection from Bolivia (Fig. 1) and a collection from Prov. Chaco in Argentina (Myndel Pedersen No. 9669, 1970, C, Fig. 2).

In the two samples from Paraguay two shoots at the same node are common, while three are rare. Two shoots issuing together are very often equally strong, but in the case of three shoots one is often stronger than the two others. At one node (M. P. No. 4105) four branches were arranged in two groups indicating a total suppression of the first order branch and two branches of second and third order.

The material of the main type has thicker and very rigid branches terminating in strong spines. Each shoot appears as a multinodal thorn. The occurrence of three branches at the same node is more exceptional; single branches occur mostly in the proximal part of the shoot system, whereas two at the same node mainly occur in the distal part. The acrotonic development is further emphasized by a decreasing length of the unbranched joints in the upper part of each shoot. The result of this type of branching is a tree with a "crown" composed of sympodial systems of photosynthetic, multinodal thorns. Apart from the size, a tree of this kind behaves like spiny, sympodial dwarf shrub, as e.g. *Vella spinosa* (Rauh 1942, Fig. 26). However, there may be even better instances of agreement with the structure of a spiny desert suffruticose like *Zilla myagroides*, which finally has a dense, sympodially branched inflorescence in which several shoot generations are produced the same year (Rauh l.c. Fig. 24 and Plate 4 Fig. 1). In the case of *Prosopis kuntzei* the anatomical studies referred to below indicate that a sympodial system of two or perhaps even three shoot generations is formed within the same year, as is also the case with South American *Adesmias* (Rauh l. c. Fig. 15).

The branch system in the specimen from Argentina in Fig. 2 may be conveyed as follows, starting from the base and omitting nodes where no branches issue:

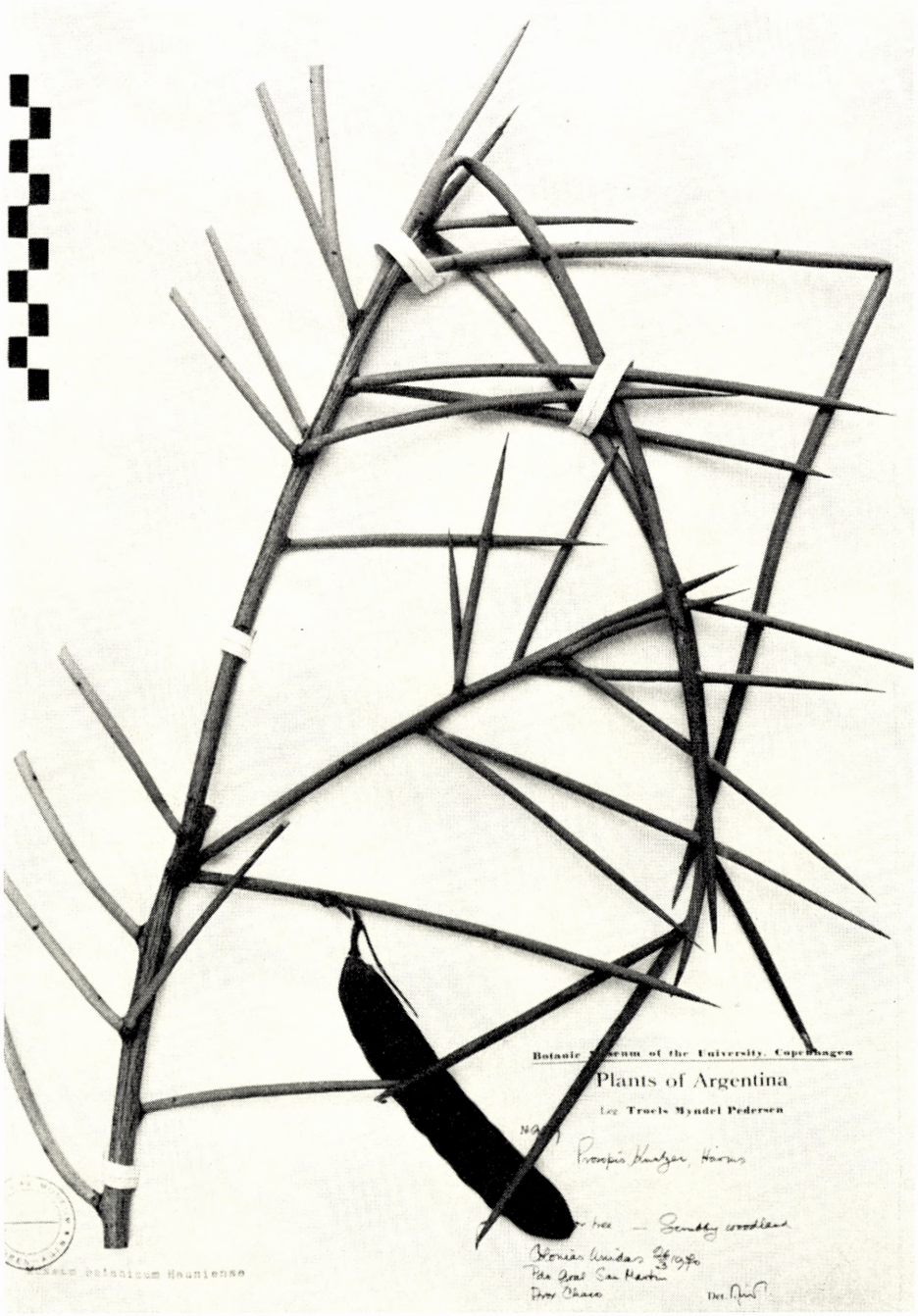

Fig. 2. *Prosopis kuntzei* from Prov. Chaco in Argentine (Myndel Pedersen 1970 No. 9669), Botanical Museum, the University of Copenhagen. The morphology of this specimen appears from the text on p. 8. Black and white squares 1 cm high. H. Elsted Jensen phot.

Shoot generation I (proximal part of unknown length, cut)

Node 1. Single branch, cut.
2. Single, rather short branch.
3. Two branches, one cut, the other issuing from the base representing the next shoot generation.
4. Two branches, a strong one and a weak one, both have been cut, the weak one turns towards the left and is probably a third order branch.
5. This node is situated below the spiny end of I, which in Fig. 2 is covered. Three shoots issue: a strong, short one which carries shoot generation II, a branch system which starts with a long unbranched part and then has four nodes each with branch pairs (of which one of the two belongs to the third generation), finally a branch which has a pod-bearing weak branch but is otherwise unbranched.
Spiny ending of I.

Shoot generation II.

Node 1. Pair of weak branches followed by a long unbranched part. One branch cut near its base, the other turning towards the left.
2. Single branch (towards the right).
3. Pair of branches, the branch turning left is cut.
4. Pair of branches, the branch turning left is cut, the other is longer and has almost the same direction as the main axis (II).
5. Pair of branches, equally strong, turning towards the right.
6. Pair of branches turning left, both cut, rather long internodium.
7. Two main shoots representing Shoot generation III a and b.
8. Two branches, one of which is long and unbranched.
Spiny ending of II.

Shoot generation III a and b.

Both shoots start with a very long unbranched part; III a has one branch pair near the spiny end (passing the pod), while III b has only one branch (pointing towards the word Copenhagen on label) near the spiny end.

The material was collected on March 26th, which is late summer in Northern Argentina, and no new shoots or leaves are found on the specimen.

3. Anatomy

A. Main structure of stem in relation to age and photosynthetic activity

As mentioned above, the small leaves in *P. kuntzei* are shed very early. They may only contribute to the production of matter during the development of the young shoots, which according to BURKART (l.c.) appear in July. Flowering is said to take place from October to December. June–July represent the coldest and driest period, which is followed by a season with incerasing precipitation and heat. During the

relativelly cold period the young shoots of *P. kuntzei* are probably less exposed to water loss resulting from transpiration. The soil may still contain enough water from the precipitation in the prededing months.

A striking structural feature in *P. kuntzei* is the thickness of the young branches. It may be of importance for the species, which grows in areas with xeric conditions, to produce thick green branches rapidly. They contain a multi-layered palisade tissue and thus represent a comparatively expanded green area exposed to radiation.

The water supply of the green tissues is secured by a rapid filling up of the central part of the branch with a tissue predominantly composed of thin-walled xylem elements. This tissue follows outside the protoxylem groups and the pith core. At the beginning the main function of the vascular system is thus probably conduction, whereas supporting or protecting tasks are undertaken by peripheral layers outside the vascular cambium. In this connection the pericyclic thick-walled tissue to be mentioned in detail later is of particular interest. It bridges the gaps between the perivascular fibre strands and thus produces an efficient support and protection against bending or compression.

While an incipient stage in the stem development is unfortunately unavailable because of the time that the material was collected, the young stage described above is marked by the vigorous palisade, which already at a short distance from the spiny end consists of 4–8 cell layers. At this stage the development of occlusing wax plugs in the stomatal caves has just started and the surface of the epidermis may appear smooth.

The next stage in the stem development is probably contemporary with the formation of a new main axis. Late in the young stage the wood contains many groups of xylem fibres but rarely vessels. The onset of the later stage is characterized by a sudden change in the wood from chiefly axial parenchyma to many vessels accompanied by a continued formation of parenchyma bands and groups of xylem fibres. At the same time the primary phloem is replaced by a layer of fibres and many sclerenchyma cells mature in the adaxial part of the pericambial layer.

The layers of palisade are maintained, and the most obvious difference from the young stage is the grater accumulation of crystals in the cells.

From an ecological point of view it is important that the palisade layers and the photosynthetic activity are maintained although the stomatal cavities now appear filled with wax (see below). Of great importance in this context is the ability of *P. kuntzei* to increase the girth in all layers outside and including the vascular cambium, and that no or few phellogens start working as long as the branch is part of the photosynthetic system.

The latest stage in the stem development was studied on sections of the oldest, basal part of the specimen shown in Fig. 2. Here the stem is 7–8 mm in diameter. It was possible, after staining the sections with fast green-safranin, to obtain reasonably good slides (Fig. 10 a). The stem in question agrees anatomically with the material from Bolivia. The vessel containing xylem, which is formed late in the first year, is continued almost without growth ring formation. It is possible to trace one growth

ring, probably corresponding to the start of shoot generation II in Fig. 2. In the phloem, however, a cylindrical layer of stone cells may indicate a relatively inactive period of the cambium. The pericambial activity has almost ceased, and the increase in girth in the extracambial layers depends mainly on tangential cell elongation. The stomatal caves are plugged with waxy material, and sometimes even small colonies of fungal cells contribute to the occlusion of the stomatal apertures. The walls in the guard cells increase in thickness and may restrain the movements of these cells in the late stage.

Already in shoot generation II phellogens may be formed along the primary fibre strands situated near the surface. The cork appears as brown stripes on both sides of the ribs (Fig. 2). The phellogens are usually initiated in the subepidermal layer, but they bend inwards and continue in the perivascular cells on both sides of the primary fibre strands, which finally become parts of narrow rhytidome stripes.

Near the points of the thorns large parts of the outer epidermis layer die and shell, while the interior layers are transformed into phellogens which produce cork, making the points brownish, and also some phelloderm layers which appear to occur as a continuation of the pericyclic layers. The phloem disappears near the points, whereas all the fibre strands continue to the points while becoming increasingly densely spaced. The parenchyma cells become filled with brownish contents. Many tracheids or groups of tracheids continue to the points where they seem to stop inside former stomatal cavities. This evidently suggests that during the growing period the points act as hydathodes. A similar function was suggested for the ends of the thorns in *Monttea aphylla* (Böcher & Lyshede, 1968, Fig. 5).

The anatomy of thorns has not been studied very thoroughly. Some few outlines are available, a short one by Schnee (1939) and a more comprehensive one by Schröder (1964), which, however, mainly deals with Central European thorny species (e.g. *Crataegus monogyna* and *Prunus spinosa*). The anatomical analysis of the latter two stresses how few points of resemblance exist between the photosynthetic thorns in *Prosopis* and the non-photosynthetic thorns of the two European shrubs. However, near the point in *Prosopis* the density of fibres is similar to that in non-photosynthetic thorns. Also the clearly demarcated protoxylem is a similarity. *Ulex eropaea* may be the only European species with photosynthetic thorns. They are multinodal with spiny leaves and are furrowed with stomata in the furrows and fibre strands in the bars, thus resembling the stem in species of *Genista* (Schröder, 1964 Fig. 24). According to Hagerup (1930), the white, light-reflecting thorns in *Acacia seyal* are possibly of importance for the photosynthesis of the green twigs.

B. Epidermis; interstomatal parts

The epidermis in *Prosopis kuntzei* is multiple. In several respects it recalls that of *P. sericantha*, but in the latter the epidermis consists of two cell layers only, of which the exterior is a palisade epidermis and the interior a hypodermoid layer. In *P. kuntzei* the epidermis resembles that of *Bulnesia retama*, where according to the previous investigations the protoderm cells, after division, develop an exterior palisade

layer of radially elongate cells and one or two hypodermoid layers. In *P. kuntzei* the outer palisade epidermis rests upon two hypodermoid cell layers and in interstomatal areas often on three layers. The exterior cells are arranged in families of 2–4 cells. originating from the same protoderm cell (Fig. 9b, Plate I j–k).

In all epidermal cell layers the walls were stained with Sudan IV. Even wall thickenings in the adjacent cortex collenchyma were sometimes stained. Minor parts of the walls of the interior epidermal cells, as well as thin interior cellulose coatings of the outer epidermal cells, alone seem to remain unstained.

The interior epidermal cell walls are also stained with Safranin but they are probably not lignified as they do not react with Phloroglucinol-HCl.

The interior epidermal walls were further stained with Ruthenium Red, which also stained the small collenchyma strands inside. Ruthenium Red did not clearly stain the isotropic layers in the outer cutinized walls, but after treatment with this stain it was possible to detect a faint reddish colour of the walls just inside the interior isotropic layer. In polarized light the cutinized layer and the cuticular flanges show up brightly, as do the epicuticular wax deposits (Fig. 3c). Two isotropic layers occur, a narrow one in connection with or just inside the cuticle and a broader one which demarcates the outer cutinized wall from the wall sections adjacent to the cell lumina. The interior isotropic layer cuts through the cuticular flanges. These flanges are clearly built up congruently on both sides of the middle lamella. With a certain rotation of the stage a black isotropic line divides the flanges in two halves which by using the red I plate show opposite signs.

Concentrated hydrochloric acid removes the wax from the walls. It is also possible to melt the wax and thereby reduce its share in the berefringence considerably. After treatment with strong acid the birefringence is maintained but is much weaker and the sign is altered to that of cellulose.

Cellulose is present in the whole wall system apart from the cuticle. Cellulose is predominant along the tapering exterior parts of the outer epidermal cells. The tapering part continues into a narrow canal or very thin plate which shows up after wax removal, but it is further stained with Phloroglucinol-HCl, Safranin, or even with Ruthenium Red. The thin plate or canal stops just below the interior isotropic layer. At this point numerous delicate strands issue which in this proximal part show the same staining properties as the canal. The delicate strands branch and soon become difficult to detect being almost submicroscopical. In most cases they are impossible to follow further without special optical remedies (e.g. interference contrast equipment). As it appears from Plate I (a and j) the strands may issue almost at right angles to the tapering cell elongations. They normally form a brush, but some observations show that they may continue into the cuticular flanges (Plate I f).

The tapering part is always terminated by the brush of delicate strands. These strands branch and become invisible exactly where the cuticular layer outside the interior isotropic band shows up in polarized light due to contents of wax. The wax generally appears as roundish, shining "clouds", one cloud for each brush (Plate I d);

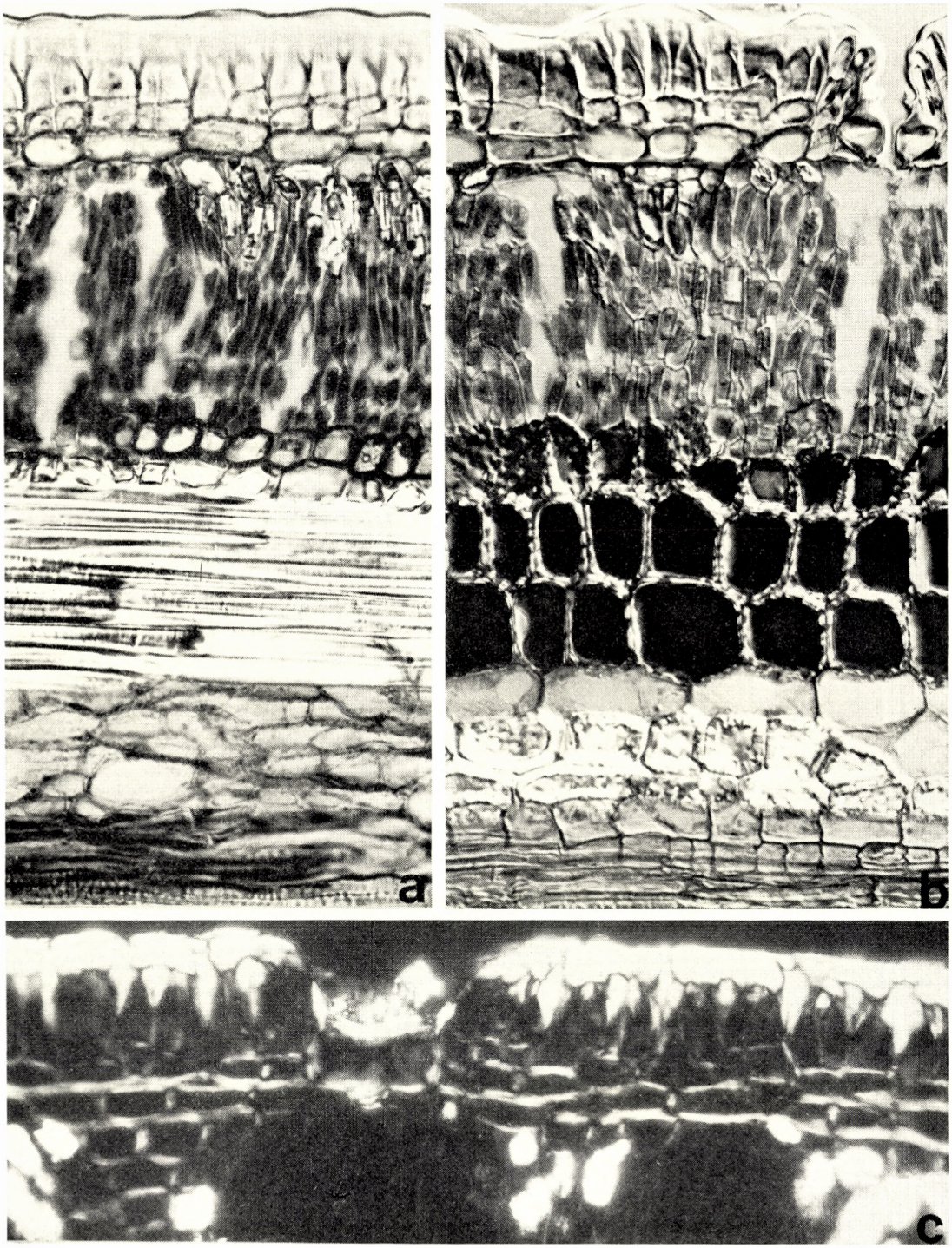

Fig. 3. *Prosopis kuntzei*. a.–b. Longitudinal sections — c. Transverse sections of stem. a. Semipolarized light b. Interference contrast. c. Polarized light. In a. twin crystals in outer palisade layers, crystal layer on abaxial side of fibre strand, phloem, cambium and the latest formed xylem. In b. cross section of stomatal apparatus, on the left initials for another stomatal complex. Inside the palisade layers there are three pericyclic layers with conspicuous pits in the thick walls. Two layers of stone cells, phloem, cambium and outer xylem. In c. there is one guard cell covered with epicuticular wax (bright), and just inside the pore a sphaerite having four bright layers. Epicuticular wax, cuticular wax in the outer walls and the flanges showing up, as do the tangential walls of the inner epidermal layers, the collenchyma (on the left) and the crystals in the palisade layers. × 500.

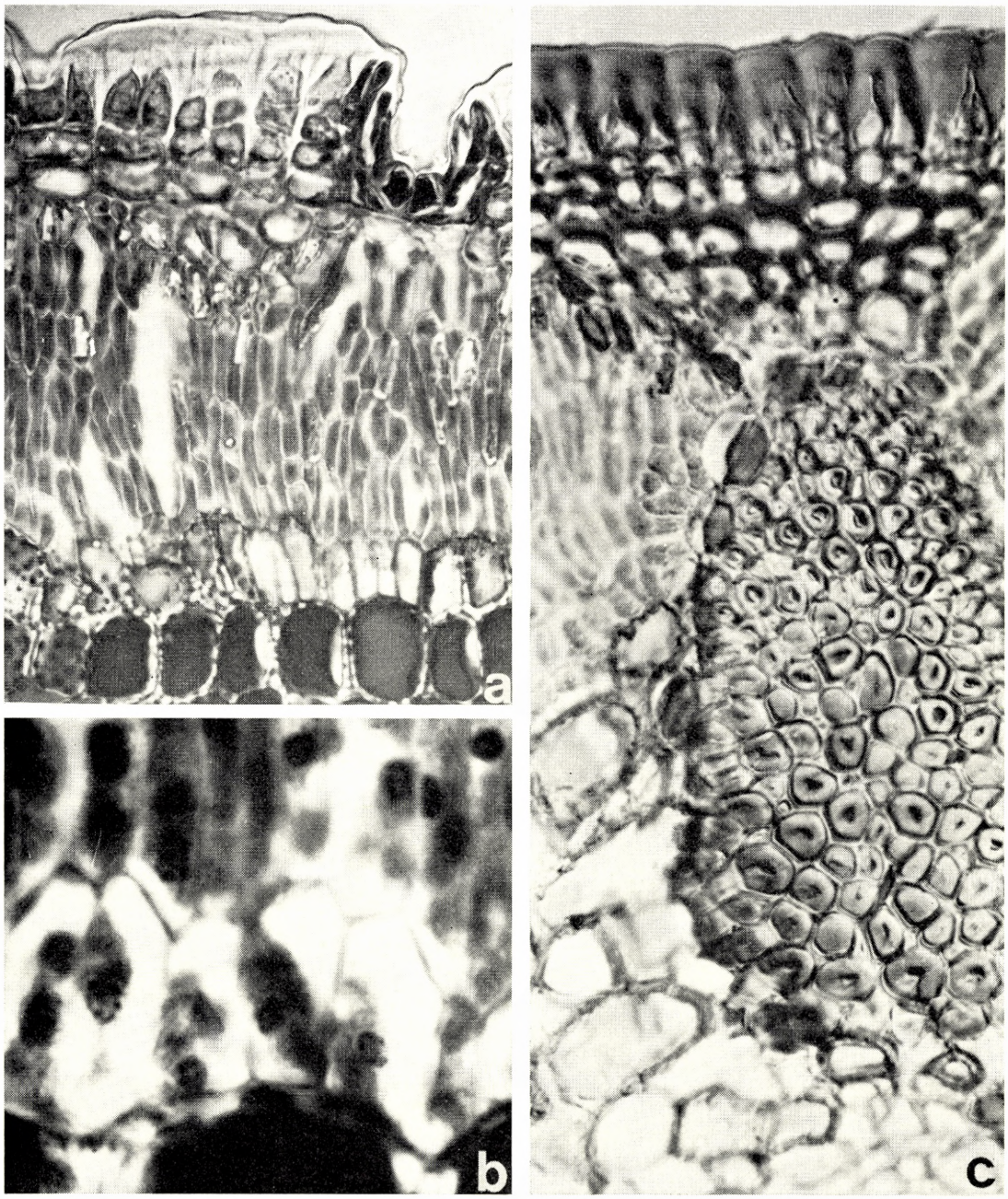

Fig. 4. *Prosopis kuntzei*. a. Longitudinal section of stem showing multiple epidermis with stomatal complex, and on the left a flat cell believed to be a precursor of a stomatal apparatus. A transitional pericambial or endodermoid cell layer on the abaxial side of the pericyclic cells. × 500. – b. The transitional, endodermoid layer of cells which have nuclei with distinct chromocentra. × 2000. – c. Cross section of primary fibre strand connected with collenchyma on abaxial side and surrounded by pericyclic cells. The tapering parts of the cells and the cuticle are distinct in the outer epidermal layer. Interference contrast, Sudan IV staining. ×500.

however, the clouds often merge. The birefringence is of a sign opposite to that of the cellulosic walls of the interior epidermal layers. In certain cases the roundish areas are replaced by crystals, which are arranged in irregular lamellated globules resembling small sphaerites. These bodies show the same type of birefringence as the "clouds", which presumably are composed of submicroscopical wax rodlets.

The microcanals or strands end presumably where the clouds are formed, but in some cases canals could be traced again on the abaxial side of the wax inclusions and seemed to continue towards the cuticle and the epicuticular wax plates (Plate I d). Other observations indicate microcanals occurring in or near the middle lamella in the anticlinal walls and running towards the surface where they branch into a brush and even seem to penetrate the cuticle (Plate I h). The most convincing pictures were obtained in slides mounted in glycerine jelly and not in Canada balsam, using interference contrast or polarized light.

The impression was that there may be two systems of microcanals, one branching near the level of the isotropic layer and perhaps giving rise to the cuticular wax inclusions (Plate I a–e), and another which branches near the cuticle and may be responsible for the epicuticular wax deposits (Plate I g–h). The majority of the microcanals in the latter system is possibly developed in or near the anticlinal walls. In younger stems wax seems to be exuded mainly outside such walls (Fig. 7 d), and in paradermal sections rows of minute bodies, which show up in polarized light, are formed in the anticlinal walls. These bodies presumably represent wax which is crystallized in the most external parts of the microcanals. In some of the transverse sections wax rodlets with opposite orientation were deposited in the outer part of the middle lamella area in the cuticular flanges.

A very striking picture is repeatedly obtained when slides mounted in Canada balsam are heated resulting in the melting of the wax. All epicuticular deposits including the wax occlusions of the stomata disappear but seem to recrystallize as minute rodlets in the balsam outside the surface. The subcuticular wax inclusions, however, were pushed out as long invisible strands, which after cooling very soon became stiff and showed up. The strands have exactly the dimensions of the microcanals observed just inside the cuticle. They are usually bent and form a network in the mounting medium. Some of them can be followed into the small pores in the outermost subcuticular part of the cutinized cell wall. The pushing out of the double refractive, unbranched strands after a short heating of the slides strongly indicates that the microcanals, as well as the tapering parts of the epidermal cells, function as pathways for wax precursors, cp. Plate II a, b.

Submicroscopical canals as the means by which wax reaches the surface of leaves were already suggested by HALL (1967). Recently WETTSTEIN-KNOWLES (1974) discusses wax extrusion through pores onto the cuticle surface in grass leaves. She assumes that the pores have 6–10 nm cores and that such pores arranged in a circle might produce a wax tube 100 nm in outer diameter. The above-mentioned birefringent strands in *Prosopis* and the microcanals (Plate I c, h, j, k) are about 0.2–0.5 μm

in diameter (200–500 nm), are thus much coarser, but almost of the same dimensions as wax rods from leaf surfaces in *Tulipa* mentioned by Clowes & Juniper (1968, Fig. 78).

The exudation spots appear very distinctly after heating of slides mounted in glycerine jelly. By strong heating all wax disappears, but after a short heating it recrystallizes on the surface of the cuticle, sometimes as small globules or sphaerites (Plate II g–h).

Another argument for this assumption about the function of the canals is that tapering cells and brushes of delicate strands occur abundantly in *P. kuntzei* and *P. sericantha*, both having an impressive wax pattern, whereas *Prosopidastrum globosum* with flat outer epidermal cells has a smooth surface, apart from small flakes due to peeling (Böcher & Lyshede, 1972, Plate XIV).

The cuticle is a very thin layer resting upon the exterior isotropic band. Generally the cuticle itself is also isotropic, but in certain areas it shows up due to contents of densely spaced minute anisotropic bodies. With Phloroglucinol-HCl the adaxial side of the cuticle stains red, probably because it contains phenolic compounds, and may thus perhaps serve as an UV-filter.

C. Epidermis; stomatal complexes

The stomatal complexes in *Prosopis kuntzei* are interesting and may in many ways be unique. They are initiated as seven-eight layered structures, but during their development the exterior three-five cell layers degenerate, disappear or are transformed, while the layer of larger cells situated inside develops into guard cells. The interior layer becomes rudimentary, or is maintained as small ledge-like protrusions on the transition to the substomatal chamber, which is formed in the abaxial part of the cortex palisade tissue.

The young stomatal complexes are clearly demarcated from the surrounding epidermis by the deviating structure of the cells. The outer ones are long and flat, but sometimes they divide, or their cell margins narrow, and the cells in the layers wedge in from opposite sides in the margins overlying one another (Fig. 6d). The complex appears as one histological entity, a rounded area inserted in the multiple epidermis and surrounded by anticlinally elongated, often tapering cells (Fig. 6).

In cross sections of the stems the complexes and the guard cells are cut longitudinally. Young complexes appear as pot-shaped structures, and the outer flat cells almost reach the surface af the tapering cells outside the complex (Fig. 6 a, b). In longitudinal sections they are likewise well demarcated and roundish, but soon get a deep median depression, which may be due to early dying away or withdrawal of exterior cells (Fig. 5 and cf. Fig. 6 a, b, g).

The stomatal complexes develop into a cell group which in the central, interior part contains the guard cells, while the marginal parts bordering the normal epidermis are composed of 2–3 layers and rows of subsidiary cells.

Usually the median depression contains no obvious remains of cells but often

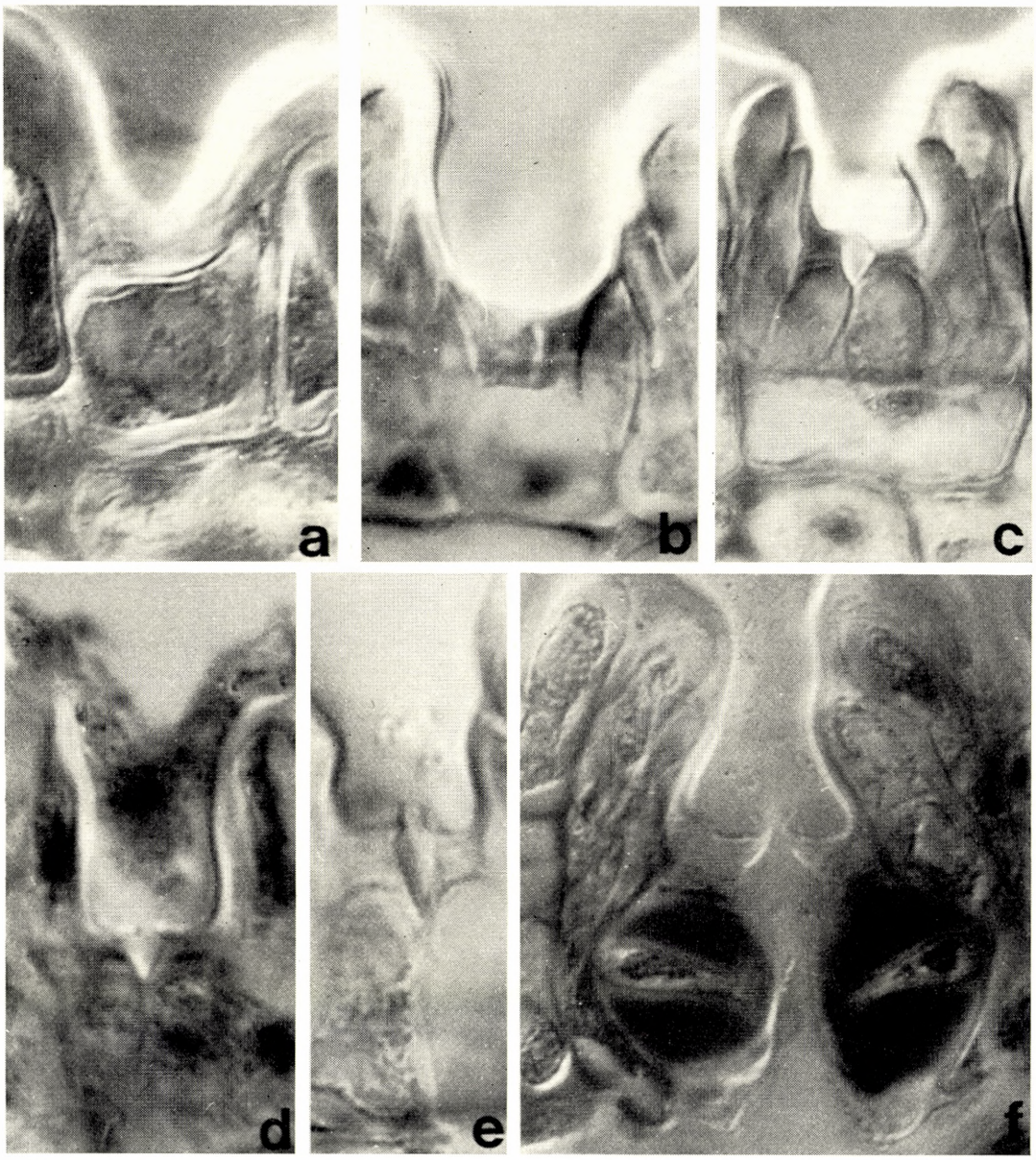

Fig. 5. Stages in the development of the stomatal complex in *Prosopis kuntzei*. Cross sections of pore. a. Formation of cavity above flat initial cell surrounded by young subsidiary cells. b. Two ledge-forming exterior cells being separated. c. Two ledge-forming small cells on top of young guard cells. d. Stomatal cavity deeper, perhaps filled with remains of decomposed exterior cells, darkly stained remains of nuclei in ledge-forming small cells. e. Young stomatal complex showing outwards bending of ledges. f. Mature stomatal complex. × 2000.

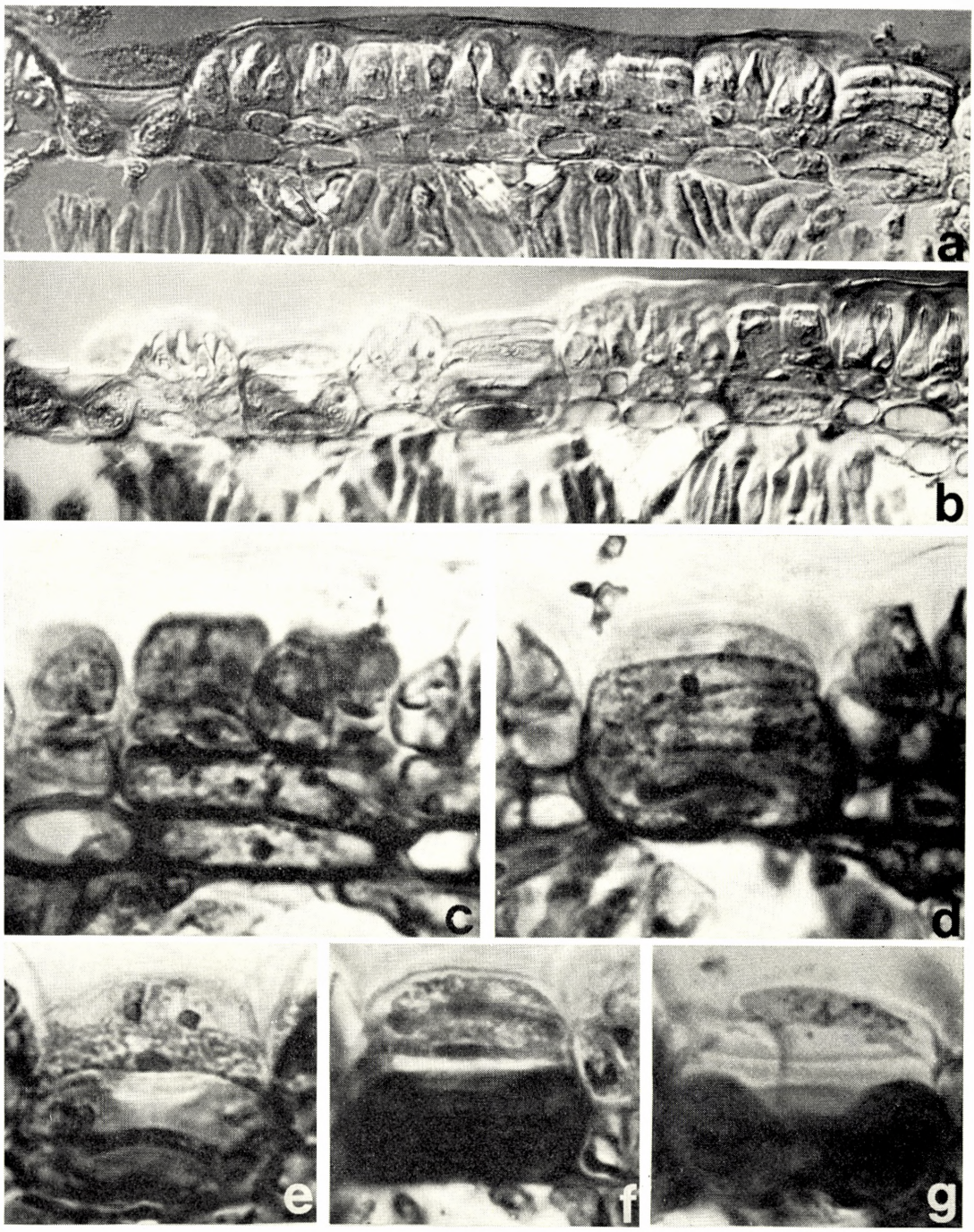

Fig. 6. Stages in the development of the stomatal complex in *Prosopis kuntzei*, longitudinal views of guard cells. a.–b. Sections of epidermis and outer cortex palisade with initial stages on the right and final stages of stomatal development on the left. Safranin-fast green, interference contrast × 500. — c.–g. Series of stages. c. initial,. g. final. In d. the young stomatal complex appears as a separate unit, in e. and f. the pseudo-ledge is distinct. × 1000.

a kind of detritus, which may be decomposed cell material and is stained by various dyes (Fig. 5 d). Sometimes it seems to be utilized by hyphae of imperfect fungi. In a few cases, however, remains of decomposed or lost outer cells occurred in the form of peculiar inwards-bending wall extensions, which together with outwards-turning, ledge-like wall remains (the outer pseudo-ledges) pretended to circumscribe a cell (Plate IV f).

The bottom of the depression is first occupied by a flat, large cell flanked by a number of narrow cells. The latter are assumed to be initials of subsidiary cell rows. The flat cell is probably the guard cell precursor. It is usually covered by a small cell which as a rule divides first. The guard cells develop after the division of the precursor. They grow perpendicularly to the surface and reach the level of the interior epidermal layer. Almost at the same time the underlying cell divides and produces a space which makes the expansion of the guard cells possible (Fig. 5).

The small outer cells are transformed into short braces for the outer pseudo-ledges, which are the walls between the small cells and decomposed or lost cells above. The braces line the flanking subsidiary cells and seem mainly to consist of wall material. In many cases, however, they contain remains of cytoplasm and even sometimes of nuclear material, which is stained by Safranin (Fig. 5 d).

Although the origin of the outer pseudo-ledges may be tangential, "separating" cell walls, they are covered by a thin cuticle similar to that in normal epidermal cells. However, on the surface of the pseudo-ledges there are rod-like wax figures, which are often arranged parallel to the surface but across the elliptic openings between the pseudo-ledges. Other wax bodies are produced along the margins. Wax is further produced in the thick walls towards the guard cells below, where it appears to be deposited in lamellae parallel to the surface (Plate IV a). The production of wax continues and finally the stomatal cavities formed between the overarching subsidiary cells are filled with more or less alveolar waxy plugs (Fig. 10 b). In old stems the surface wax develops an almost continuous covering, which may coalesce with or eventually even partly cover the stomatal wax plugs (Plate VII b).

The production of wax is particularly great outside the subsidiary cell rows and along the margins of the pseudo-ledges. The exterior cells do not taper as the normal epidermal cells, but they carry, scattered on their outer surfaces, several short delicate strands, which may serve as pathways for wax precursors.

A wax production by the subsidiary cells in *Prosopis kuntzei* is supported by the fact that the subsidiary cell rows have direct connection to palisade cells. As the wax precursors probably are produced by the photosynthetic cells, a pathway for wax from the palisade cells to the subsidiary cell rows appears to be a likely possibility. The nuclei in the subsidiary cells are large and often placed where wax exudation is assumed to take place. In this connection it is of importance to point out that delicate strands, perhaps plasmodesmata, traverse the wall near the corner from where the pseudo-ledges issue (Plate IV g), and that the wax bodies, which are arranged regularly on the surface of the pseudo-ledges, are formed just outside these strands. This means

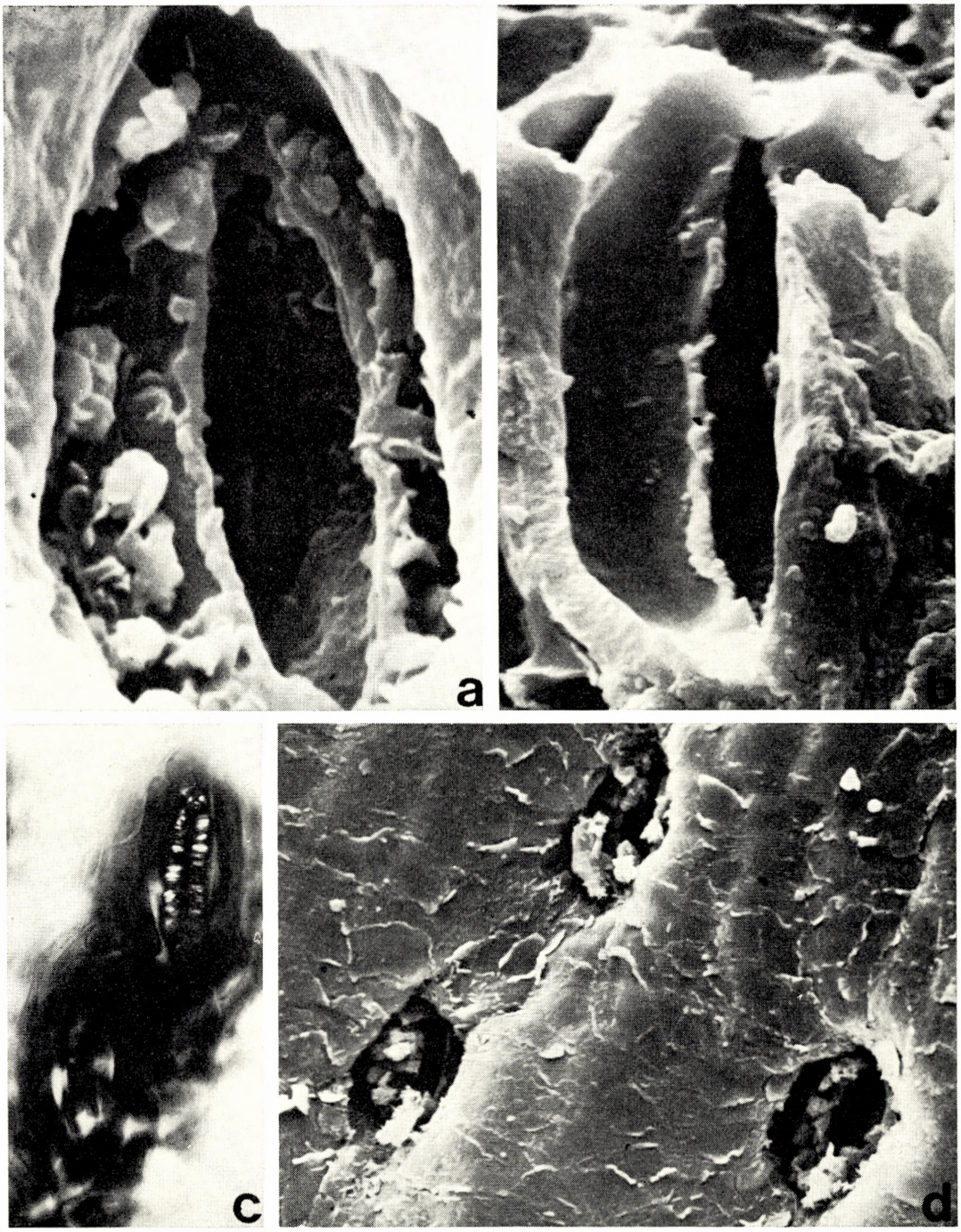

Fig. 7. Stomatal complex in *Prosopis kuntzei* a, b SEM micrographs (× 2800). a. Outwards-bending pseudo-ledges with many wax bodies surrounding stomatal antechamber and stomatal pore. — b. Left-hand part of stomatal cavity severed, the outer parchment-like pseudo-ledge being laid bare. — c. Two stomata belonging to the same cavity, the upper one with wax crystals arranged on both sides of elliptic opening between the pseudo-ledges (polarized light × 500). — d. Surface with three stomatal cavities partly filled with wax bodies. Further, wax appears to be exuded outside anticlinal epidermal walls. Part of the epicuticular wax cover was removed beforehand as a result of alcohol treatment. SEM micrograph × 700.

that the filling up of alveolar wax in the stomatal cavities may also be due to the activity of the subsidiary cells, and that the guard cells, in spite of their being covered by wax, do not temselves produce wax.

The structure of the stomatal apparatus in *P. kuntzei* is clearly connected with the occurrence of a multiple epidermis. The closely related *P. sericantha* has neither multiple epidermis nor anything like the outer pseudo-ledges. On the other hand, the guard cells are here provided with genuine outer ledges (see Böcher & Lyshede 1972 Fig. 41 and Plate XVI b; compare further Esau 1953 Fig. 7,2).

The connection between a multiseriate epidermis and the pseudo-ledges is strongly supported by the stomatal structure in *Prosopidastrum globosum*. In this species, which was also mentioned in the previous paper, there are likewise pseudo-ledges and a multi-layered epidermis. The pseudo-ledges here form a thin diaphragma with circular openings leading down to the antechambers. The cells giving rise to the pseudo-ledges are small and flat, and from the surrounding cells they may receive substances of importance for the formation of the diaphragmata, which especially in young stages tend to keep the stomatal apertures closed, cp. further p. 35.

The small cells which cover the guard cells and take part in the formation of the pseudo-ledges are interesting for their imitation of the guard cells. Similar guard cell imitators, placed inside outer and inner ledges, were discussed in the previous paper (Böcher & Lyshede 1972 pp. 32–33, 93–96) in *Diostea juncea* and *Discaria articulata*, which also seem to have pseudo-ledges. Lengthwise views of the stomatal complexes in *Prosopis kuntzei* show that the small outer cell has a dumb-bell shape similar to the larger guard cell below; even the nucleus seems to be stretched out occurring as a narrow strand between the swollen parts. At a later stage the small cell degenerates and in longitudinal views its remains may be found as two small. more or less triangular or wedge-shaped bodies outside the swollen ends of the guard cells. The pseudo-ledges, however, are clearly walls between the small cells and two overlying, early disappearing cells, which undoubtedly are more responsible than the small cells for the formation of the separating walls that develops into the pseudo-ledges (cp. Fig. 6e, f, and Plate V a).

At an early stage an intercellular space seems to arise regularly between the pseudo-ledge and the later degenerating exterior cell (or cells). Sometimes wax is exuded in the space. Assuming that the pseudo-ledge is the remains of the interior thick wall of the overlying disappearing cell (or cells), two smaller and thinner ledges might be formed as remains of walls bordering intercellular spaces on both sides of the small cells on the abaxial side of the guard cells. Such thin walls would protrude from the margin of the antechamber as set pieces or wings in a theatre (cp. Plate VII a, and dark lines in Fig. 5f).

In some cases the stomatal complexes develop in a manner similar to that described in *Prosopidastrum globosum*. The pseudo-ledges keep merged over an extended area producing a diaphragma through which a roundish or elliptic hole leads to the antechamber. Plate VIII d shows a normal stomatal cavity where the pseudo-

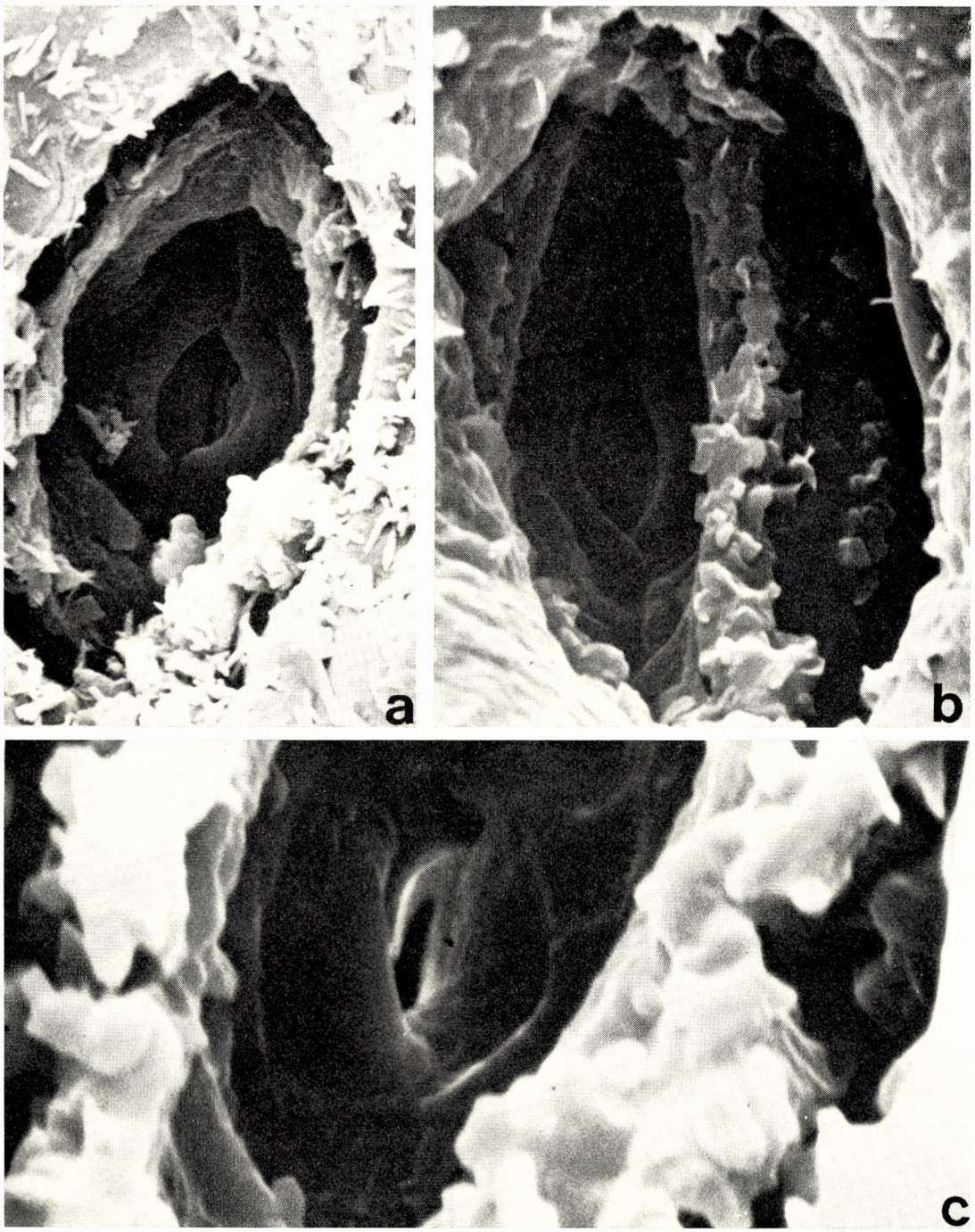

Fig. 8. SEM micrographs of stomatal cavities in *Prosopis kuntzei*. a.–b. Entire cavities. In a. the interior, narrowest part of the pore is visible, in b. the cavity is apparently divided by the outwards bent margin of the pseudo-ledge, the margin being densely covered with wax configurations (× 2800). — c. Central pore with interior narrow opening to rear cavity surrounded by the two pseudo-ledge margins and on the left and right side of the two cavities between the ledge margins and the lining subsidiary cells. (× 6000).

ledges have outwards-bending margins and surround an elliptic opening. Other SEM micrographs make it evident that the margins can coalesce over an extended area leaving one (or two) openings into the antechamber (Plate VIII a–c, e–f).

It is puzzling that the apertures between the guard cells are fairly often displaced in relation to the elliptic opening to the antechamber (Fig. 7 a, Plate VIII e). This could be a result of the dilatation growth. Evidently, an increase in cell number and size also takes place in the interior epidermal cell layers adjacent to the guard cells.

Most stomatal cavities contain one pair of guard cells only. The often deep cavities are surrounded by elongate subsidiary cells. The interior row of subsidiary cells usually consists of two cells at the long sides of the cavity and one (rarely two) cell at the ends, in all about six or seven cells (Plate V c).

In a number of cases the same cavity may contain two stomata, while five or eight may be exceptional. In Fig. 9 a are cavities with two or five stomata arranged almost end to end but probably not entirely contiguous as the guard cell pairs are separated by low cell ridges composed of two or four cells (Fig. 9 b). The arrangement in rows supports the idea that stomata in the same cavity belong together ontogenetically. The cavities shown in Fig. 9 a are framed by epicuticular wax crystals which are particularly densely spaced above the surrounding subsidiary cells.

In the thickest branch (Fig. 2) paradermal sections unveiled the occurrence of long cell rows (of up to 16 cells) which were evidently formed in continuation of the cells at the ends of the guard cells. The cells in the rows had a shape and size similar to that of the terminal subsidiary cells and were orientated parallel to them. They were clearly comparable to the cells in the short separating cell ridges shown in Fig. 9 b, being inserted between two stomatal apertures of which one seemed to be older (longer and more dilated) than the other. The stomata were usually arranged end to end in the same groove, which again was orientated across the axis of the thorn. But in rare cases two stomata were placed parallel in the same groove indicating that initiation of new stomata may occasionally be brought about by transformation of some of the sidelong arranged subsidiary cells. Obliquely forward orientation of two stomata in the same row was also observed (Fig. 7 c).

In this connection the SEM picture, Plate VIII e, is of particular interest. Here the cavity has, at the bottom, two small ± elliptic openings in a diaphragma. Below, it is possible to see two elliptic stomatal apertures, the one on the right being somewhat displaced. In the middle is an extended area, perhaps a part of the diaphragma formed later, which appears to join the diaphragmata outside the two densely spaced apertures. A major question concerns the formation of new stomatal cavities. Maybe the process is accompanied by a cracking of the cuticular layer and a production of new cuticular material that gradually smoothens the cavities (cf. Plate VIII b).

The increased number of stomata is a natural consequense of the increase in girth and of the dilatation growth of the extra-cambial cell layers. However, there is no clear connection between the photosynthetic activity and the increase in stomata because in older and thicker stems so many of them become blocked with alveolar wax plugs.

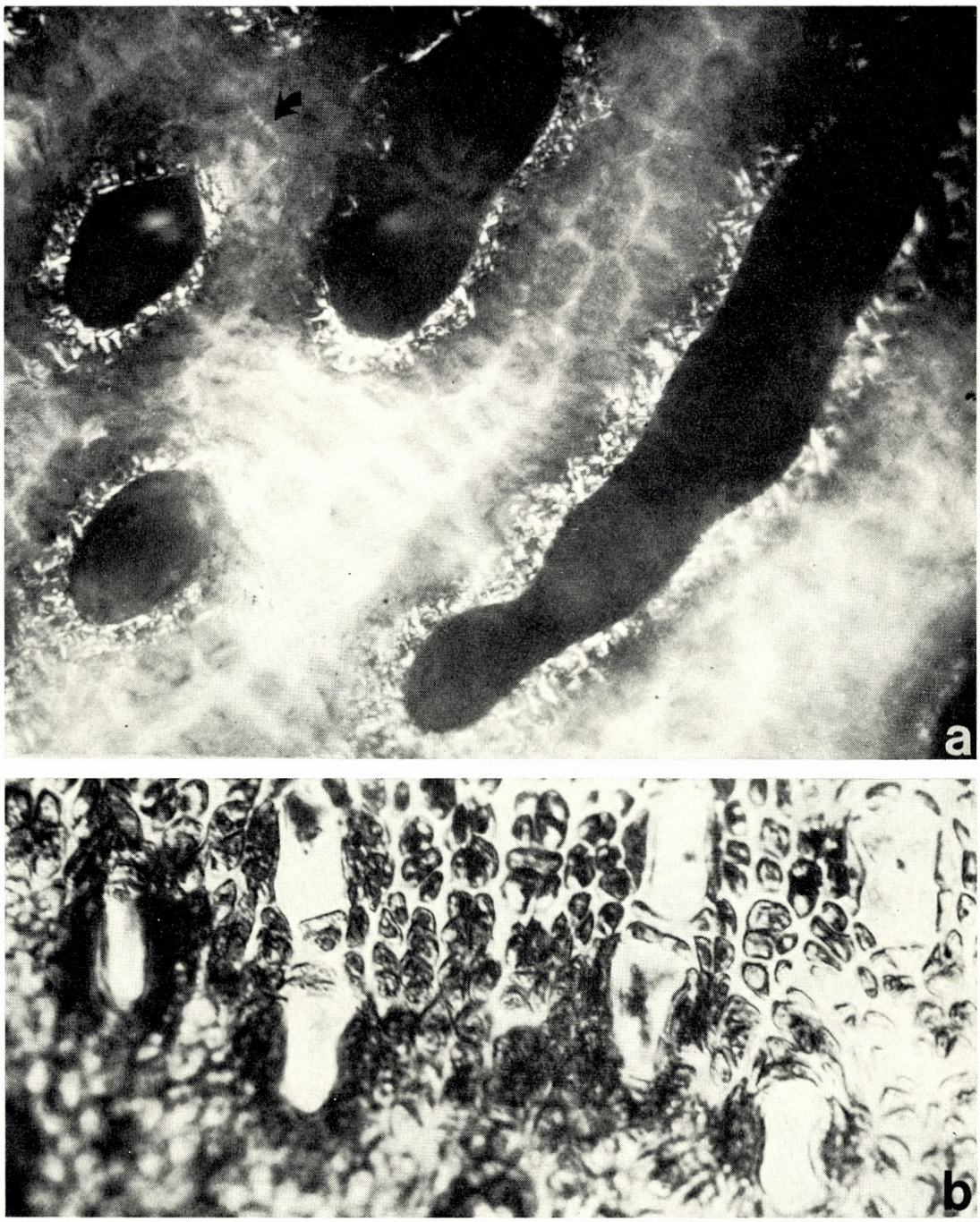

Fig. 9. a. Surface of older branch of *Prosopis kuntzei* as seen in polarized light. The stomatal cavities are framed by epicuticular wax deposits. The two cavities on the left have one stomatal opening each, the middle one has two openings, while the long cavity on the right has five in a row. Many anticlinal walls appear light and some of them (see arrow) contain small, bright bodies, probably due to wax. — b. Epidermis with several stomatal cavities, two cavities with two stomata and separating cell ridges. Epidermal cell families conspicuous. × 500.

The incipient stage in the plugging is the above-mentioned formation of wax rods on the surface of the outer pseudo-ledges (Plate IV a, c). This stage is followed by a filling up of the stomatal cavity by irregular wax crystals or rectangular wax blocks developing at right angles to the surface (Plate IV b). The final stage is a complete filling by loosely packed wax crystals which do not block the pores completely but probably restrict the gas exchange considerably (Fig. 10 b, Plate VI c, d, VII b, c). The formation of wax varies in intensity. Young stomatal cavities may be without wax (Plate VIII), in other cases an early filling is obvious (Plate VI b). The differences may be due to environmental or racial divergencies.

Another kind of plugging occurs in connection with the small interior pseudo-ledges. As appears from Plate III a and IV d these are independant short cells and develop below the guard cells and the interior layer of subsidiary cells. The interior pseudo-ledges are more intensely stained, e.g. by Safranin, and sometimes they contribute to a narrowing of the adaxial interior part of the stomatal aperture (Plate III b).

At a short distance from the interior mouth of the central pore the two interior pseudo-ledges merge. In many cases the wedge-formed rear cavity, which as already mentioned is more or less narrowed by the interior ledges, is filled with a sphaerite, probably composed of concentrically arranged calcium oxalate crystals. The sphaerite formation starts from the narrowest part of the pore and continues its growth into the rear cavity and further on to the substomatal air chamber (Plate IV h, V f). Finally the sphaerite is pressed against the outer parts of some of the palisade walls, which swell slightly here (Plate III c), or it penetrates into the outer intercellular spaces in the palisade tissue. In the younger stems sphaerites are few and small, but in older stems thay are numerous and sometimes even present inside most stomatal openings.

Similar sphaerites were observed by Böcher & Lyshede (1972: 76) in *Prosopidastrum globosum*, where they were found to issue from cell walls in different kinds of tissues. In *Prosopis kuntzei* they are also initiated at a cell wall but always develop into intercellular spaces and almost always in close connection with the stomatal pore. They are built up of birefringent, optically negative crystals arranged in concentric layers with intervals consisting of isotropic material. The bright layers occur as irregular zig-zag bands. Sometimes, however, the isotropic layers appear as chains of small bright bodies, each surrounded by a dark, not double-refractive, circle. The isotropic layers might be connected with radiating isotropic lines. Frequently the centres of the sphaerites are the rear cavities; in a few cases the centres are situated in the cell walls of the palisade cells next to the substomatal chamber. 7–10 bright layers are common in the sphaerites.

The site of the sphaerites strongly suggests a connection with the stomatal activity. It is natural to assume that calcium and bicarbonate ions are transported to the stomatal apertures by the transpiration flux. Here Ca ions are combined with oxalic acid to form calcium oxalate crystals while carbon dioxide is liberated and probably assimilated by the green cells. Calcium crystals are further produced in the green cells in great numbers and especially in the outer palisade layers in intervals between

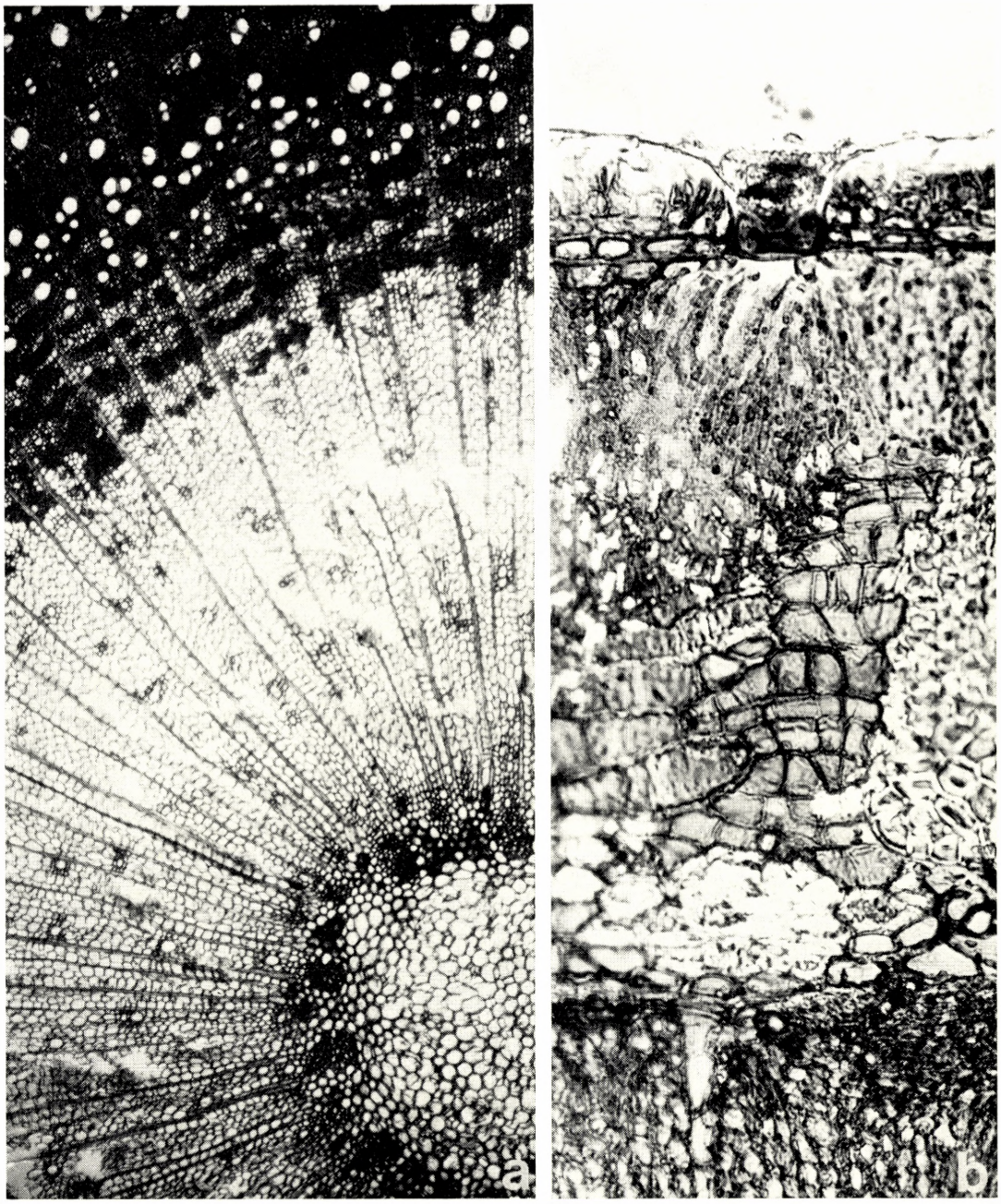

Fig. 10. a. Older branch of *Prosopis kuntzei* (Argentinian material, cf. Fig. 2). Cross section showing pith, protoxylem (dark), early wood with scattered solitary vessels surrounded by parenchyma. The later formed wood with bands of fibres alternating with parenchyma bands. The latest formed wood contains many solitary vessels or groups of two vessels surrounded by parenchyma. × 50. — b. Cross section of outer part of older branch (Bolivian material), semipolarized light. The epidermis contains one stomatal cavity filled with alveolar wax. Many crystals in the palisade layers. Between fibre strand (on the right) and pericyclic cells inserted area of young pericambial cells making an increase in girth possible. Inside these, groups of stone cells and phloem ray. × 320.

the stomatal apertures (Fig. 3). Old branches contain more crystals distributed in all palisade layers (Fig. 10b).

The sphaerites undoubtedly slow down the water loss through the stomata. At the same time they may act as very modest CO_2-suppliers. They disappear after treatment with hydrochloric acid, as do the intercellular calcium oxalate crystals.

D. Periderm

Periderm formation takes place by transformation of interior epidermal cell layers into phellogen cells. The outer tapering cells and the cells below get suberized walls while two-three phelloderm layers may be formed adaxially. In the younger parts periderm formation is limited and localized, often occurring near primary fibre strands (see Fig. 2) or near the thorn points. Sometimes stomatal complexes are transformed. The first sign is a subdivision of the outer cells that cover the guard cells (Plate V b). The next stage is a suberization of the cell walls. Finally the whole complex develops into a small cork which often becomes part of a wider peridermal spot.

E. Cortex

The cortex is almost exclusively a multi-layered palisade tissue only interrupted by collenchyma, which fills out the areas between the epidermis and the primary fibre strands. If the layer of crystal cells, which usually surrounds the fibre strands abaxially, can be homologized with an endodermis, the crystal layer will also be cortical. However, the problem of endodermoid tissues in *Prosopis kuntzei* is intricate and so far it remains probably unsolved. In *P. sericantha* a similar crystal-cell layer is present and was considered to represent an endodermis by Böcher & Lyshede (1972: 77 and Plate XV d). While the crystals in the endodermoid layer in both species are usually simple and tetragonal, those in the palisade tissue are almost exclusively solitary twin crystals.

The intercellular air spaces in the palisade tissue deserve comment. In paradermal sections they appear to be as numerous as the stomatal openings (Plate VI a), but in cross sections it is obvious that they are more or less displaced in relation to the stomatal apertures. However, there is clearly a connection between the small substomatal chambers and the larger air spaces, which frequently in cross sections appear as narrowly elliptical or spindle-shaped cavities penetrating almost all the green palisade layers (Fig. 3–4). The displacement of the air spaces may have the effect that the water vapour contained in the spaces does not escape so easily when the stomata open. On the other hand, the air spaces will also accumulate CO_2 produced by e.g. all the non-photosynthetic cells in the central part of the stem.

The water supply of the green cells raises some questions, as does the transfer of assimilated carbohydrates to the phloem. A satisfatory explanation of the pathways for water and food-conduction between the vascular system and the photosynthetic tissue may depend on a full understanding of the function of the pericyclic cell layers.

F. Pericycle

Members of the *Mimosaceae* frequently have a pericycle containing a continuous, more or less composite ring of sclerenchyma. According to METCALFE & CHALK (1957) the pericyclic fibres are often mucilaginous or unlignified. *Prosopis kuntze i* differs from *P. seriantha* by possessing a very distinct layer of cells which deserves the designation of pericycle. It occurs just inside the above-mentioned endodermoid layer of crystal cells which adjoin the perivascular fibre strands on their abaxial side. However, a continuation of the crystal cell layer between the fibre strands was doubtful. In any case such crystal cells were very scattered and never occurred in a separate cell layer between the strands. The fibre strands, on the other hand, were regularly connected by a multi-layered tissue of parenchymatous, mostly thick-walled cells with many conspicuous simple pits and dark contents. This layer was interpreted as a pericycle. It clearly corresponded to layer No. 7 in *Cassia aphylla* and layer b in *Discaria articulata* (cf. BÖCHER & LYSHEDE, 1972, pp. 15 and 34). The primary fibre strands in *P. kuntzei* almost reach the surface of the stem, being only separated from the epidermis by collenchymatous cells and the layer of crystal cells (Fig. 4c). These primary strands may border the pericyclic thick-walled tissue in the adaxial part, while smaller secondary strands are as a rule almost totally embedded in this tissue (Fig. 11a).

The pericyclic tissue layer is not uniform and the dominating cell type is not always so thick-walled as in the material from Bolivia; thus the thick branch from Prov. Chaco in Argentina (Fig. 2) has tangentially more elongate and not particularly thick-walled cells. However, these cells had been exposed to some stretching due to dilatation. They often occurred in groups originating from one ± tapering, tangentially stretched cell subdivided into a short row of cells.

In the Bolivian material the youngest parts are situated near the sharp points of the thorns. Here the pericyclic layers are fewer and the cell walls rather thin. At some distance from the point the cells have thick unlignified walls with canal-like pits and numerous, conspicuous and densely spaced plasmodesmata through the pit-fields (Plate IV i). The primary walls are stained by Safranin but do not react with Phloroglucinol-HCl.

At this stage the thick-walled cells border or cover the fibre strands and there are few signs of cell divisions. A number of the pericyclic cells have transverse thin walls. In older and thicker branches a thin-walled parenchymatous tissue is commonly found inserted between the fibre strands and the thick-walled tissue (Fig. 10b). These insertions bring about a dilatation growth. The thin-walled cells have meristematic character and may be termed pericambial (cf. GUTTENBERG, 1943). The meristematic nature appears also from the fact that the thin-walled insertions in some cases are transformed into phellogens which may surround the fibre strands and make them parts of a very local rhytidome.

In spite of becoming thick-walled, the pericyclic cells maintain their nuclei and thus stay alive. Sometimes they even have two nuclei. The cells which were fixed in alcohol are usually highly plasmolyzed but the dark protoplasma often sticks to the

wall, probably because the pit-fields with their plasmodesmata resist the withdrawal from the wall. With iodine the plasma-filled pits turn dark brownish.

Usually the pericycle consists of three to five cell layers. The cells are shorter in cross sections than in longitudinal sections. The pericycle adapts to the increase in girth by the insertions at the fibre strands, but it may also produce an additional abaxial layer. In this layer the cells appear to be derived from the bordering green cortex cells, which here increase their width and sometimes divide. The nuclei often have particularly distinct chromocentres and two adjoining cells may have the same shape and size (Fig. 4b).

A cell layer situated between the green cortex palisade and the pericyclic thick-walled cells may, however, fulfil a special physiological task. It is situated where an endodermis would be expected (Fig. 4a) and it is always single-layered. Mostly the cells adjoin several rows of palisade cells. Hence the cells in the layer may act as assembling units or as a kind of transfer cells, being modified endodermis cells. In *P. sericantha* the endodermoid layer consists of large, thin-walled, chloroplast-containing cells each bordering on one to three radial palisade cell rows. Against the assumption that these cells function as assembling or transfer units speaks the fact that locally the layer is not developed in *P. kuntzei*. Sometimes it contains few thin-walled cells, inserted between the thick-walled, and thus perhaps cells comparable to passage cells.

On the adaxial side of the dark, thick-walled pericyclic cells some of the parenchymatous cells may gradually attain a similar stucture and get thick walls or dark contents. However, in this part of the stem it is more common that cells in one or two layers sclerify and become lignified stone cells (Fig. 3b).

A well developed pericycle is more common in roots. Porsch (according to Guttenberg 1943 Fig. 162) pictures a pericycle of thick-walled, densely-pitted cells adjoining the endodermis in roots of *Philodendron*. This tissue resembles the tissue discussed in the *Prosopis* thorns. The best example of a pericycle in a stem is the well-known layer of fibres in *Aristolochia sipho*, which, being a woody liana, in many ways has a deviating stem structure.

As already mentioned, *P. kuntzei* differs significantly from *P. sericantha* by the cell type and the extent of the pericycle. Both species belong to the Chaco-formation and are presumably ecologically closely related. It is difficult, perhaps impossible, but most tempting to find an explanation of the difference between the two species. The most obvious point is connected with the fact that *P. kuntzei* is a tree whereas *P. sericantha* is a shrub. The water economy, and with it the water supply of the green cortex cells, may be the crucial factor. *P. kuntzei* seems to be more xeromorphic than *P. sericantha*. It has deeper sunken stomata and several structures effecting a narrowing of the stomatal pore or a stomatal occlusion. The epidermis has one or two more cell layers and all paradermal walls in the epidermis are cutinized. The palisade tissue is denser and the substomatal air chambers are slightly dislocated.

Still, the physiological role of the pericycle is obscure. The mechanical properties

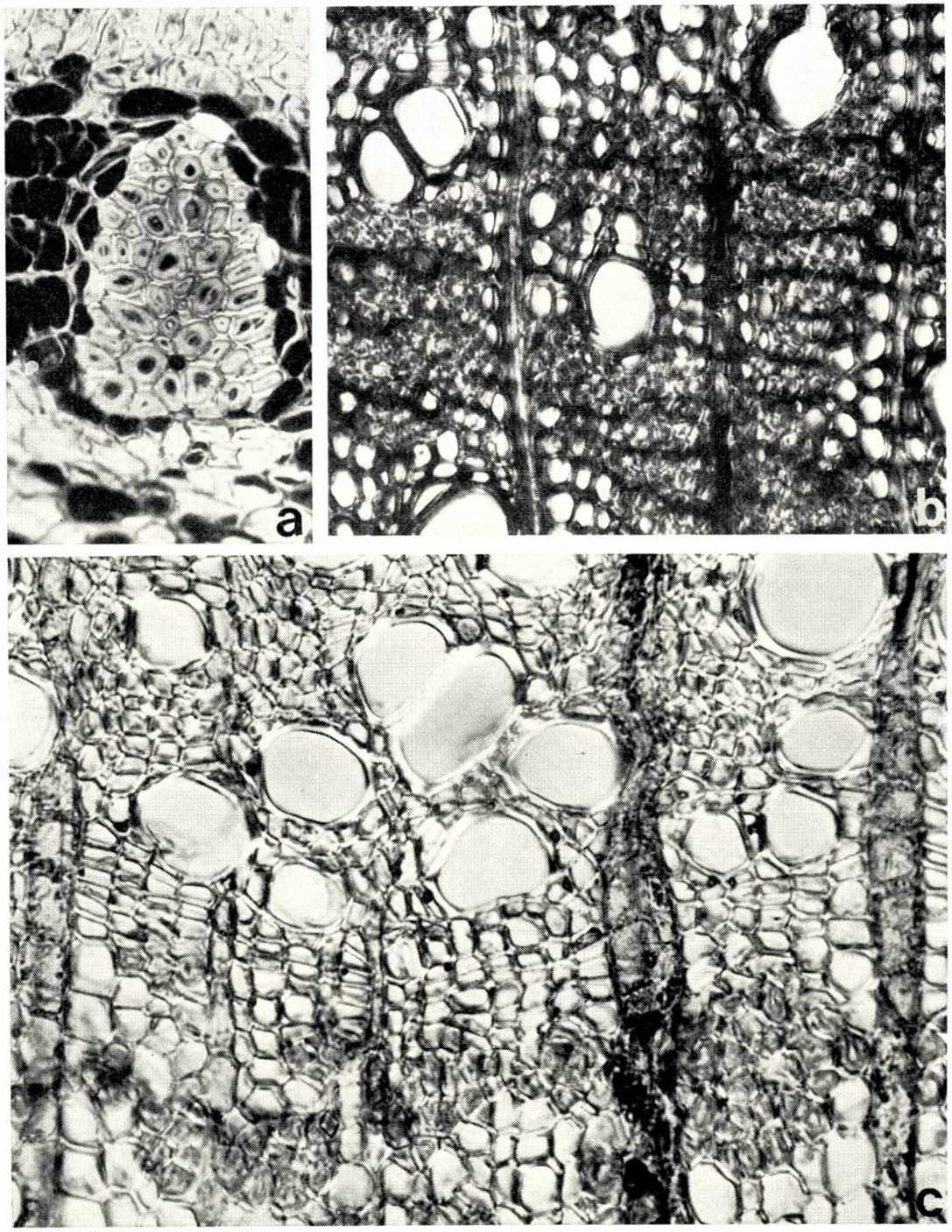

Fig. 11. a. Cross section of young stem; fibre strand surrounded by pericyclic cells (dark), quadruple staining, semipolarized light. — b. Cross section of later formed wood showing vessels and paratracheal parenchyma, also parenchyma rows accompanying the rays. — c. Cross section of later wood in semipolarized light. On the right, two rays, the biseriate one has some cells with dark contents, the uniseriate one is accompanied by axial parenchyma which is interrupted by few fibre cells in connection with a fibre band placed between two parenchyma bands. (× 500).

of the pericycle may be most important. The pericyclic cells together with the fibre strands, acting as buttresses, produce a very strong supporting system, which further is firmly connected with the multiple epidermis by collenchyma (Fig. 4c). In *P. sericantha* collenchyma is absent and the many fibre strands are not connected by a continuous and strong pericyclic cylinder.

However, the many canal-like pits and plasmodesmata in the pericycle cells make it likely that products of phtosynthesis can pass these cells and reach the phloem. Water may easily pass the other way and also utilize the thick unlignified cell walls as pathways. It is a striking fact that the whole cylinder of photosynthetic tissues has no direct connection to the vascular cylinder, e.g. by small veins as in leaves. The pericyclic cylinder might thus be compared to the so-called transfusion tissue in conifer needles, although this latter tissue is provided with transfusion tracheids.

G. Vascular cylinder

The phloem appears to raise no particular problems. In older branches one finds the primary phloem cells crushed or sclerified, whereas the secondary phloem appears stratified with bands of fibres. The dilatation of the rays is conspicuous and the peripheral ray cells have connection to young parenchyma cells in the pericyclic cylinder, e.g. the insertions accompanying the fibre strands (cf. Fig. 10b).

In young branches the cambium is cylindrical, but in older ones its outline is more or less sinuous. The bulges occur where groups of wide vessels are initiated.

The protoxylem is clearly demarcated by its small and comparatively thick-walled cells which project into the pith making the latter stellate in outline (Fig. 10a).

As already mentioned, the first formed secondary xylem consists mainly or almost exclusively of axial parenchyma, tracheids and ray cells. In the thick branch from the Argentine (Fig. 2), however, the first formed xylem contains scattered vessels, wide and solitary, surrounded by parenchyma (Figl 10a).

The later formed xylem is characterized by irregular, tangential bands of fibres and parenchyma. In the latest stage, the xylem finally contains groups of wide vessels in bands of parenchyma alternating with bands which mainly consist of fibres.

The vessels in the later formed xylem are solitary or grouped in small clusters of two. The axial parenchyma is paratracheal, confluent, the bands being broad (often four or five seriate), see Fig. 12. A characteristic feature is the occurrence of rows of parenchyma along the rays. A physiological importance of this structure may be seen from the fact that the species is without any small veins that would connect the vessels with the photosynthetic tissues.

The rays represent the easiest pathway from the xylem to the cortex. Water may not only be conducted through the vessels and tracheids and further on to the rays through the contact parenchyma. The structure of the early wood indicates that water may be transported through the axial parenchyma and pass into the rays from the surrounding parenchyma. This is inferred from the fact that vessels are not found in

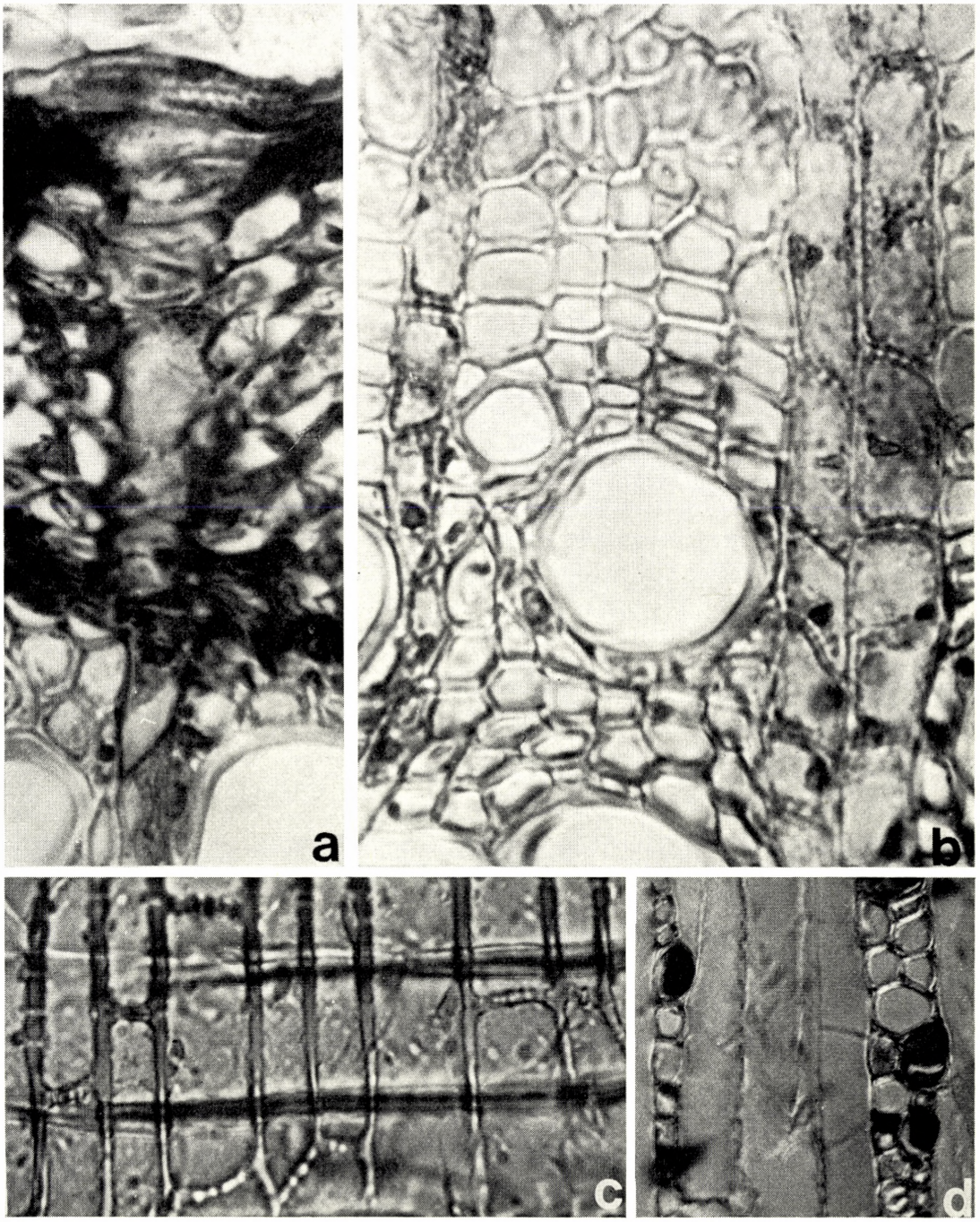

Fig. 12. Wood rays in *Prosopis kuntzei*. a. Cross section of ray through late xylem, cambium and phloem. On both sides of dilatated part of the ray crushed primary phloem. — b. Cross section of uniseriate and biseriate ray in late wood. — c. Radial section showing pitting and alterations in height of the ray cells (a.–c. × 1000). — d. Tangential section of uni- and biseriate ray with surrounding parenchyma and scattered cells with dark contents. (× 500).

the early wood in the Bolivian material and they are few and scattered in the early wood in the thicker Argentinian branch (Fig. 10a).

The rays are mostly uniseriate but may be three cells wide. They are heterogeneous and frequently two rays, lying above one another, fuse, at the same time being slightly shifted in a tangential direction (Fig. 13a)

The heterogeneity is not of the usual type with procumbent and upright cells but relates to the cell size and the cell contents. In *Prosopis sericantha* there are clear signs of a heterocellular ray structure with procumbent and upright cells (Böcher and Lyshede 1972, Plate XVe), but in *P. kuntzei* a similar structure was rare although many of the marginal cells were large and tapering (cf. Fig. 13b). In the *Caesalpiniaceae* clear cases of heterocellular rays were described by Huber (1949, see further Braun 1970 Fig. 143).

In the early wood (Fig. 13b) some of the ray cells appear to be swollen and not rarely the uppermost and lowest cells are particularly large and resemble axial parenchyma cells. Some of the swollen cells may simply be inserted parenchyma cells dividing the ray into sections.

In the later formed wood some of the swollen cells are transformed into idioblasts with dark contents (perhaps tannin).

In cross sections of the latest wood the rays near the cambium may be two to four cells wide and composed of rows of wide cells with conspicuous pits in the very thick tangential walls and rows of narrower cells with thin walls. Sometimes one row of wider cells has dark contents, while the adjacent row has narrow cells without the dark contents. The content of starch is often great in the rays as well as in the pith parenchyma and the axial parenchyma (Fig. 13c)

In radial and tangential sections the differences in size between cells in the same ray is conspicuous. There seems to be alternation e.g. between one broad and two narrower cells (Fig. 12c). The broad cells often contain more cytoplasm along the walls. The number of cell rows as counted in radial sections amounts to as many as 50, but such high numbers are probably generally due to fusion of two (or more) rays.

4. Discussions and Conclusions

Prosopis kuntzei belongs to the terete apophyllous stem photosynthetic plants but its primary cortex fibre strands divide the green palisade layers into sections. The later formed fibre strands, however, are placed inside the chlorenchyma so that the result strongly resembles the structure of e.g. *Bulnesia retama*, which has a cylindrical, continuous cortex chlorenchyma. Went (1971: 211–215) recently referred broom-like species with a continuous, many-layered palisade to a special type, the *Cercidium* type, which according to him has developed mechanisms enabling growth in thickness without injury to the bark. He exemplifies the type by *Cercidium torreyanum*, *Dalea spinosa*, and *Asclepias subulata* from Southern Californian deserts. Growth in thickness in these species involves tangential cell divisions of both chlorenchyma and epidermis resulting

Fig. 13. Tangential sections of wood in *Prosopis kuntzei*. a. and c. later formed wood, b. early wood. Parenchyma cells along rays and vessels (a.). In c. a septate tracheid, starch in both kinds of parenchyma, and farthest to the left a fibre cell which is bright in semipolarized light. In a. the ray to the left in the picture is composed of two fused rays, the lower one having two large, dark cells. (× 500).

in a smooth green surface even after many years of growth. Only five – ten-year-old stems develop a cork layer.

WENT mentions seven species from the deserts in Southwestern USA showing this anatomical structure. His main point, however, stresses that the *Cercidium* type is restricted to North American deserts as it does not include any of the broom-like plants of the Negev Desert (EVENARY et al. 1971: 294–296).

The investigations of the South American apophyllous species make it clear, however, that in any case some of the West Argentinian species have smooth green stems, multiple epidermis and a multi-layered green palisade (*Bulnesia retama, Bredemeyera colletioides*) or other types of epidermis which can follow the increase in girth (*Stillingia patagonica, Cassia aphylla,* and *Monttea aphylla*).

A multiple epidermis is found in Negev Desert plants like the articulated *Chenopodiaceae,* but these plants have a thick water-storing parenchyma below the green cells (FAHN & DEMBO, 1964).

It is evident that broom-like plants are anatomically rather heterogeneous, but they can be divided into three or more subtypes (or sub-lifeforms) according to their anatomical structure (BÖCHER & LYSHEDE 1968, 1972). It would be amazing if these subtypes were not connected with transitional structural types and *Prosopis kuntzei* is just transitional, having a smooth green surface but maintaining primary cortex fibre strands probably because this character is important for the support of the young branches. Each of the subtypes and the transitional types as well have their own eco-physiological and phylogenetic background. In some of the groups that belong to *Fabales,* the genetic constitution may make an evolution of the *Cercidium* type particularly easy.

If the morphological evolution started with plants having a terete green stem, a one-layered epidermis, and a cortex of normal green parenchyma cells, we might imagine developments of the different apophyllous sub-lifeforms to be further, more specialized adaptations. The *Cercidium* type would be one of these, another would be the grooved type, which occurs in e.g. *Casuarina, Retama, Neosparton,* and *Aphyllocladus.*

However, in the case of *Prosopis kuntzei* another evolutionary possibility or sequence is to be discussed. In the genus *Prosopis* the majority of species are foliate trees among which several show different kinds of adaptation to xeric conditions (e.g. *Prosopis spicigera* (BHATT & LAHRI 1964), *P. cineraria,* and *P. chilensis*). Hence, one might imagine a line starting with foliate trees, continuing with low trees with green bark and then apophyllous trees like *P. kuntzei,* and ending with apophyllous shrubs like *P. sericantha* or *Prosopidastrum globosum.*

I have had the opportunity to see the "Algarrobal" in Argentina during the dry season. It is an open, low, xerophilous woodland composed e.g. of *Geoffraea decorticans, Cercidium australe,* and *Prosopis alpataco.* When the trees have shed their leaves, the light green branches are very conspicuous, especially in *Cercidium* and *Geoffraea.* Both have a periderm composed of living, choroplast-containing cells (cf. ROIG 1970, p. 108). Exactly the same type of living periderm was described in the African *Acacia seyal* by

HAGERUP 1930. In the latter species the cork is translucent and covers a many-layered green cortex. The cortex cells are stretched tangentially and are divided by anticlinal radial walls. Curiously enough HAGERUP says nothing about stomata.

During the expedition to Argentina in 1955–56 I collected material of a green branch of *Cercidium australe* and fixed it in alcohol. This material has now been investigated and exhibits a kind of periderm consisting of living cells with chloroplasts, nuclei and comparatively thick, tangential, cellulosic walls. Inside follows a cortex of green cells. The periderm has five or six layers of cells and harbours guard cells formed in the periderm. In the first stage they are covered by the exterior cell layers. After some of the overlying cells have died away, the guard cells appear sunken and their middle part is stretched out to a thin strand. This is a result of the dilatation of the periderm layer. All stomatal apertures are orientated across the axis of the branch. The cells just outside the guard cells are also stretched and form a thin plate, which develops into a diaphragma made up of tangential wall material.

Of particular interest are the conspicuous intercellular spaces developed in the green tissue inside the stomatal apertures, and that this spongy parenchyma is surrounded by green cells containing enlarged nuclei.

Prosopidastrum globosum is a shrub, which resembles the algarrobal trees by its phellem-like multiple epidermis, but its cortex is composed of radially arranged green palisade cells; here the epidermis also has stomatal apertures which are adapted to the phellem-like structure. It fills out a gap between the woody species with green bark and the apophyllous woody plants like *Prosopis kuntzei*. If the suggested evolutionary system touches on something essential, *Prosopidastrum* might be more primitive whereas the two species of *Prosopis* would be more advanced by their development of the external epidermal layer as a palisade epidermis of wax-exuding tapering cells.

Accepting the two apophyllous *Prosopis* species as specialized, we may regard *P. kuntzei*, which retains remains of cell tiers including the guard cells, as more primitive, while *P, sericantha* might be even further derived having almost abandoned a multiple epidermal structure.

But this would probably be to carry speculation too far. We must be content with pointing out possible evolutionary trends and exemplifying the trends by facts about certain species.

The comparison of the three apophyllous species of *Prosopis* – *Prosopidastrum*, however, raises another question regarding the orientation of the stomatal apertures. In the two species of *Prosopis* they are orientated at right angels to the longitudinal axis of the branches whereas in *Prosopidastrum* they are parallel to the axis.

According to experience gained from the study of many species with green stems and reduced leaves, it is possible to conclude that an orientation parallel to the branch axis is found in primitive groups like *Psilotum nudum, Equisetum hiemale* (DE BARY 1877 Fig. 24), several species of *Ephedra* and in monocotyledons like *Asparagus stipularis*. In the dicotyledons there are many examples of parallel orientation but in addition a good deal of transverse orientation; among these examples are the articulated

Chenopodiaceae, discussed by FAHN and DEMBO (1964), to which I can add *Allenrolfia vaginata*.

It is a striking fact that there are cases of diverse and opposite orientation within the same systematic groups. Besides *Prosopis* – *Prosopidastrum* mention should be made of the following closely related taxa:

Parallel orientation	*Transverse orientation*
Gymnophyton isatidicarpum	*G. robustum* and *polycephalum* (BÖCHER 1972)
Verbena scoparia	*Verbena (Junellia) glauca* (BÖCHER & LYSHEDE 1972)

and in the following genera belonging to the same order we find similar divergences:

	Parallel orientation	*Transverse orientation*
Fabales	*Spartium junceum*	*Cercidium australe*
	Spartocytisus nubigenus (according to verbal information by O. LYSHEDE)	*Cassia aphylla* and related taxa
	Dalea spinosa	*Carmichaelia petriei*
Asterales	*Bebbia juncea*	*Vernonia juncea*

It is not surprising that species from the same family show the same orientation, thus e.g. *Polygala virgata* and *Bredemeyera colletioides* (both with parallel orientation) and *Colletia ferox* and *Discaria articulata* (both with transverse orientation). But it is remarkable that the two orientations occur in the same sub-lifeform; thus the furrowed type with the stomata confined to furrows in the stems. The two *Neosparton* species mentioned in the previous paper, as well as *Casuarina*, illustrated by SOLEREDER in METCALFE & CHALK, have transverse orientated stomata, while *Aphyllocladus spartioides* has parallel orientation. (Fig. 60 and Plate XXIIIa in BÖCHER & LYSHEDE 1972).

It is not immediately understandable why and how the divergence in orientation arose within related groups. Some apophyllous species have different, "mixed" orientations, e.g. *Stillingia patagonica* and *Genista aetnensis*, and consequently one might explain parallel and transverse orientation as results of two oppositely directed specializations. Plants with mixed orientation may keep this character because it is not harmful, being perhaps due to genes which are responsible for a similar orientation in the leaves. On the other hand, a fixed orientation is possibly due to some kind of adaptive force.

The transverse orientation was discussed by FAHN & DEMBO (1964), who consider it a characteristic feature in articulated plants and also mention *Tamarix aphylla* studied by VOLKENS (1887). They say that the orientation is due to the pattern of division and the differentiation of the protodermal cells. The development of these cells proceeds in one direction – from the base and upwards – and the orientation of the cell divisions in the stomata-producing cells is constant.

Returning to the case of *Prosopidastrum–Prosopis* we can exclude any connection with the occurrence of a multiple epidermis, because this is present in both genera. But we may discuss a possible connection with the secondary growth and the increase in girth. *Prosopidastrum* has very strong ribs closely connected with the primary fibre strands dividing the green cortex palisade into distinct sections. The increase in girth in the green stratified cortex and in the multiple epidermis with its multiseriate stomatal complexes takes place in the inter-rib areas exclusively. Hence, parallel orientation along the ribs seems to be most suitable. Some observations indicate that the stomata in *Prosopidastrum* are arranged in irregular rows parallel to the ribs; but here the precursors of the stomatal complexes are multiseriate cell groups and are probably initiated at fixed, though sometimes short, intervals.

In the two species of *Prosopis* the inter-rib areas are occupied by transversely arranged stomata. Sometimes, in *P. sericantha*, the stomata are densely spaced so that the short inter-rib areas resemble step-ladders (Böcher & Lyshede 1972, Plate XV a–b). In *P. kuntzei* a number of stomatal apertures may occur in lengthy depressions (p. 22), and here it is evident that new epidermal cells are inserted at the ends of already formed stomata and are arranged parallel to the cells limiting the stomatal cavities at their ends. Some of the new cells develop into precursors of new stomatal complexes which therefore assume the same orientation as the original ones. This may be so, but it gives no explanation of the transverse orientation of all stomata.

The transverse orientation may be advantageous because during the thickening of the branches the apertures are exposed to stretching and not to transverse forces, which would tend to draw the guard cells apart. This stretching of the guard cells appears strikingly in *Cercidium australe*.

The development of stomatal complexes in *P. kuntzei* is not only a matter of cell differentiation in limited parts of a multiple epidermis. Also the cortex is involved as the cortical air chambers are formed before or simultaneously with the differentiation of the stoma complex precursors. In *Cercidium* cortex air spaces are only formed in connection with stomata. The determination of stomatal spheres in both epidermis and cortex is made particularly intricate by the fact that the process is not completed but seems to proceed concurrently with the increase in girth.

The very different structure of the stomatal complexes in three related apophyllous species deserves comment. The outer ledges in *Prosopidastrum globosum* and *Prosopis kuntzei* are remains of cell walls between cells in the exterior cell layers in a multiple epidermis. *P. sericantha* has overarching subsidiary cells like *P. kuntzei*, but here the guard cells are provided with small genuine outer ledges. Both species, however, produce a kind of interior pseudo-ledge, the cells inside the stomatal pore forming protruding ridges which narrow the substomatal chamber. The most important differences between the species are summarized in Table 1.

The majority of South American stem-photosynthetic species are provided with palisade tissue, often arranged in several layers of radially elongate cells. The stems resemble cylindrical leaves like those of *Hakea*. In both cases the cylindrical palisade

TABLE 1.

Cell layer from surface inwards	*Prosopidastrum globosum*	*Prosopis kuntzei*	*Prosopis sericantha*
1	flat cells, cuticle peeling	flat cells, some disappearing	non-existent
2 & 3	small flat cells, separating cell wall almost persisting as a diaphragma (pseudo-ledge)	small flat cells, some disappearing, parts of separating cell wall persisting mostly as pseudo-ledges	—
4	guard cells, walls striated and with grooves (BÖCHER & LYSHEDE 1972 Fig. 39) outer walls traversed by delicate strands	guard cells, striation in wall as a rule less pronounced, but sometimes distinct (fig. 5f, Plate III).	guard cells with short genuine outer ledges and sometimes very short interior ledges too
5 & 6	small flat cells producing ledge-like protrusions	small cells producing short, sometimes curving, ledge-like protrusions	small cells hardly forming ledge-like protrusions

cells are not, as in flat dorsiventral leaves, arranged parallel to the angle of incidence of light. The elongate structure of the cells therefore seems primarily not to be an adaptation to the direction of the light. It is rather the conduction of water and the products of photosynthesis that are decisive. In several iso-lateral leaves elongate palisade cells form rows which radiate from the central veins, and many grass leaves with C_4-photosynthesis have radiating mesophyll cell rows around the bundle sheaths. Important is also the fact that the rate of DNA synthesis is higher and continues longer in palisade cells than in other mesophyll cells. Apart from *Cercidium australe* spongy mesophyll is absent in green stems, and the intercellular spaces between the palisade cells are sometimes very narrow. In *P. kuntzei*, however, the dense palisade tissues are regularly interrupted by larger spaces, which, as mentioned, are somewhat displaced in relation to the rear cavities inside the stomata.

In *P. kuntzei* young branches have five to nine palisade layers, a number similar to that in *P. sericantha*. *Prosopidastrum* usually has five layers. In *P. kuntzei* older thicker branches have not increased the number of layers but the cells per layer have clearly increased, the breadth of the cells being unaltered. This probably means that an increase in palisade layers would not imply a higher production of matter, the light reaching the interior layers being perhaps too dim.

In *P. kuntzei* production probably decreases as age increases, despite the maintenance of the green cortex. The stomatal apertures are to a great extent plugged. The plugging is clearly a stage in the metabolic development of the branch. In the next stage photosynthesis will be reduced to a minimum due to increasing rhytidome formation.

Regular stomatal occlusions by wax plugs have been reported by JEFFREE, JOHNSON & JARVIS 1971 for *Picea sitchensis*. They found wax tubes in the antechambers of the stomata and calculated the extra resistance for diffusion of water vapour and carbon dioxide. The wax-filling was aaumed to reduce the rate of transpiration by about two-thirds but the rate of photosynthesis by one-third only.

Plugs also occur in the *Winteraceae*. According to BAILEY & NAST (1944) and BONDESEN (1954) they consist of alveolar cutin, or alveolar cutin externally covered by homogeneous cutin. BONGERS (1973) also refers to the stomatal occlusions in the same family but says that the plugs consist of alveolar cutinaceous material or wax. The question of stomatal plugging is old, dating from the beginning of the nineteenth century. The literature on the subject was reviewed by TH. WULFF in 1898.

Not only occlusions by wax plugs in stomatal antechambers reduce the traspiration. RENTSCHLER (1974) studied water loss in leaves of *Brassica napus*, *Tropaeolum majus*, and *Chelidonium majus* and found that a thick, felt-like wax covering of the epidermis and the stomata reduces water loss considerably. Leaves from plants grown in dry air with thick wax cover were compared with leaves from plants grown under humid conditions with a thin cover. The loss in weight after drying up for one hour was ten per cent in the first case and more than fifty per cent when the cover was thin.

According to experience gained from studies on American xerophytes, including the present study, it seems clear that permanent and total occlusion always precedes a cessation of the photosynthetic activity, e.g. by development of a cork layer in a stem or by degeneration or wilting of leaves. In the species in question there are no signs of complete stomatal closure but many indications of partial closure which will slow down the evaporation considerably and probably reduce the CO_2 uptake. A partial occlusion may be due to: 1) filling of the stomatal cavities with more or less loosely packed wax crystals, 2) filling of the same cavities with fungal hyphae, 3) filling of the substomatal chambers with sphaerites, 4) narrowing of the entrance by growth of subsidiary cells and outer pseudo-ledges. A permanent closure is obtained if the epicuticular wax covering covers the alveolar wax plugs, but in many cases cracks occur in the wax cover just outside the plugged stomatal cave or the wax cover may appear to be perforated by many minute pores (Plates VI–VII).

In *Prosopis kuntzei* a slowing down of stomatal diffusion is clearly increased as age and wax occlusion increase, but it must be low even without wax plugging simply because the pathway by which transfer of gases by diffusion takes place is so long and so frequently narrowed by various ledge-like protrusions.

Before repeating the structural features which restrict gas diffusion, we might try to consider the gas exchange situation in a branch like the one found in *Prosopis kuntzei*. It is evident that the interstomatal surfaces are very effectively protected against water vapour loss and other gaseous penetrations. Hence almost all transfer of gases uses the stomatal pathway. This also applies to oxygen. With the increasing age of the branch oxygen liberation during photosynthesis will probably decrease while the oxygen requirement of the living cells in the wood will increase. This implies an

increasing demand for access to oxygen. The stomatal pores may therefore increasingly act as lenticels. On the other hand, the occlusions will also slow down the diffusion and removal of CO_2 produced in the branch during respiration.

In older branches the CO_2 uptake from the atmosphere may therefore be of minor importance if the green cortex are sufficiently supplied with CO_2 from the cells in the central parts, e.g. pith, xylem, and phloem. The dislocation of the cortical air chambers in relation to the stomata may as already hinted be considered an adaptation to accumulation of CO_2 received from the interior tissues, but it must be admitted that water vapour also will be restrained.

According to JEFFREE, JOHNSON & JARVIS (1971) the presence of an antechamber in Sitka spruce stomata increases the length of the pathway for the transfer of gases and the presence of wax in the antechambers increases the tortuosity of the passway and reduces the cross sectional area available for diffusion. They estimated the diffusion resistance of pore and a funnel-shaped antechamber to double the resistance of the pore alone.

Before roughly trying to estimate the diffusive resistance in a stomatal system as that in *Prosopis kuntzei*, we must first survey the structural obstacles to diffusion presented by structures constantly present:

1) The length of the pathway through the stomatal complex. The pathway has four sections: rear-cavity, pore, front-cavity (or antechamber), and stomatal cave which corresponds to the extra front cavities or the epistomatal cavities discussed in the previous paper (1972: 126). The total length, if the narrowing produced by the exterior subsidiary cells is used as the outer limit, is 30–35 μm (cf. Fig. 5 f, Plates III a–c, IV d–g). In the Sitka spruce the length (depth) of the pore + antechamber is stated to be 24 μm.
2) The number of bulges and protrusions which narrow the pathway. We may here anticipate three important narrowings, viz. the interior pseudo-ledges, which may reach the interior entrance to the pore (Plate III b), the bulging guard cells, and the outer pseudo-ledges.

Both structures, 1) and 2), vary much. The epistomatal cavity may in some cases be so wide that its outer part should be disregarded with regard to the length of the pathway. In other cases the subsidiary cells from both sides narrow the outer entrance considerably (Plate III a and d) so that we have to reckon with four important narrowings.

There are several structures that frequently contribute to the restrictions:

a) Sphaerite formation almost filling substomatal chamber and rear-cavity (Plates III c, IV h, V f).
b) Striation of guard cell walls facing rear-cavity (Plate III b).
c) Two short, extra pseudo-ledges (wall remains) between pore and outer pseudo-ledges (Plate VII a).

d) Occasional extra pseudo-ledge at entrance to epistomatal cavity (Plate IV f).
e) Wax filling of epistomatal cavity (alveolar wax), cf. Plate IV b, e, forming wax plug.
f) Partial covering of epistomatal wax plug of extracuticular surface wax (Plate VII b).

According to JEFFREE et al. the presence of the wax tubes in Sitka spruce reduces the area of cross section available for diffusion, but also the voids for diffusion are reduced almost to the mean free path of the molecules. Hence, the free diffusion coefficient of water vapour in air is no longer applicable.

In the stomatal complexes of *Prosopis kuntzei* the pathway for water vapours is restricted in so many ways and the restrictive structures are subject to so much variation that it is hardly possible to make any calculations. However, if the reduction in the rate of transpiration in the Sitka spruce is about two-thirds as a result of wax plugging, we may probably reach much higher reductions in *Prosopis* and especially when the wax plugs are partly covered (f). In any case the stomatal complexes in *Prosopis kuntzei* may well hold the record for anti-transpirant structures.

In a xerophytic tree water economy may sometimes be particularly problematic. It is evident that the closely related *P. sericantha* is not so advanced in anti-transpirant adaptations. Also, it is a shrub. The pathway for gases through the stomatal complex is not much shorter, but *P. sericantha* has no pseudo-ledges to produce a partial closure of the antechamber. *Prosopidastrum globosum* is also a shrub and here the pathway is shortened because there is no overarching by subsidiary cells and thus no stomatal cavities.

The water economy of *Prosopis kuntzei* was briefly referred to in connection with the xylem structure. As the species, like most other stem photosynthetic species, lacks small veins leading from the xylem to the green cells, it was assumed that radial water conduction mainly takes place through the rays and further through the pericycle to the green cortex. The connection between rays and axial parenchyma as well as vessels was found to support this view. Also in *Prosopidastrum* the vessels are in contact with axial parenchyma and rays. In a species like *Bulnesia retama*, which may reach five or six meters in height and is tall shrub or a low tree, the diffusely arranged vessels occur in groups which accompany the rays (BÖCHER & LYSHEDE 1968 Fig. 14).

In *Psila spartioides* water, from the xylem to the green cortex, has to pass a well developed endodermis of wide cells with suberized cell walls. This passage seems to be restricted to non-suberized passage cells ((BÖCHER & LYSHEDE 1972: 61–62). *Psila spartioides* is connected with saline soils. This is mentioned mostly in order to emphasize the comprehensiveness of the eco-physiological side of the stem structure in apophyllous species and to remind us how far we are from a versatile or real understanding.

Institute of Plant Anatomy and Cytology
University of Copenhagen

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PLATE I

Outer epidermal layer in *Prosopis kuntzei*. a–i. cross sections, j–k. paradermal section, in j. the left part of the section is closer to the surface than the right part. a., b., f., g., h. interference contrast, c.–d. polarized light, i.–k. quadruple staining. – Tapering part of the cells continues in brushes of delicate strands. Strands reaching the cuticle and the surface are seen in c., g., and h. For further details see text (× 2000).

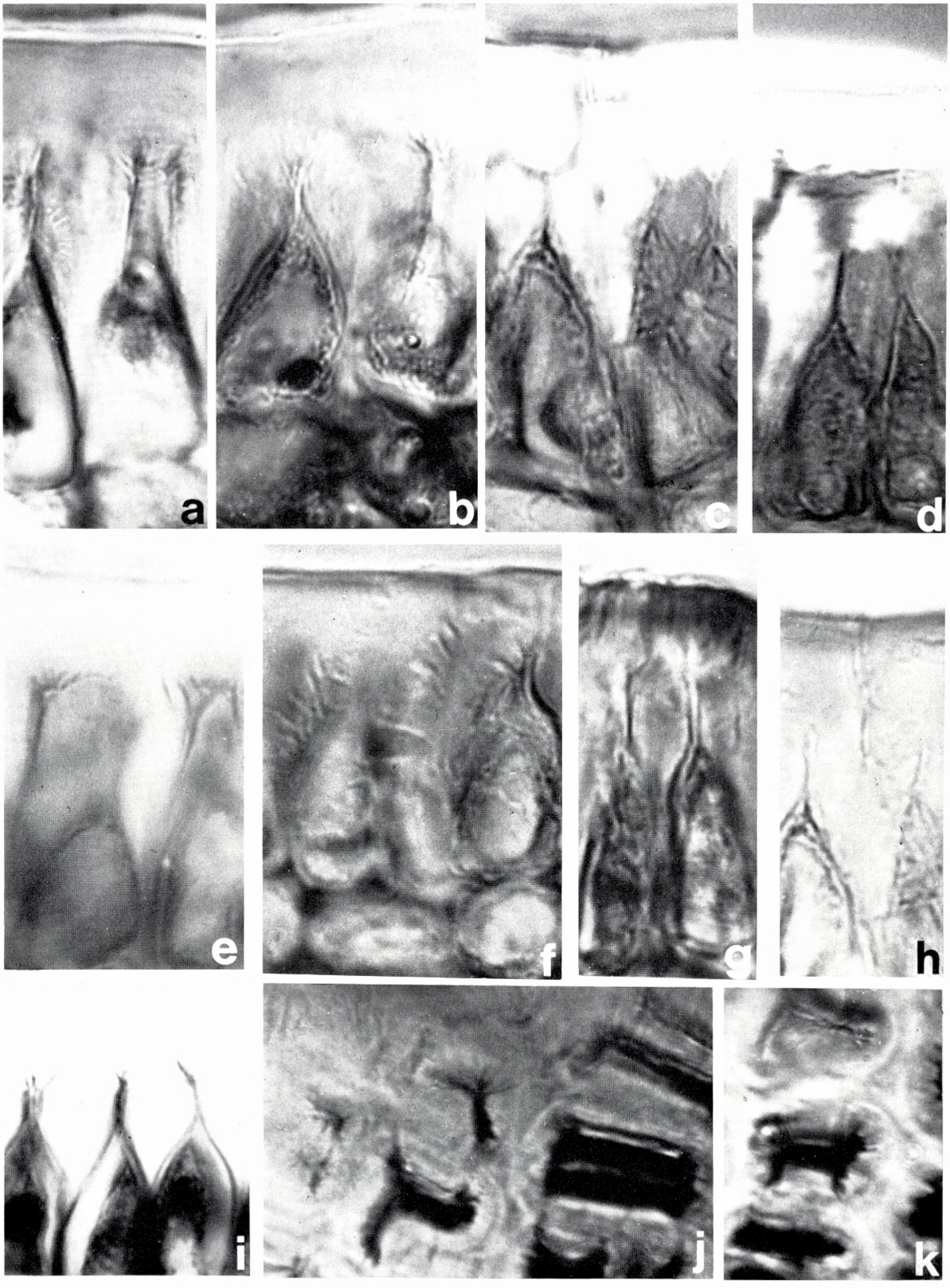
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PLATE II

Epidermis in *Prosopis kuntzei*. a.–b. Slides mounted in Canada balsam after heating. Wax has disappeared from most parts of the cuticular layer but recrystallization has taken place in the mounting medium as delicate birefringent strands and short strands where the canals penetrate the cuticle. Some wax not exuded during melting remains in the cuticular layer or flanges as bright spots. Polarized light × 500. — c.–d. Longitudinal view of young stomatal complex with wax plug. c. before, d. after heating, melting, and recrystallization in hemispherical wax bodies. Mounted in glycerine jelly, polarized light, ×1000. — e.–h. Slide mounted in glycerine jelly, in e.–f. before heating, g.–h. after heating, melting, and recrystallization of wax as small globules. e. and g. show the same area. Note many cracks or delicate canals in outermost wax-containing part (e.–f.) and globules formed outside the cracks or canals in g.–h. Polarized light, ×1000.

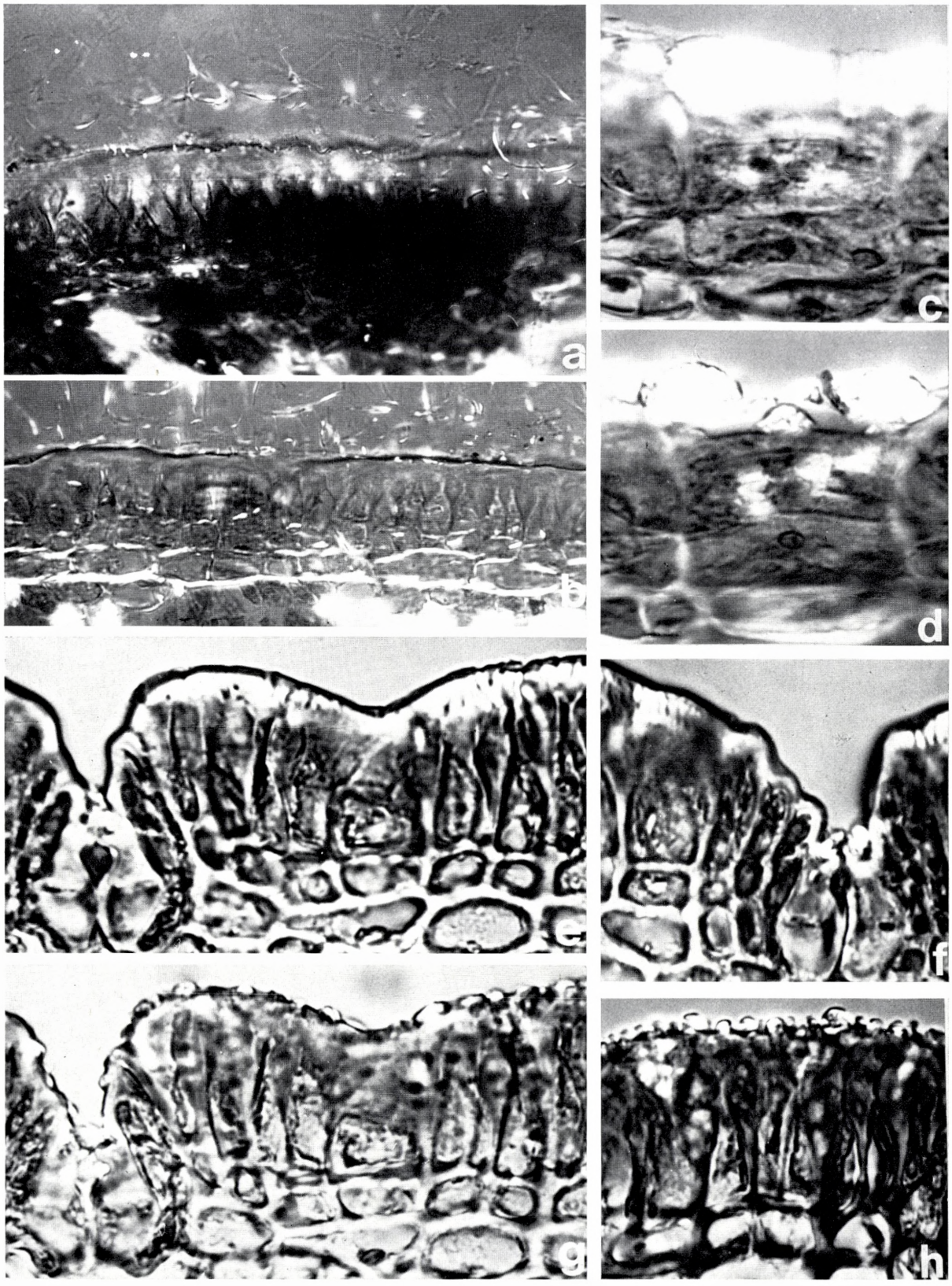
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PLATE III

Stomatal complex in *Prosopis kuntzei*. In a.–c. the interior ledges are visible, in c. also calcium oxalate sphaerite filling substomatal chamber and coming up against outer palisade cells. a. and d. adjoining of exterior parts of subsidiary cells resulting in closure or narrowing of entrance to epistomatal cavity. Interference contrast, × 2000.

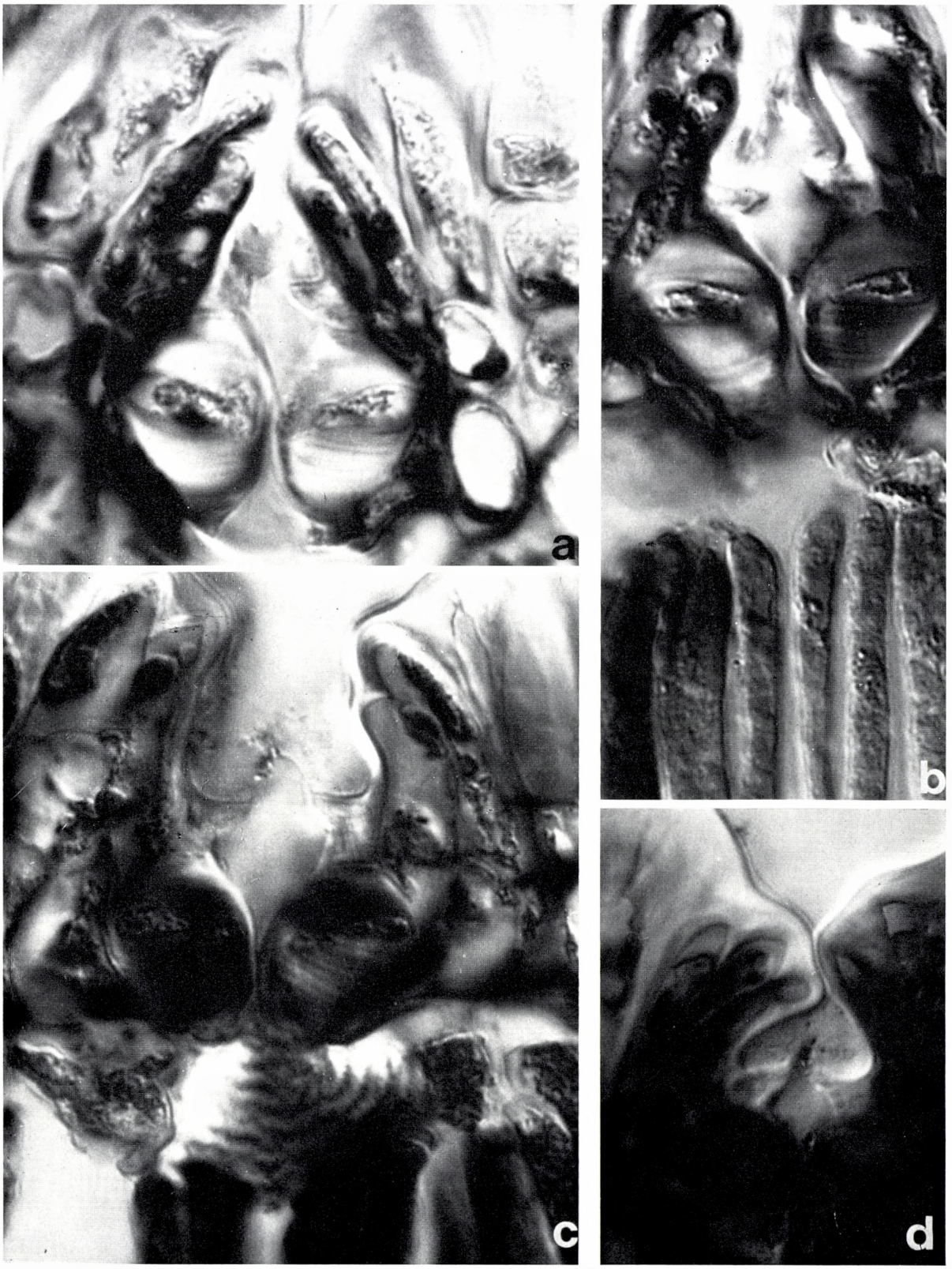
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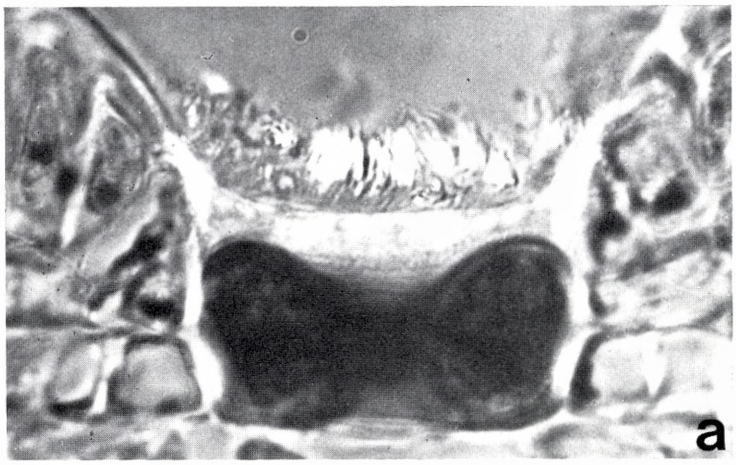
PLATE IV

Stomatal complex in *Prosopis kuntzei* and single pericyclic cell (i) showing pit canals and staining of primary wall (Johansen's quadruple staining); at the top connections to several palisade cells. — a.–b. Longitudinal section of guard cells, a. as observed in polarized light, wax bodies show up as well as layers of waxy material in wall on the abaxial side and cellulosic anticlinal walls, b. as observed with interference contrast. — c. outer pseudo-ledges with wax crystals, paradermal view, semi-polarized light. — d.–g. Cross sections of stomatal aperture with surrounding cells, interference contrast. In e. wax filling of stomatal cavity outside outer pseudo-ledges. In f. two ledges indicating a "lost" exterior cell on right side of stomatal cavity. In g. two delicate strands traverse wall outside nucleus in left-hand subsidiary cell. — h. Large sphaerite formed inside stomatal pore, narrow middle part of guard cell seen above centre of sphaerite, in the right corner overarching subsidiary cells, ×about 1000.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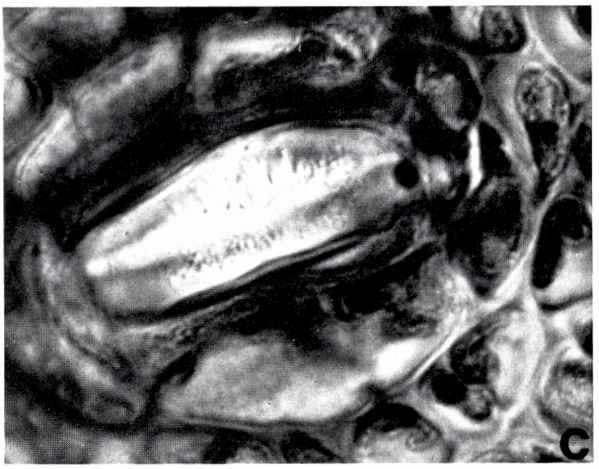
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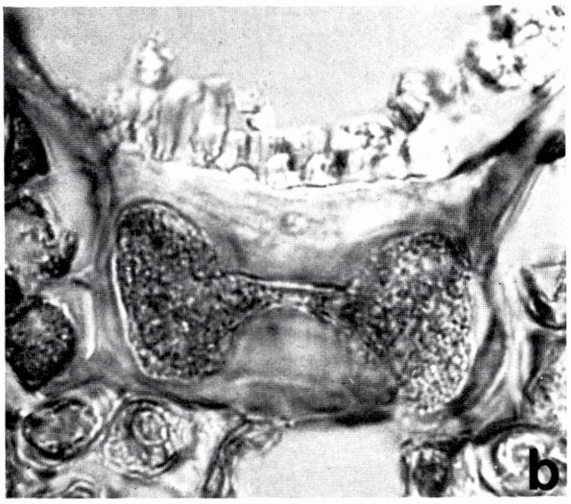
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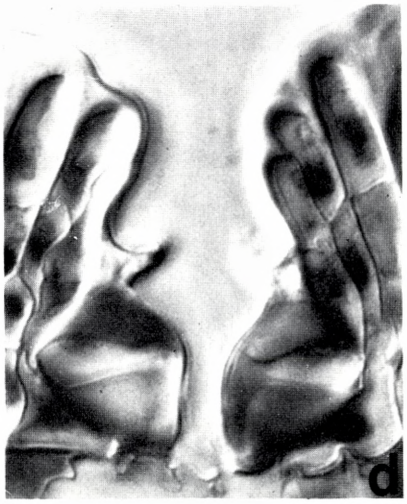
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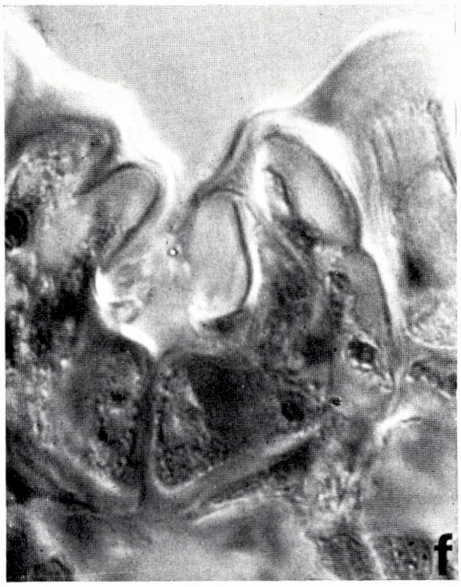
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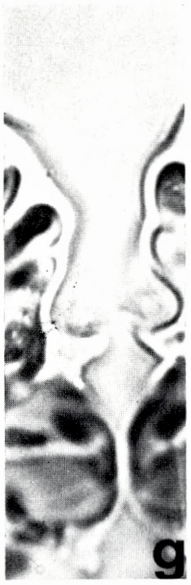
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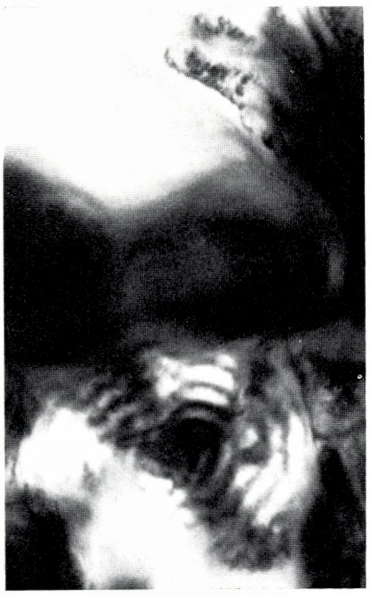


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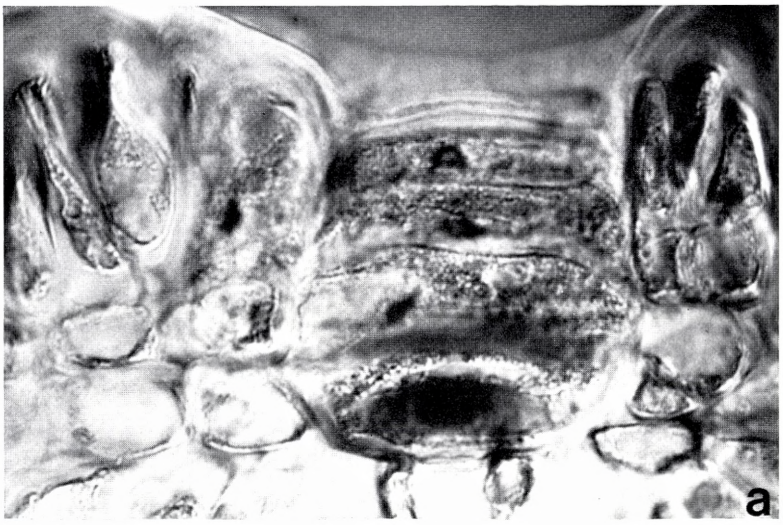
PLATE V

Stomatal complex in *Prosopis kuntzei*. a.–b. Longitudinal views, a. normal case with multiple, multi-layered, initial cell complex, the nucleus in the lowest cell is seen below the arching narrow part of a young guard cell, two palisade cells in substomatal chamber. — b. Stomatal complex showing subdivision of outer cells which develop into local phellem plug. — c. Paradermal view of guard cells. — d. Guard cell lengthwise; remains of outer cells outside pseudo-ledge. — e. Cross section of stomatal complex, the two guard cells covered by five cell layers, cf. Fig. a. — f. Cross section of guard cells, not mature, the small ledge-forming cells partly covered by wax, substomatal chamber with sphaerite. Interference contrast. × about 1000.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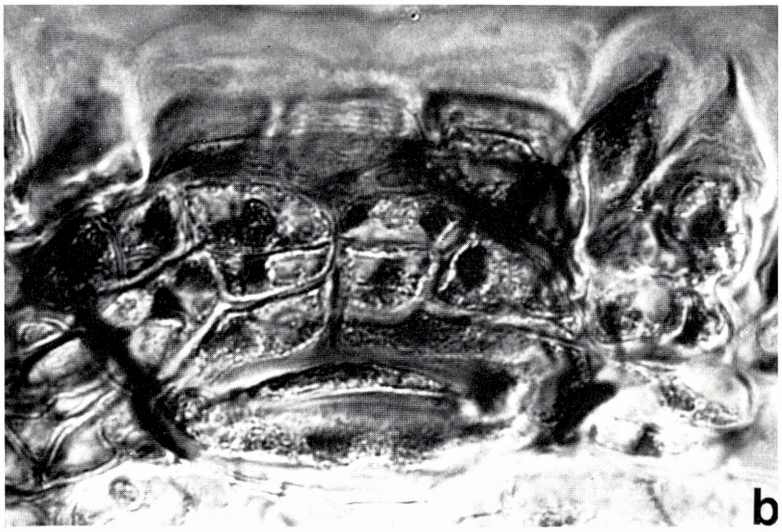
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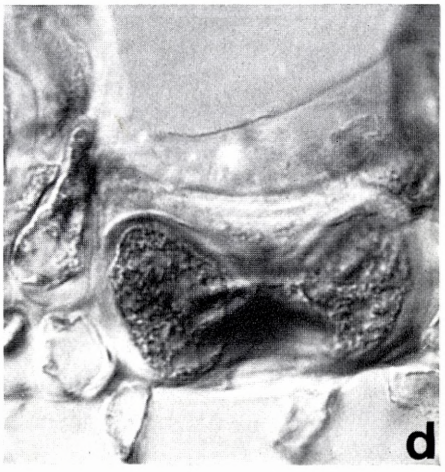
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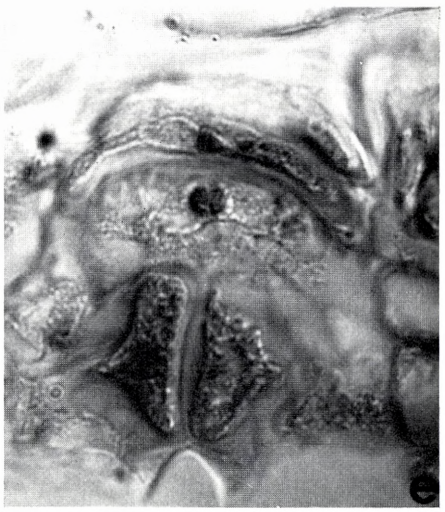
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PLATE VI

Stomata and epidermis in *Prosopis kuntzei*. — a. Oblique, almost paradermal section showing from the right (deepest part of section), two substomatal air spaces surrounded by palisade cells with crystals, next three stomata cut at the level of the bulbous guard cell ends, surrounded by interior epidermal cells, on the left two stomata cut at the level of the outer ledges, surrounded by outer epidermal cells arranged in families, × 320. × b. Surface of young branch, epicuticular wax cover partly removed due to alcohol fixation. SEM micrograph × 300. In the upper row one cavity covered by epicuticular wax (except along margin). Conspicuous wax production along the pseudo-ledge margins in two of the outer stomatal cavities. The ledge margins appear more or less fused in some of the cavities (arrows). — c. Old branch from Argentina. Stomatal cavities filled with alveolar wax or overflown by epicuticular wax leaving only small openings to alveolar plug below (arrow), SEM micrograph × 300. — d. As the preceding, but only part of plug with two pores leading down to guard cells, × 2800. NB. The axis of the branch is from left to right in the picture.

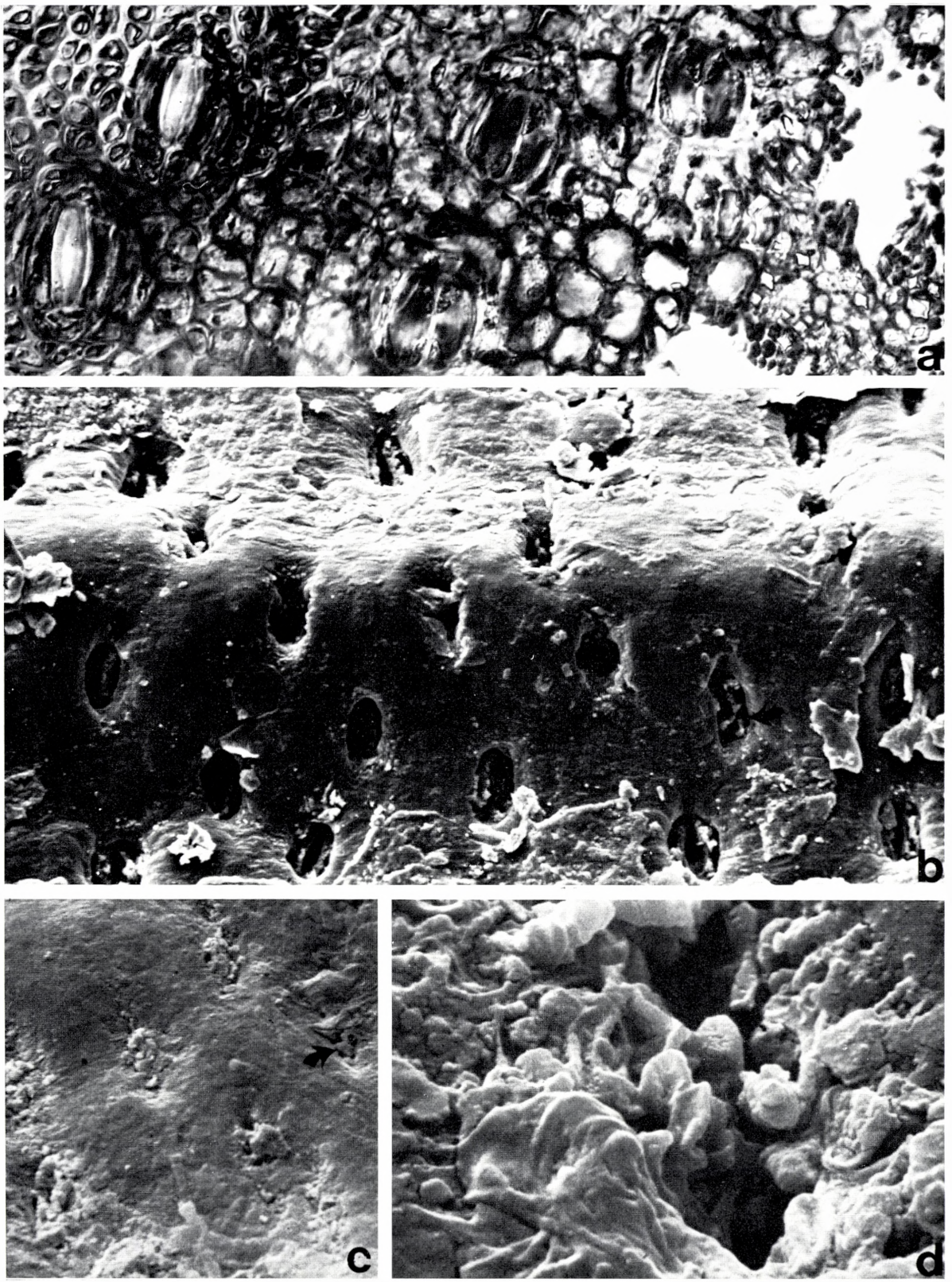
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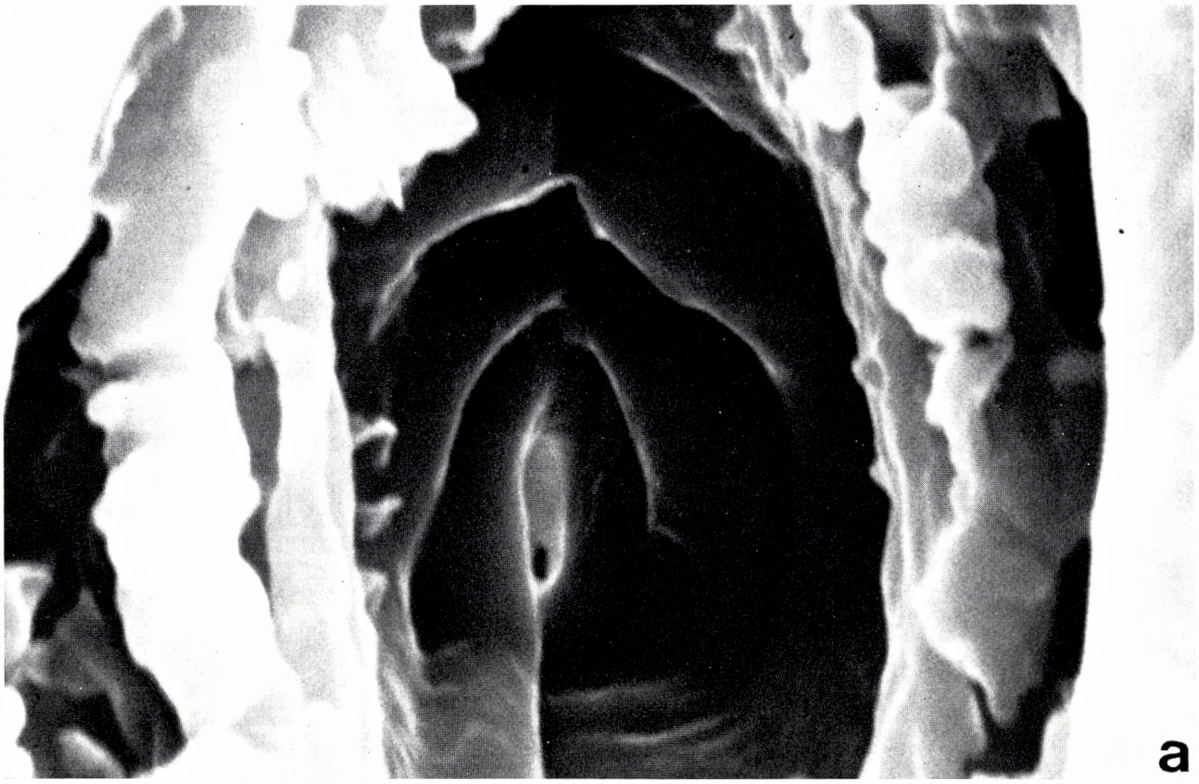
PLATE VII

Stomatal openings in *Prosopis kuntzei*. SEM micrographs. — a. Slightly oblique view through the whole complex from the bulging pseudo-ledge margins (with wax) and margins of cavity (farthest right and farthest left) through the central pore to the interior ledges and the narrow fissure leading to the substomatal chamber. The various "set pieces" are difficult to explain but some are due to remains of some cell walls separating the cells which originally covered the guard cell precursor (cf. text and Fig. 5f). The riffled "floor" may be due to wall layering (cf. Plate II b), × 6000. — b. Epicuticular wax cover partly overflowing alveolar wax plugs in stomatal cavities, × 1400. — c. Alveolar wax plug in old elongate cavity, × 700. Axis of branch is from left to right, all cavities being orientated across the branch axis.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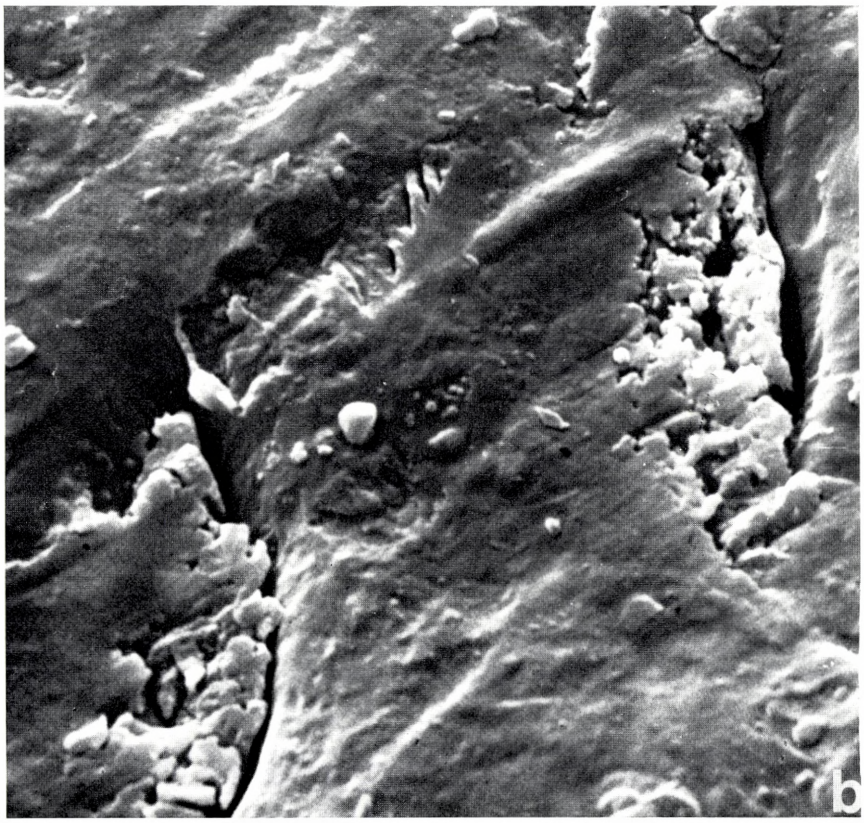
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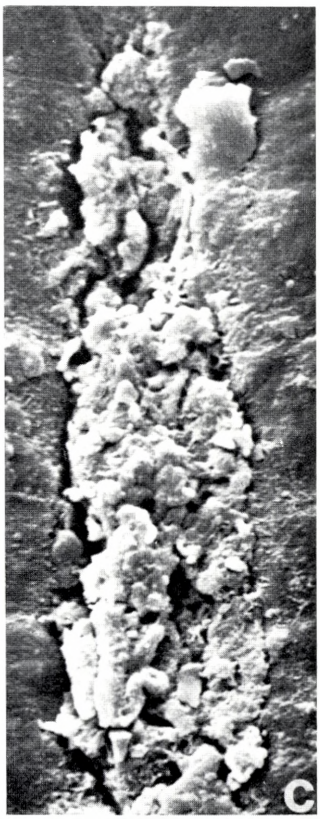
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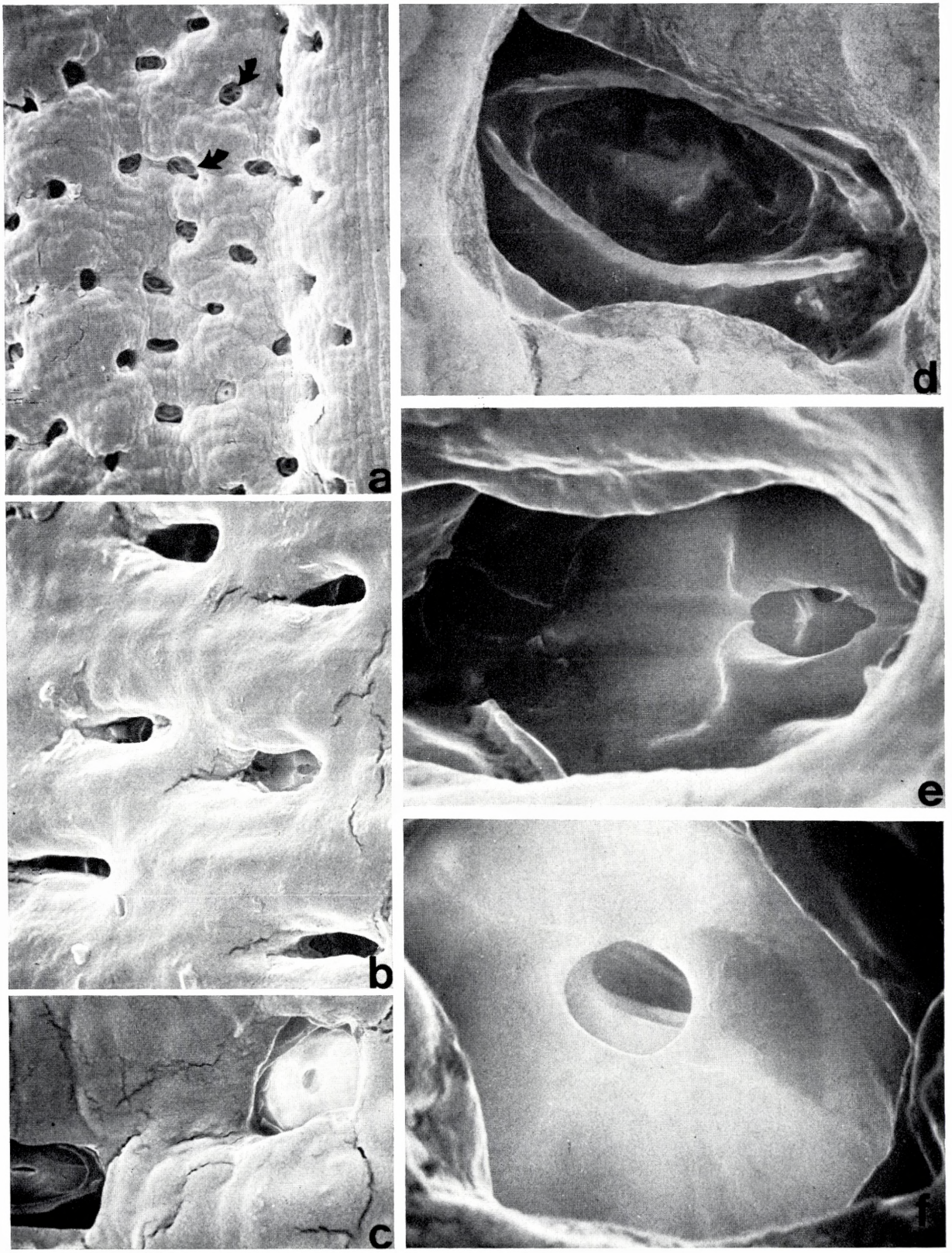
Plate VIII

Surface of Bolivian material of *Prosopis kuntzei;* young branch with sparse, epicuticular wax cover. SEM micrographs. — a. Part of branch near low ridge on the right (probably supported by primary fibre strand below). Some of the cavities contain two stomatal entrances (arrows), two are partly covered by overflowing wax (on the left). Some of the cavities are placed in the same groove (lower arrow) and may belong together ontogenetically, × 220. — b. As the preceding; two lower cavities very narrow. One or two contain two entrances (cf. e), × 450. — c. Two cavities in the same groove, the stomatal pore distinct in that farthest to the left, the other has a diaphragma with a circular hole (cf. f). — d. Cavity and outer pseudo-ledges with margins bending upwards, × 3100. — e. Cavity with two entrances through diaphragma (partly merged pseudo-ledges), the stomatal pores visible below, the one at the right side is displaced, × 3100. — f. Diaphragma with circular hole leading down to stomatal pore, which is partly covered but just visible below as a dark, crescent-shaped area, × 5300. Axis of branch is from top to bottom of the picture.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