COMPARATIVE ANATOMY OF THE LEAF-BEARING CACTACEAE, XV

SOME PRELIMINARY OBSERVATIONS ON THE OCCURRENCE OF "PROTEIN BODIES"

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IN THE NINTH PAPER of this series (1963c), I called attention to the occurrence of filamentous bodies in the vessels of *Pereskia sacharosa* Griseb., *P. grandifolia* Haw., *P. bleo* DC., and *P. tampicana* Web. which are rarely, if ever, present in the vessels of other categories of *Pereskia* or in those of *Pereskiopsis* and *Quiabentia.* These structures differ markedly in size and form. Many of them are tenuous threads a fraction of a micron in diameter, which are variously elongated, oriented, and aggregated (FIGS. 1 & 3). Others are coarse strands up to ten micra in diameter and of varying lengths and formations (FIG. 4). Some of the smaller more compact aggregations of slender filaments resemble the filamentous forms of "protein bodies" originally found and intensively investigated by Molisch (1885, *Figs. 3 & 5*) in the outer parenchymatous living tissue of young stems of *Epiphyllum.*

In the secondary xylem of this anatomically most primitive category of pereskias (Bailey 1962, 1963c) globular forms of bodies may occur in parenchymatous cells adjacent to vessels (FIGS. 5 & 6), in the first-formed unlignified parts of rays in roots and infrequently in lignified ray cells which contain abundant starch. Filamentous and stellate bodies are in general of rare occurrence in parenchymatous cells of the xylem except at times in the innermost parts of the rays in roots.

In the main stem and larger roots of *P. sacharosa* collected for me by Carenzo and Legname, from a tree in its native habitat in Jujuy province of northern Argentina, large deposits of material occur in the unlignified parenchyma of the broad zone of secondary phloem. These bodies vary considerably in form. The last formed cells of the multiseriate rays commonly contain abundant minute more or less spiny granules with frequent transitions to relatively large stellate bodies composed of short, slender, radially oriented filaments (FIG. 2). Cells in the outer modified parts of the rays and of the inner cortex frequently contain nearly globular bodies.[2] Filamentous forms resembling those which occur in the vessels of the secondary xylem are of sporadic occurrence in ray cells of the phloem, but may be present at times in the axially oriented parenchymatous cells of parts of the phloem which contain or once contained functioning sieve tubes.

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[2] In using the term globular, I include some deviations from perfectly spherical form to hemispherical, ovate, and irregularly roundish compact bodies.

The only collections of *P. tampicana* available to me are young stems of cultivated plants obtained by Dr. Boke at Victoria in Tamaulipas, Mexico. These small stems with scanty xylem and phloem have filamentous bodies in their vessels and globular ones in parenchyma adjacent to vessels (FIG. 6). Although most of the unlignified parenchyma of the phloem and cortex does not contain stellate or globular bodies, some of the ray cells and axially oriented ones (more or less closely associated with sieve tubes) are filled with deposits of diversified forms. These vary from slender filaments to coarse strands (as in the vessels of the xylem) and exhibit various transitions to homogeneous appearing masses of diversified forms, sizes and orientations (FIG. 7).

At present there are serious taxonomic difficulties and uncertainties in dealing with *P. grandifolia*, *P. bleo*, and such putative segregates as *P. moorei* Britt. & Rose and *P. bahiensis* Gürke. These uncertainties are much intensified in the case of plants growing in greenhouses and botanical gardens remote from their native habitats. Regardless of whether these plants are ultimately shown to be closely related distinct species or merely geographical variants of a single widely ranging taxon, it is significant that the anatomy of their stems and roots is remarkably similar (Bailey 1961, 1963c). Therefore, in discussing the occurrence of "protein bodies" I shall confine myself at this time to collections from mature plants growing in Brazil, Venezuela, and Costa Rica.

In material of *P. grandifolia* kindly collected for me by Dr. Castellanos in Brazil, filamentous bodies occur in the vessels of stems and roots. The phloem of the larger roots contains abundant deposits of bodies in its ray and axially oriented parenchyma. These bodies are more or less closely aggregated minute hollow appearing globules or small rings, but exhibit frequent transitions to stellate forms as in *P. sacharosa*. The bodies in the phloem of the main stem are of similar forms but less abundant.

In specimens of a mature plant, either *P. grandifolia* or *P. bleo*, kindly collected for me by Dr. Steyermark in Venezuela, filamentous bodies occur in the vessels of both stems and roots, and globular ones in parenchymatous cells adjacent to vessels. The ray cells in the broad secondary phloem of the main stem and larger roots are densely packed with granular and globular contents, as are the unlignified cells in the first formed parts of the rays of the xylem in roots. In the tangentially expanded cells of the cortex, there are transitions to ring-shaped bodies (FIG. 9) and to frothy- or vacuolated-appearing formations (FIG. 10). In these cortical cells coarsely filamentous bodies are of sporadic and infrequent occurrence.

In material of an adult plant, either *P. grandifolia* or *P. bleo*, kindly collected by Dr. Rodríguez in Costa Rica, there is a tendency at times for fewer of the vessels to contain filamentous bodies than in comparable material of the two preceding collections from Brazil and Venezuela. The ray cells and axially oriented parenchyma in the broad zone of the secondary phloem of the main stem contain very abundant globular bodies. Where excessively abundant, they tend to fuse in highly diversified formations, viz. convoluted coarse strands, massive rings, and lobate formations (FIG. 8). Slender filamentous bodies are of sporadic and infrequent occurrence. The phloem in large roots of this plant contains less abundant deposits in finely globular forms, hollow appearing bodies, or rings of diverse sizes which when very abundant fuse to form extensive vacuolated or frothy deposits.

In a second category of pereskias [3] which includes *P. aculeata* Mill., *P. humboldtii* Britt. & Rose and allied taxa, and *P. pititache* Karw. (*P. conzattii* Britt. & Rose) and related species, globular bodies tend to occur in the xylem and phloem of stems and roots, filamentous forms being absent in my collections of these plants.

Within this category of pereskias, *P. pititache*, *P. autumnalis* (Eichlam) Rose and *P. nicoyana* Web. exhibit less divergent trends of anatomical specializations except in the basal parts of their large trunks (Bailey, 1963b). Globular bodies are of less abundant occurrence in the secondary xylem except at times in the first-formed parts of the rays in roots. In ray cells and axially oriented parenchymatous ones of the phloem, the globular bodies although varying considerably in size and number, commonly tend to be comparatively few and of relatively large size. However, they may fuse at times into beaded or lobed formations (FIG. 11).

In *P. humboldtii* and related taxa of Peru and Bolivia, which are of more or less reduced and modified stature, advanced trends of divergent anatomical specializations occur in the roots (Bailey, 1963a); those species being characterized by forming much enlarged tuberous swellings. The size and the distribution of globular bodies in the secondary xylem and phloem of the stems of these plants resemble those that occur in *P. pititache* and allied taxa. However, in some stems the parenchymatous cells of the phloem and cortex tend to form extensive massive deposits, but without the transitions to filamentous structures such as occur in *P. tampicana*. In parts of the root system, bodies of globular forms are abundant in the rays of the secondary xylem. Where closely aggregated, they may at times exhibit transitions to lobed formations or to short rods.

From ecological and physiological points of view, it is particularly significant that the soft succulent tissue in the large tuberous swellings is devoid of globular bodies as well as starch, but contains numerous large cells with mucilaginous contents and others with druses of calcium oxalate. This suggests that the tubers may function at least seasonally in the retention of moisture rather than as organs for the storage of starch and other food substances.

The scandent *P. aculeata* is anatomically the most divergently specialized representative of the genus *Pereskia* (Bailey, 1962). The variations in the structure of the stems and roots are so extensive and highly variable as to necessitate more adequate collections of this species than are available to me at present before reliable generalizations regarding the occurrence of globular bodies can be attained. In available material of stems and roots, the parenchymatous cells of the pith and cortex as well as those

[3] For a description of three anatomically distinct categories of pereskias see the second paper of this series (1961).

of the xylem and phloem contain abundant starch. Few of them contain granular and globular bodies. Filamentous and stellate bodies do not occur in the secondary phloem, nor in vessels of the xylem. The tyloses in vessels frequently contain abundant starch but infrequently globular bodies in addition. In some stems and roots, the cells of the phelloderm are devoid of starch and contain abundant granular and small globular forms of material.

Pereskias of the third category, viz. *P. guamacho* Web., *P. colombiana* Britt. & Rose, *P. cubensis* Britt. & Rose and *P. portulacifolia* Haw., tend to form trees under favorable environmental conditions. Divergent trends of anatomical specializations are largely confined to the roots (Bailey, 1963d), but without leading to the formation of tuberous enlargements such as characterize the pereskias of Peru and Bolivia. In the xylem and phloem of stems and roots of the four species, the bodies when present tend to be of globular forms, stellate and filamentous ones being absent. In some collections of *P. guamacho*, where the globular bodies become very abundant and closely aggregated in cells of the secondary phloem, transitions to more massive irregularly rounded deposits and frothy- or vacuolated-appearing ones occur in the ray and axially oriented parenchyma (FIG. 10).

It should be noted in passing that in the first category of pereskias, which characteristically tend to form filamentous bodies in many of their vessels, some of the fully matured lignified vessels may contain merely vestiges of protoplasmic contents and at times globular bodies. In the second and third categories of pereskias, which do not form filamentous deposits in their vessels, vestiges of protoplasmic contents may likewise persist in some of the fully matured vessels. Occasionally globular deposits may occur in a vessel as well as in parenchymatous cells adjacent to it.

In striking contrast to *Pereskia* the cells in comparable stems and roots of the relatively succulent and anatomically more divergent genera *Pereskiopsis* and *Quiabentia* (Bailey, 1964) do not contain filamentous, stellate or massive deposits of material. This is true regardless of whether collections were obtained from greenhouses, botanical gardens or from adult plants growing in their native arid environments. Although diversified forms of "protein bodies" do not occur in my extensive collections of stems and roots of *Pereskiopsis* and *Quiabentia* as they do in *Pereskia* when preserved in FAA,[4] conspicuous spindle-shaped ones occur at times in the epidermal and sub-epidermal cells of living leaves of *Pereskiopsis* [*Boke B-18*] when grown in a greenhouse at Norman, Oklahoma. These bodies closely resemble in size and form those encountered by Molisch (1885, *Fig. 1*) in living tissue of *Epiphyllum*. In the epidermis the bodies, commonly one to each cell (FIG. 12), are oriented parallel to the surface of the leaf, whereas in the mesophyll they are arranged more or less at right angles to the surface. As in *Epiphyllum*, the spindles are composed

[4] Forty per cent formaldehyde, five parts: glacial acetic acid, five parts: 50 per cent ethyl alcohol, ninety parts.

of more or less closely compacted aggregations of slender filaments arranged parallel to the long axis of the body. In living cells, the bodies are conspicuously anisotropic in polarized light, indicative of crystalline composition. In some leaves, the bodies consist at times of diffusely aggregated strands of coarser texture. Bodies of similar size and form occur in some living leaves of *Pereskia aculeata* and *P. grandifolia*.

Such occurrences in living leaves of *Pereskia* and *Pereskiopsis* suggest that material conveniently available in greenhouses should provide favorable specimens, not only for a reconnaissance of the occurrence of spindle-shaped bodies in various species of *Pereskia* and *Pereskiopsis*, but also critical material for detailed cytological, physiological, and biochemical investigations.

DISCUSSION

The structures dealt with on preceding pages obviously are extraordinarily variable in size, form, abundance, and distribution in different parts of a plant, but commonly exhibit transitions from one form to another, not only in neighboring cells of the same tissue, but also within the limits of a single cell. This is suggestive of possible fundamental similarities in their composition.

In the case of living tissue of *Epiphyllum*, which contains many of the essentially similar diverse forms of these bodies, Molisch's extensive tests of the solubilities, color reactions, and general behavior of the bodies in acids, alkalies, and various other chemical reagents provided much cumulative evidence in substantiation of proteinaceous composition.

In my collections of stems and roots preserved in FAA the diverse forms of the bodies give consistently rapid positive tests with Millon's reagent, as do the spindle-shaped ones in living leaves of *Pereskiopsis* prior to their longitudinal contraction, lateral expansion, and ultimate solution. Unfortunately this test, *by itself*, is not conclusive proof of solely proteinaceous composition. However, in stems and roots fixed in FAA, the bodies of crystalline and amorphous forms give a positive violet coloration with the Biuret test. The crystalline spindle-shaped bodies in living leaves dissolve so rapidly in copper sulphate and sodium hydroxide that it is difficult to obtain a reliable positive test with the Biuret reagents. But, when the living tissue is killed with the aldehyde fixatives now utilized in light and electron microscopy, the bodies persist for a number of days giving a positive violet coloration with the Biuret test. Furthermore, when sections are treated with acid fuchsin the bodies stain as do other cell constituents of known proteinaceous composition. In addition, the consistent similarity in the staining of the bodies and that of cell inclusions of known proteinaceous composition when treated with toluidine blue, Haedenhein's iron alum haematoxylin, and other staining reagents, provides much cumulative corroborative evidence. Thus, there appears to be no valid objection to concluding that the bodies in question are at least of partly proteinaceous composition.

Of course, in dealing with the color reactions of the smaller forms of the diversified bodies, it is essential to differentiate them with care from nuclei, coagulated protoplasm, plastids, and from deposits of "slime" in sieve tubes.

The common occurrence and abundance of the diverse forms of protein bodies raises a number of fundamental questions regarding metabolic phenomena in cells of the Cactaceae. In my extensive collections, such bodies do not occur in cells concerned in the formation of mucilage or crystals of calcium oxalate. Nor do they occur in the lignified libriform fibers which function at least seasonally in the storage of starch. Although not invariably absent in lignified parenchyma containing starch or invariably present in unlignified cells devoid of starch, there appears in general to be an inverse correlation between metabolic activity in cells concerned in the storage of starch and that during the deposition of abundant protein bodies.

In the case of plants growing in their arid native habitats, the abundance of proteinaceous bodies in the basal part of the main stem and larger roots raises questions of much ecological, physiological, and biochemical significance. Are such occurrences indicative of at least seasonal accumulation of reserves of utilizable proteinaceous substances? Molisch concluded that the protein bodies are reserve products whereas Chmielewsky (1887) regarded them as excretions. Neither of these investigators provided conclusive evidence in support of their generalizations. The filamentous bodies in fully matured vessels of the xylem and those in crushed dead parts of the phloem may no longer be potentially available to the plants and thus might be regarded as excretory. The only evidence available to me at present in favor of Molisch's interpretation is in two plants having affinities to *Pereskia sacharosa*. In the case of specimens obtained by Carenzo and Legname from a plant growing in its native habitat in northern Argentina, the collections were made during the resting, "winter" season after abscission of the leaves. In this plant, as indicated earlier in this paper, abundant protein bodies occur in the basal part of the main stem and larger roots. On the contrary, in comparable material collected for me by Dr. Cárdenas during the active growing and flowering season, but from a plant at somewhat higher elevation in Bolivia, filamentous bodies are present in the vessels, but diverse forms of proteinaceous bodies are absent or of sporadic occurrence in the phloem, cortex, and phelloderm. In spite of possible taxonomic and environmental uncertainties, this at least suggests that proteinaceous material in living cells of the phloem and cortex, present during the resting season, may be utilizable during seasons of active growth. More adequate and conclusive evidence can be obtained only by detailed investigations of living plants of the same taxon growing together in the same habitat and at different seasons of the year.

Evidence obtainable from my preliminary investigations of the form, distribution, and abundance of protein bodies in more or less haphazardly available collections indicates much variability in different tissues, not only in different collections of the same taxon, but also in stems of different sizes and stages of development within the same plant. For adequate clarification of the significance of this variability it will be necessary to study the living tissues of stems and roots of plants growing in their native arid environments. From significant ecological, physiological, and biochemical points of view it is essential that this be done, particularly in adequate correlation with metabolic phenomena involved in the production of mucilage, of large amounts of organic acids and their calcium salts, and also in the accumulation and possible seasonal depletion of unusually abundant starch in the secondary xylem of large stems and roots.

It should be noted in conclusion that my collections from adult plants growing in their native habitats were preserved in FAA. The diverse forms of protein bodies in stems and roots have not dissolved after prolonged treatment in this fixative and subsequent mounting in diaphane for permanent slides. However, the possibility exists that changes in form may be induced by fixation in FAA. Molisch (1885) found that in the case of living cells of ***Epiphyllum*** the elongated and spindle forms of the bodies contracted longitudinally and expanded laterally prior to dissolving in acids and other reagents. The spindle-shaped bodies in living leaves of *Pereskiopsis* behave similarly. Even in the least drastically modifying modern methods of fixation, the spindles may expand, or they may contract more or less laterally and at times even longitudinally. Transitions from anisotropic crystalline composition to isotropic amorphous form occur in some reagents.

The persistence of anisotropic filaments and elongated bodies in association with isotropic globular ones in the same section of a stem or root makes it appear unlikely that all of the amorphous globular and more massive forms are due to modifications of crystalline filamentous ones in FAA. I had hoped to test the possibility of such changes by obtaining living material from easily accessible greenhouses. Thus far none of the numerous stems from the unnatural environments of greenhouses contain protein bodies after fixation in FAA with exception of two collections from the Missouri Botanical Garden. Unless I can obtain living stems of such sporadically occurring specimens in greenhouses, it will be necessary to study the behavior of globular forms of bodies in living stems and roots of plants growing in their native habitats.

The presence of filamentous bodies in the vessels of one category of pereskias and their absence in the vessels of two other categories, and of *Pereskiopsis* and *Quiabentia* provides some evidence of considerable taxonomic significance. However, the occurrence, distribution, and diversified forms of the protein bodies in haphazard collections of the second and third categories of pereskias are highly variable. Before utilizing conspicuous differences in the form and distribution of protein bodies as reliable diagnostic criteria in differentiating species, extensive collections of different plants must be obtained and studied in detail. Such an extensive investigation is hardly justifiable from a solely taxonomic point of view unless significant ecological, physiological, and biochemical data are obtained at the same time.

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EXPLANATION OF PLATES

(All "protein bodies" stained with Millon's reagent or Haedenhein's iron-alum haematoxylin for photographic purposes.)

PLATE I

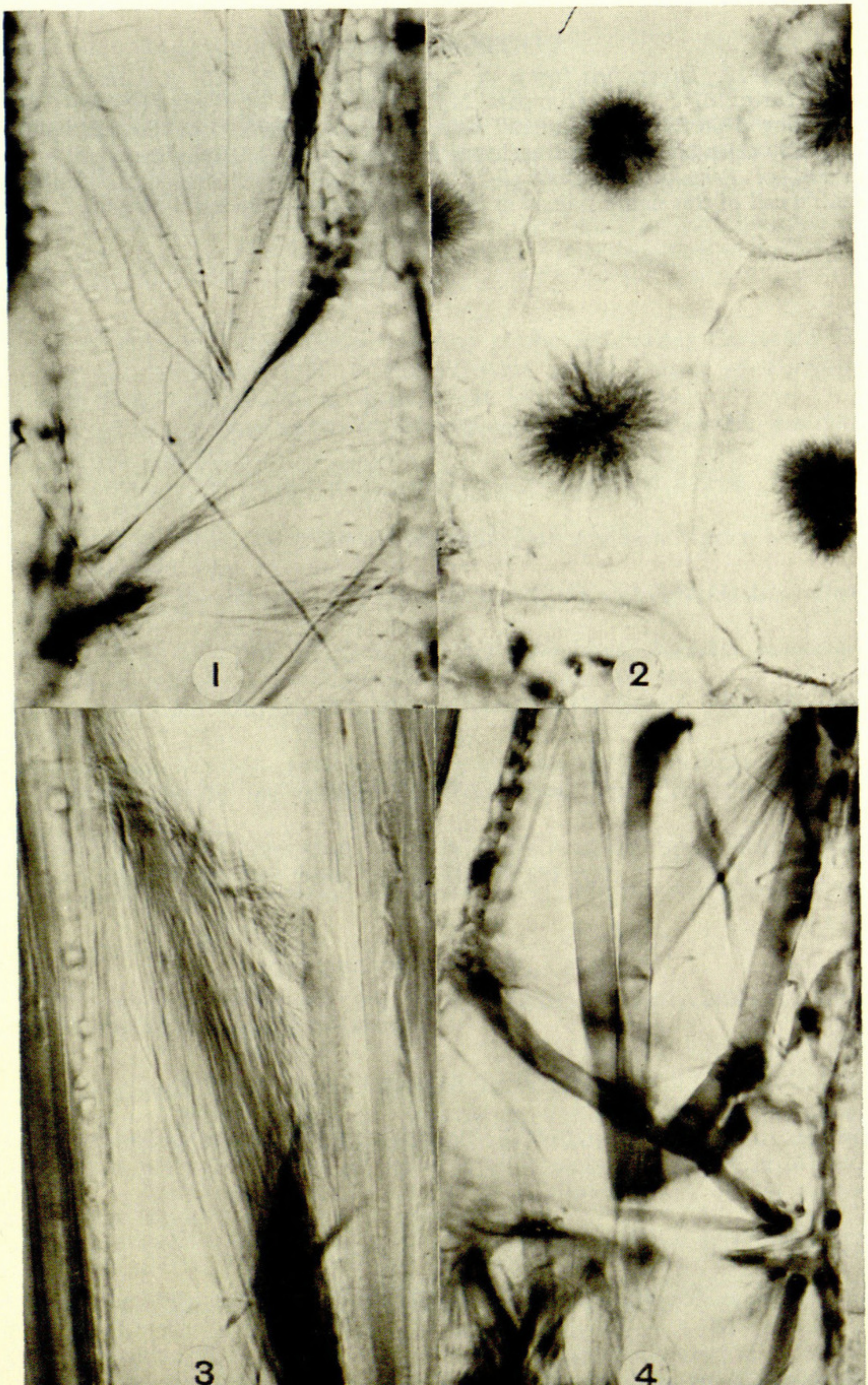
FIGS. 1–4. "Protein bodies" in *Pereskia*, × 510. 1, Diffusely distributed tenuous filaments in vessel of *P. grandifolia*. 2, Stellate aggregations of tenuous filaments in unlignified phloem parenchyma of *P. sacharosa*, stained in Millon's reagent. 3, Aggregated filaments in vessel of *P. tampicana*. 4, Coarse strands and tenuous filaments in vessel of *P. grandifolia*.

PLATE II

FIGS. 5–8. "Protein bodies" in *Pereskia*, FIGS. 5, 7, and 8 × 510, FIG. 6 × 1130. 5, Globular bodies in axially oriented wood parenchyma adjacent to vessel of *P. tampicana*. 6, Globular bodies in ray cell adjacent to vessel of *P. tampicana*. 7, Transitions from tenuous filaments to massive plates in phloem parenchyma of *P. tampicana*. 8, Spherical and hemispherical bodies in phloem parenchyma of *P. bleo*; bodies adhering to walls of cells with transitions to coarse convoluted strands.

PLATE III

FIGS. 9–12. Diversified forms of "protein bodies" × 510. 9, Large rings in outer cortex of *P. bleo* (compare Molisch *Fig. 2*). 10, Vacuolated or frothy appearing deposits in phloem ray cells of *P. guamacho*. 11, "Protein bodies" in axially oriented phloem parenchyma of *P. pititache*. 12, Spindle-shaped bodies in leaf of *Pereskiopsis* [*Boke B-18*], nucleus with nucleolus near spindle in central cell of this figure stained in Haedenhein's iron-alum haematoxylin.



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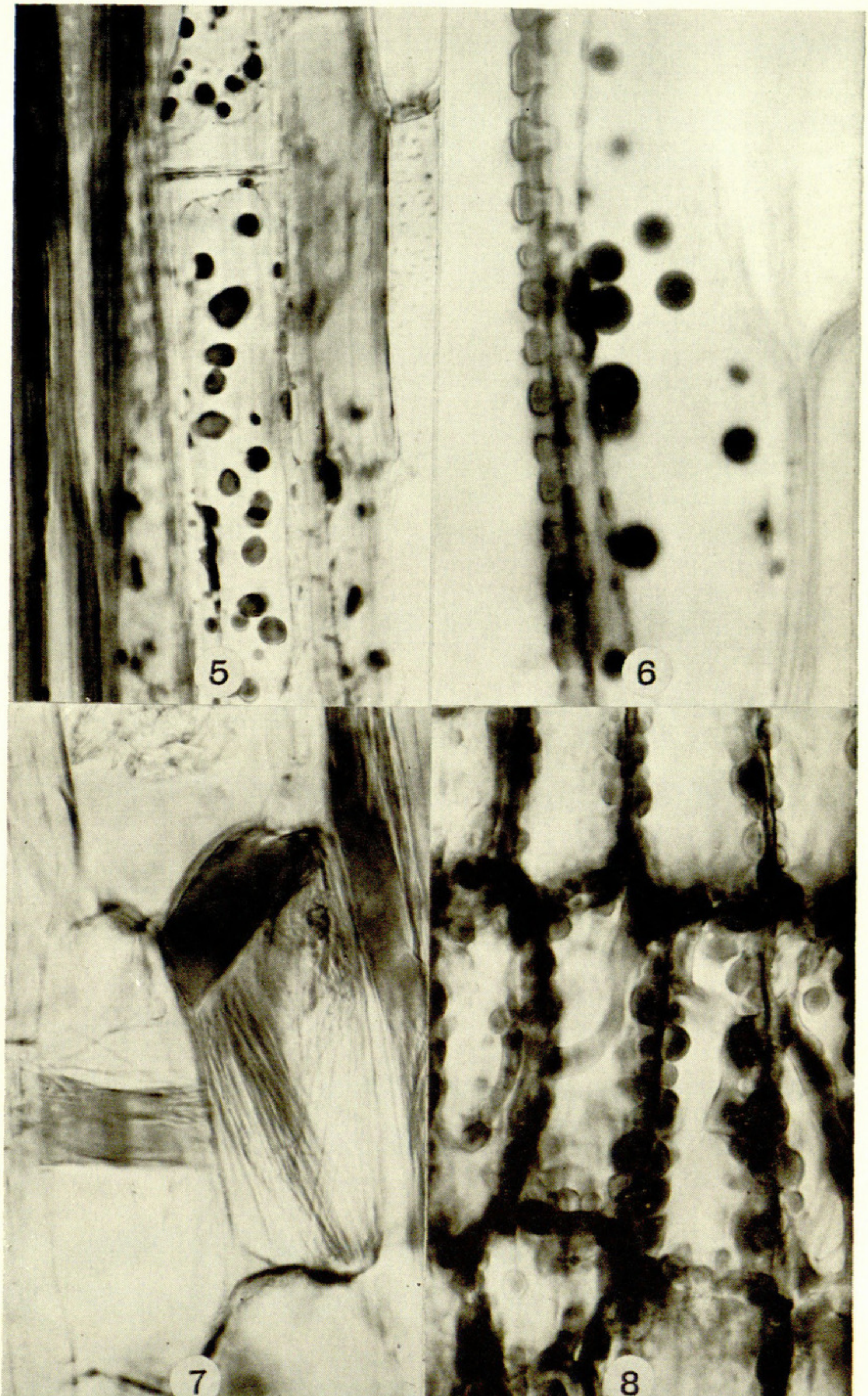

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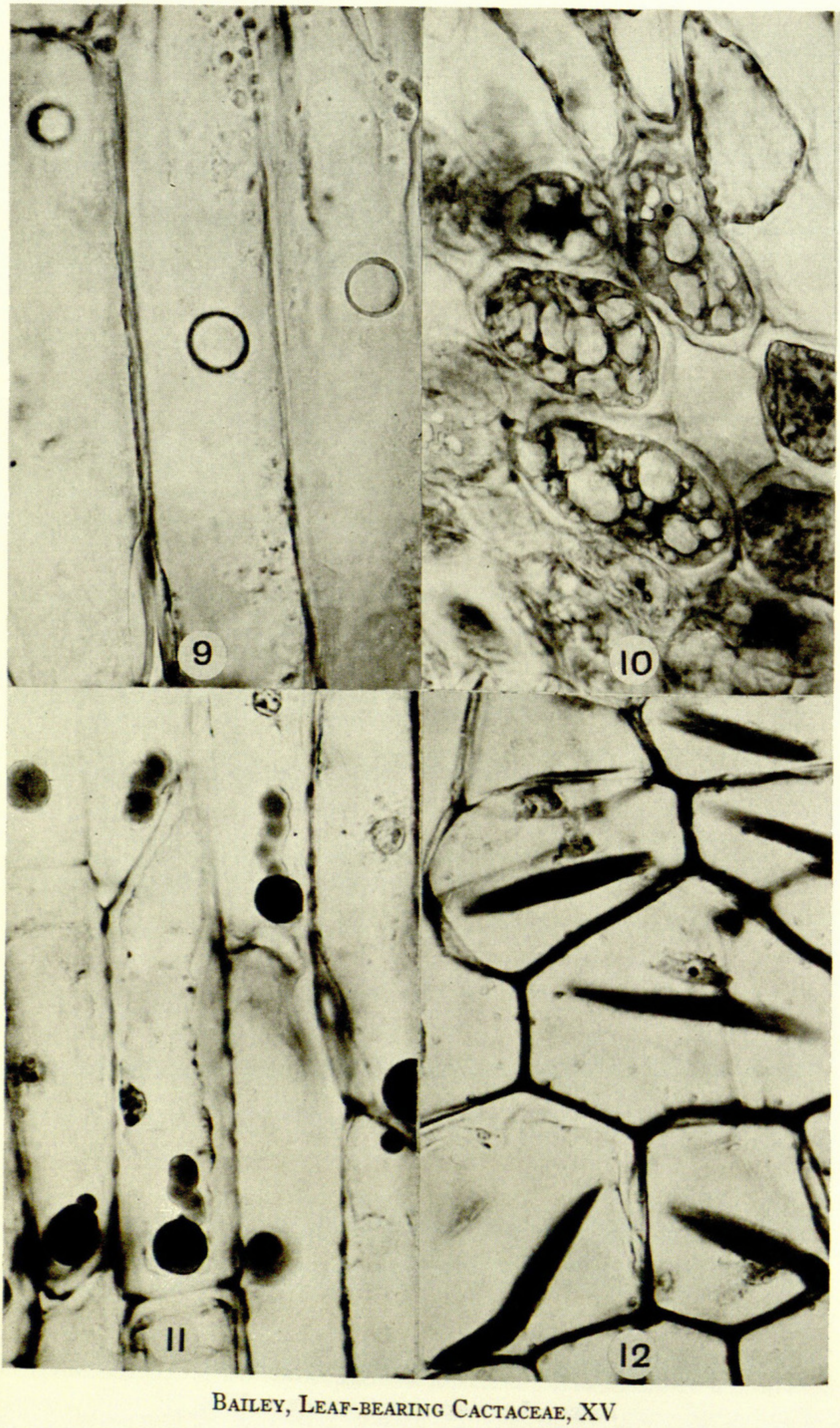

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