

# RESEARCH PAPERS

## THE STRUCTURE OF THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

BY S. ROHATGI AND J. W. FAIRBAIRN

*From the Pharmacognosy Research Laboratory, School of Pharmacy, University of London*

Received March 1, 1950

### INTRODUCTION

THE flowering tops of *Hyoscyamus niger* have been included in the British Pharmacopœia since 1864 as part of the official drug except during the period 1914 to 1932. Although the British Pharmacopœia, 1948, provides a brief description of the gross morphology of the flowers, it gives only scanty details of the histology. A more complete description of the gross morphology of the flower is given by Bentley and Trimen in "Medicinal Plants,"<sup>1</sup> and Moll and Janssonius in their "Botanical Pen Portraits"<sup>2</sup> give an account of the histology of the flower, which is not illustrated by drawings. The histology of the seed appears with drawings in the Anatomischer Atlas of Berg<sup>3</sup> and in that of Tschirch and Oesterle<sup>4</sup>.

It was decided therefore to prepare adequate illustrations of the histological characters; this made it necessary to reinvestigate the histology of the flower. This has been done and new descriptions are given to explain the details of the drawings. A careful investigation of this kind was also required for the purpose of making a comparative study of the flowers of other closely related plants used as drugs. The gross morphology has also been reviewed as an introduction to the histological work. In the course of this work, certain details not hitherto recorded have been noted and incorporated in the new descriptions now given.

### MATERIAL

For the purpose of this work, 4 samples of the biennial plant, cultivated during different years and in different places, were examined to provide material as representative of various habitats as possible. The following is the list of samples used:

1. Flowers cultivated at Long Melford, 1923.
2. Flowers from plants grown at Mill Hill, July, 1938.
3. Flowers and fruit from plants grown at Mill Hill, September, 1945.
4. Flowers from plants grown at Mill Hill, collected in June and July, 1949.

### GROSS MORPHOLOGY

The inflorescence is described by Bentley and Trimen<sup>1</sup> as a two-ranked, unilateral raceme, whereas Sachs<sup>5</sup> describes the inflorescence as a scorpionoid cyme and Tschirch and Oesterle<sup>4</sup> refer to it as cymose. The coiled inflorescence elongates and straightens out as the fruits develop, forming an axis about 10 cm. long, carrying about 10 to 20 sessile or very shortly

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

stalked flowers, each very slightly displaced from the axil of a large, leafy spreading bract; the flowers being arranged in two vertical rows on the axis with the oldest flowers at the base.

The flowers are hermaphrodite and slightly irregular.

*Calyx* : (Fig.1, A). *Sepals* 5, gamosepalous, inferior, forming a somewhat urceolate green calyx, covered with long clammy trichomes, abundant on the outer surface of the basal half of the tube; *tube* 4 to 15 mm. long; *lobes* triangular, 2 to 5 mm. long, slightly spreading and each terminating in a sharp apical spine; *æstivation* free; calyx persistent in the fruit.

*Corolla* : (Figs. 1, A and 4, A). *Petals* 5, alternating with the sepals, gamopetalous, campanulate-infundibuliform, forming a narrow *tube* about 10 to 25 mm. long; *lobes* 5, slightly unequal, rounded, 2 to 4 mm. long, inner surface velvety, colour yellow with purple veins, merging into

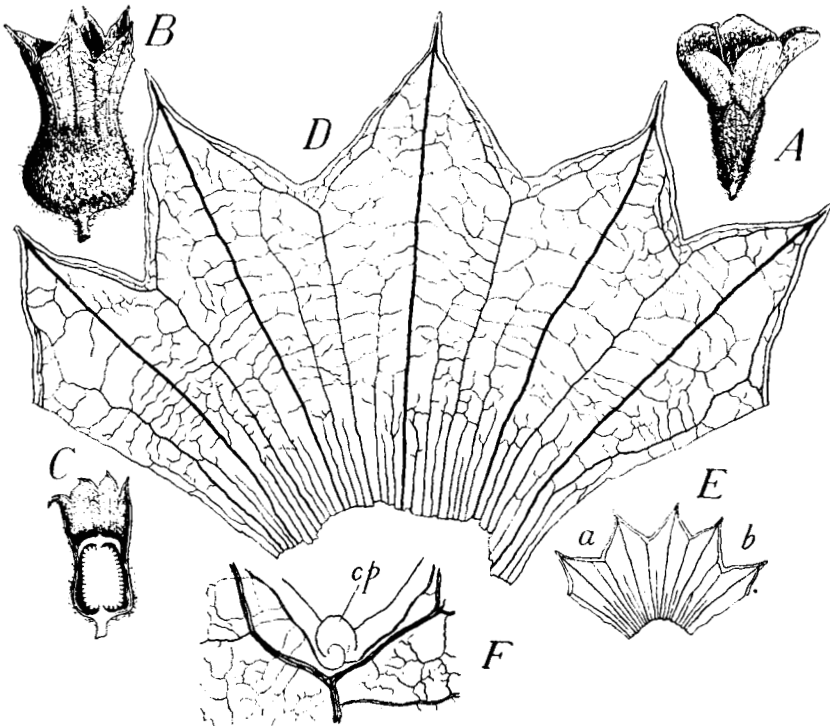


FIG. 1.—*Hyoscyamus niger* Linn., A, Flower ( $\times 1$ ). B, Fruit ( $\times 1$ ). C, longitudinal section through a partly mature fruit ( $\times 1$ ). D, Calyx, spread out, showing the 5 lobes and the venation ( $\times 4$ ). E, Calyx showing absence of fusion between the lateral veins leading to the sinuses *a* and *b* ( $\times 1$ ). F, details of venation at the apex of a sinus showing an early stage in the development of a cusp ( $\times 12$ ). *cp*, cusp.

a general bluish-green colouration at the lower part of the tube which is white at the base; trichomes frequent on the outer surface of the lobes, absent on the inner surface, rare on the tube; *æstivation* imbricate; corolla fugaceous.

*Andræcium* : (Fig. 4, A). *Stamens* 5, alternating with the corolla lobes,

epipetalous, the free parts of the filament about 10 to 14 mm. long, the part adherent to the corolla being about 5 to 10 mm. long and covered with abundant trichomes. The 5 filaments which are gently curved away from the petals, vary in length and in the degree of adnation to the corolla. The length of the adherent portion varies inversely as the length of the free portion. *Anthers*, deep purple, dorsifixed at a point slightly lower than the middle, slightly curved with the convex surface inwards; dehiscence introrse along two longitudinal lateral splits before the flower is fully opened.

*Gynæcium*: (Fig. 7, A). *Carpels* 2, syncarpous, superior. *Ovary* (Fig. 1, C) sphæro-conical, about 3 to 5 mm. in length and 2 to 3 mm. in diameter at the widest part; bilocular, with an external equatorial groove corresponding to the line of dehiscence of the fruit, becoming more marked as the ovary matures, placentation axile, ovules numerous. *Style*, arising from the apex of the ovary, about 2 cm. long, curving in the upper part which is purple, towards the anterior petal. It terminates in a fleshy, rounded, capitate *stigma* (Fig. 7, B.), which has a funnel-shaped hollow in the centre, extended on either side as a shallow groove reaching almost to the margin.

*Fruit*: (Fig. 1, B). The pyxis or fruit proper is surrounded by the enlarged, persistent and markedly urceolate calyx, which is from 20 to 27 mm. long, most commonly 25 mm., the sepals being stiffer than in the flower, the inner surface glossy, the outer surface hairy. The spine at the apex of each lobe becomes more pronounced and a small cusplike development at the apex of each sinus between the lobes (Fig. 1, F). The lower  $\frac{3}{4}$ th of the pyxis is thin walled, but the lid, which separates by circumscissile dehiscence, is thicker and woody. The fruit contains numerous small, brown, somewhat reniform seeds.

#### HISTOLOGY OF THE CALYX

*Outer or lower epidermis*: The cells of the outer epidermis vary along the length of the sepal. On the lobes, the cells have very wavy anticlinal walls except over the veins, the margin and the tips, where they are axially elongated with less wavy and sometimes nearly straight anticlinal walls (Figs. 2, A and B). Further down the calyx the cells have progressively less wavy anticlinal walls and become smaller and thicker walled until, at the base, they are small, sub-rectangular and thick walled (Fig. 2, D). *Dimensions*: On the lobes, L and T = 15 to 70 to 125  $\mu$  and R = 11 to 21 to 53  $\mu$ \*; over the vein, however, L might be as much as 400  $\mu$ . At the base, L and T = 6 to 21 to 53  $\mu$  and R = 17 to 18  $\mu$ . The odd subsidiary cell adjoining the stomata in the cruciferous arrangement may be as small as L and T = 6  $\times$  15  $\mu$ . The cells under the larger trichomes are less wavy, sometimes straight walled and larger than the neighbouring cells, occasionally measuring as much as 75  $\times$  90  $\mu$ . Flowers from plants cultivated during a dry

\* R, T and L indicate the measurements made in the radial, tangential and longitudinal directions respectively; the use of these symbols is suggested by Moll and Janssonius in their "Botanical Pen Portraits." In cases where some cells in a given tissue show elongation in the longitudinal and others in the tangential direction, readings for L and T have been combined.

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

season were found smaller in size with the cellular dimensions correspondingly smaller. Typical cruciferous (anisocytic)<sup>6</sup> stomata occur and are usually slightly raised above the surface of the epidermis (Fig. 2, G). They are numerous on the lobes but gradually become less frequent in the middle region of the calyx until at the base they are rare or absent.

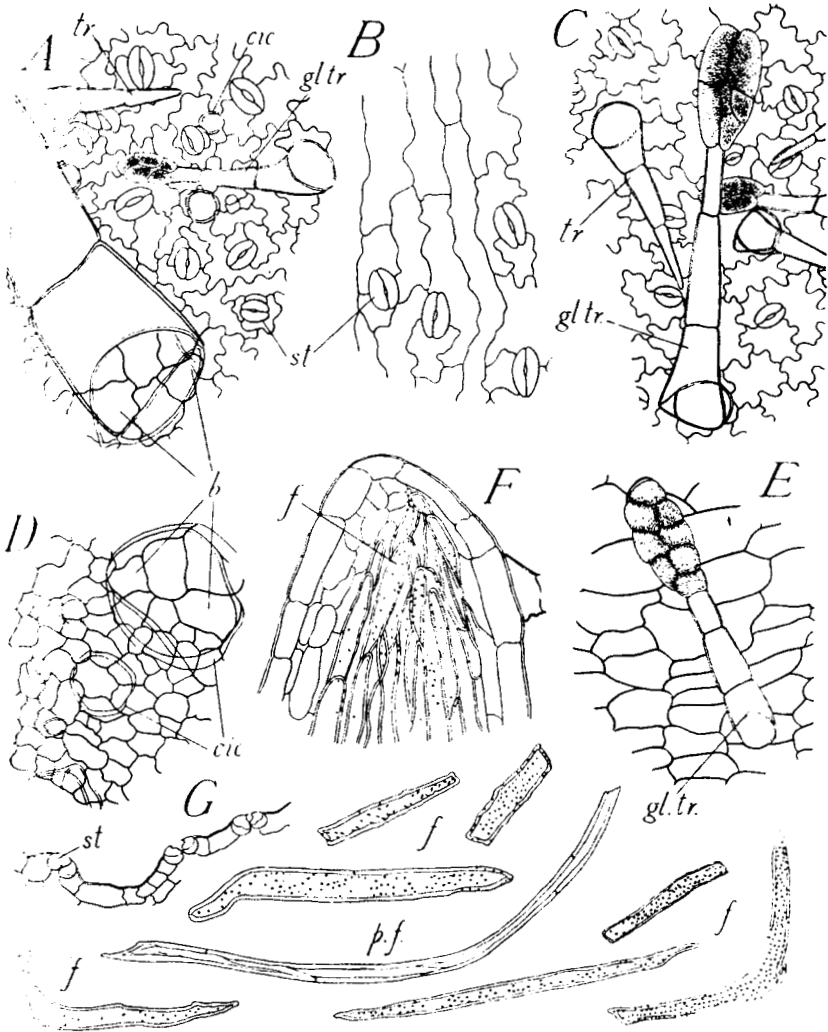


FIG. 2.—*Hyoscyamus niger* Linn., Calyx. A, Outer epidermis of a lobe. B, Outer epidermis over midrib near the tip of a lobe. C, Inner epidermis of a lobe. D, Outer epidermis at the base of the tube with the trichomes removed. E, Inner epidermis at the base of the tube. F, Longitudinal section through the tip showing the bundle of fibres strengthening the spine. G, Transverse section through the middle of a sepal showing details of the stomata. *b*, large epidermal cells under a trichome; *cic*, cicatrix; *f*, xylem fibres; *gl. tr.*, glandular trichome; *p.f.*, part of a pericyclic fibre, half in length; *st*, stoma; *tr*, covering trichome. All  $\times 150$ .

Numerous covering and glandular *trichomes* occur on the entire epidermis, but are more crowded and longer towards the base of the calyx.

*Inner or upper epidermis*: The cells are similar to the corresponding cells of the outer epidermis (Fig. 2, C), except at the base where they are comparatively larger (Fig. 2, E), measuring about  $L$  and  $T = 9$  to  $40$  to  $140 \mu$  and  $R = 7$  to  $12 \mu$ . Numerous cruciferous stomata appear in all parts of the epidermis, although they are not so frequent at the base of the tube. *Trichomes*, similar to those on the lower epidermis, occur on the upper but are not so numerous. Very large trichomes similar to those on the outer surface of the base of the tube are absent, the basal portion being nearly glabrous.

*Trichomes*: Two types of trichomes occur on the calyx, glandular and covering. (a) *Glandular trichomes*, which are more numerous and resemble those of the foliage leaves, usually have a 1 to 4-celled uniseriate stalk and a large multicellular, ovoid or club-shaped glandular head containing yellowish-brown contents. The larger glandular trichomes occur chiefly on the lower part of the calyx and have a uniseriate stalk of up to 12 cells. The glandular trichomes frequently contain small crystals of calcium oxalate in the form of prisms, rosettes, single or double pyramids, the latter sometimes with truncated ends, micro-crystals of indeterminate shape and abundant minute globules of volatile oil. The oil appears as globules which stain yellowish-brown with iodine solution and orange-yellow with Scarlet R solution. The globules are soluble in alcohol and can be reprecipitated by addition of water; the tests indicating presence of volatile oil. The oil globules are present in dried specimens, but are absent from samples preserved in alcohol which has dissolved the oil. The majority of the glandular trichomes measure about 270 to 900 to 2160  $\mu$  in length, the larger ones being sometimes as long as 1 cm.; diameter at the base being 50 to 180  $\mu \times$  40 to 90  $\mu$ . The cicatrix left by a large glandular trichome appears as a prominent ring in surface view and may cover one much enlarged cell or 2 to 12 large epidermal cells, the latter being larger than the adjoining ones and having a less wavy outline (Figs. 2, A and D). (b) *Covering trichomes*, short, erect, uniseriate, 1 to 4-celled and tapering, are numerous over the entire calyx; occasional longer uniseriate trichomes have a stalk of up to 12 cells, the terminal cell of these long trichomes being either bluntly conical or cylindrical and rounded at the tip. They occur chiefly on the outer surface of the base of the tube. The covering trichomes are 105 to 175 to 228  $\mu$  long, the very long trichomes attaining a length of about 3.5 mm.; diameter at the base approximately 25 to 35  $\mu$ .

*Mesophyll*: This consists of 4 to 7 layers of thin-walled parenchyma irregularly arranged, the number of layers and the size of individual cells decreasing from apex to base, where the cells are also more closely packed, so that the calyx is thinner at the base than at the apex (Fig. 3). Calcium oxalate *crystals* are abundant in the mesophyll of the calyx. They occur as prisms or irregularly shaped crystals, either singly or in small groups and increase in density from apex to base. Idioblasts, containing a few large triangular shaped crystals together with a mass

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

of micro-crystals of various forms, triangular ones being more frequent, also occur in the tube and become more frequent towards the base. In the fruit, the calcium oxalate crystals increase in the calyx, appearing as clusters and micro-sphenoidal masses in the tube and as isolated square prisms or groups in the lobes. The increase in the number and dimen-

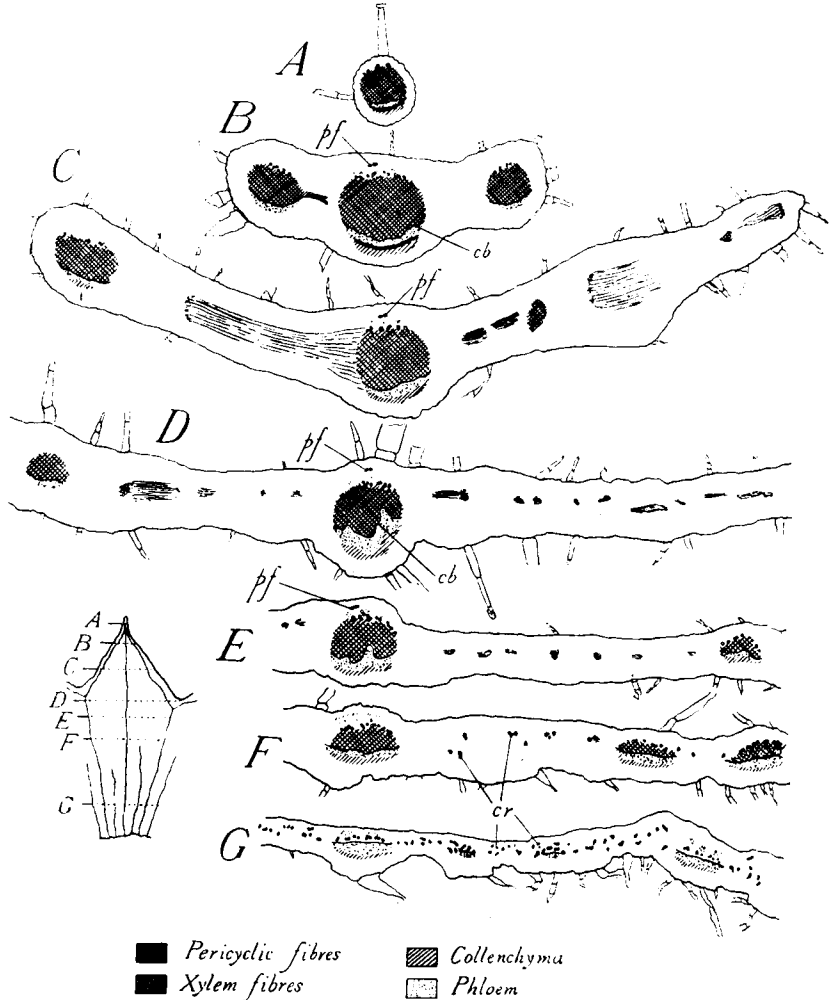


FIG. 3.—*Hyoscyamus niger* Linn., Calyx. Series of 7 transverse sections through a sepal showing diagrammatically the thinning of the lamina from apex to base, the distribution of tissues in the midrib and of the microsphenoidal crystal idioblasts at the base. The diagram in the corner at the bottom indicates the position on the sepal at which each section was cut. *cb*, cambium; *cr*, crystal-sand idioblast; *p.f.*, pericyclic fibre. All sections  $\times 25$ .

sions of the calcium oxalate crystals as the calyx grows older agrees with the findings of Anselmino and Gilg<sup>7</sup> who examined foliage leaves of gradually increasing ages with a similar result as to the accumulation of calcium oxalate.

*Venation* : (Fig. 1, D). Each sepal has a well marked midrib which terminates in the spine of the lobe; between the midribs is a further well marked vein ending at the sinus between the lobes and joining with the prominent marginal vein, the latter running up to and strengthening the spine from either side. Between this prominent marginal vein and the actual margin of the calyx, there is a much more delicate marginal vein connected with the stronger one by numerous very slender branches. Beyond this more delicate marginal vein there are only a very few projecting vascular elements. As the two marginal veins approach the spine of the calyx lobe, they unite to form a single strong vascular strand. Branches also enter the cusps which are usually well developed in the fruit and contain strongly lignified elements (Fig. 1, F). Occasionally, in place of the single well-marked vein between the midribs, there occur two parallel veins; these represent the lateral veins of the sepals which, although normally fused, have escaped fusion<sup>8</sup> (Fig. 1, E). Several short, thinner veins arise at the base of the tube running vertically and parallel to the main veins and ending at different levels in the lower half of the tube. All the veins anastomose freely forming a dense network (Fig. 1, D).

In transverse sections (Fig. 3) the vascular strand of each midrib is small and oval in outline at the base of the calyx, gradually becoming larger and more circular in the lobes due to the gradual development of a mass of lignified xylem fibres with bluntly pointed ends. At the tip itself, the midrib is further enlarged by fusion with the two marginal veins from either side; the group of xylem fibres becomes enlarged and pericyclic fibres arise in the otherwise collenchymatous pericycle. The resulting cylinder of fibres forms the bulk of the spine and makes it remarkably stiff (Fig. 2, F). In the persistent calyx of the fruit, a development of lignified xylem fibres usually takes place in the vascular strands of the other larger veins also.

#### HISTOLOGY OF THE COROLLA

*Outer or lower epidermis*: The epidermal cells vary greatly from apex to base. On the lobes, the anticlinal walls have well-marked infoldings which are very characteristic (Fig. 5, Ao). Over the veins, the cells are elongated, do not have infolded anticlinal walls and are slightly papillose (Figs. 5, Av and 4, B). The infoldings gradually disappear towards the base, the cell walls becoming merely sinuous and finally straight; the cells at the base being sub-rectangular and comparatively thick walled (Figs. 5, Do, Eo and Fo). Each cell possesses a well-marked nucleus which is centrally placed on the inner wall, except at the base of the corolla, where it lies against one of the side walls. On the lobes of the petals, the epidermal cells measure approximately L and T = 25 to 40 to 63  $\mu$  and R = 11 to 14 to 25  $\mu$ ; over the veins some of the cells may be as long as about 125  $\mu$ . Further down, near the lower part of the lobes, the cells are larger. At a height of about 1/3rd from the base of the petal, the cells measure approximately L = 90 to 100 to 163  $\mu$ , T = 15 to 30 to 40  $\mu$  and R = 13 to 20 to 35  $\mu$ . In the basal part of

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

the tube, the cells measure about,  $L = 30$  to  $63$  to  $90 \mu$ ,  $T = 15$  to  $25$  to  $38 \mu$  and  $R = 40$  to  $45$  to  $55 \mu$ . Occasionally, cruciferous *stomata*, slightly raised above the general surface of the epidermis, occur towards the base of the corolla, but are rather rare on the lobes. The stomata measure approximately  $38\mu$  long and  $20\mu$  broad. Short stalked glandular trichomes with a 1 to 4 celled uniseriate stalk and a small head

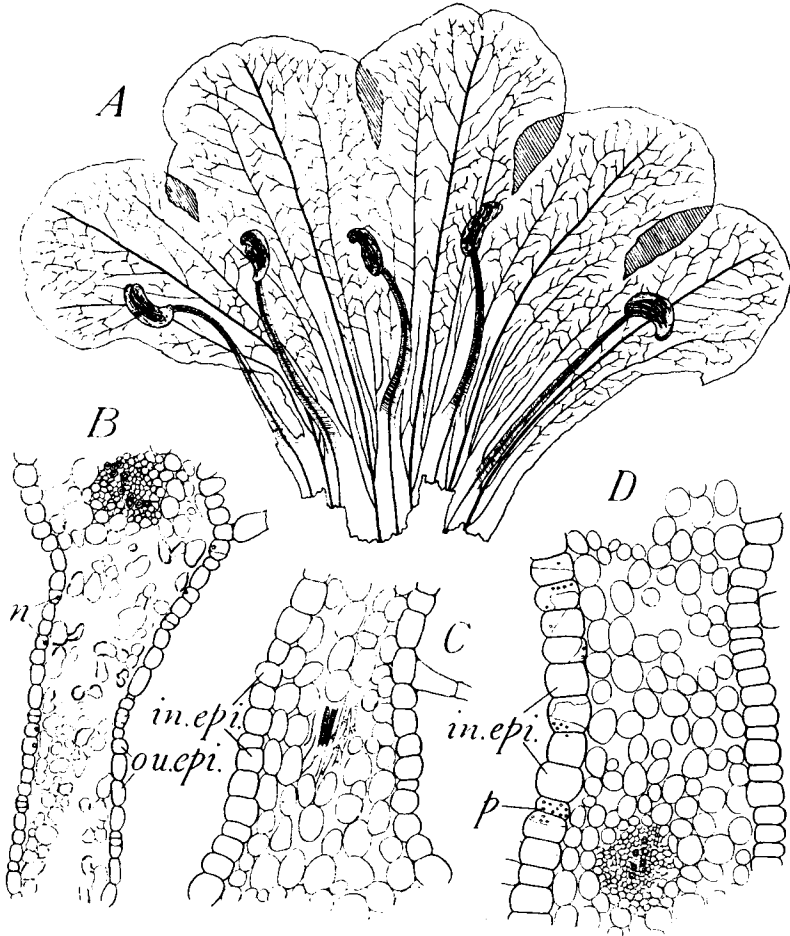


FIG. 4.—*Hyoscyamus niger* Linn., Corolla. A, Corolla spread out showing venation and stamens ( $\times 3$ ). B, Transverse section through the lobe of a petal. C, Transverse section through the middle of a petal. D, Transverse section through the tube at a position just above the actual base. *in. epi.*, inner epidermis; *n*, nuclei; *ou. epi.*, outer epidermis; *p*, pits on anticlinal walls of the inner epidermis. All  $\times 100$  except A.

of 1 to 4 cells or, more commonly, a large head of 10 to 30 cells filled with yellowish-brown contents are numerous, but much less so than on the calyx. The stalks of these glandular trichomes are longer towards the base of the tube. The trichomes often contain minute oil globules.



Scattered uniseriate covering trichomes, about 1 to 3 to 4 celled, also occur, but are comparatively rare. The glandular trichomes measure about 144 to 270 to 630  $\mu$  long, have a diameter of about 36 to 54 to

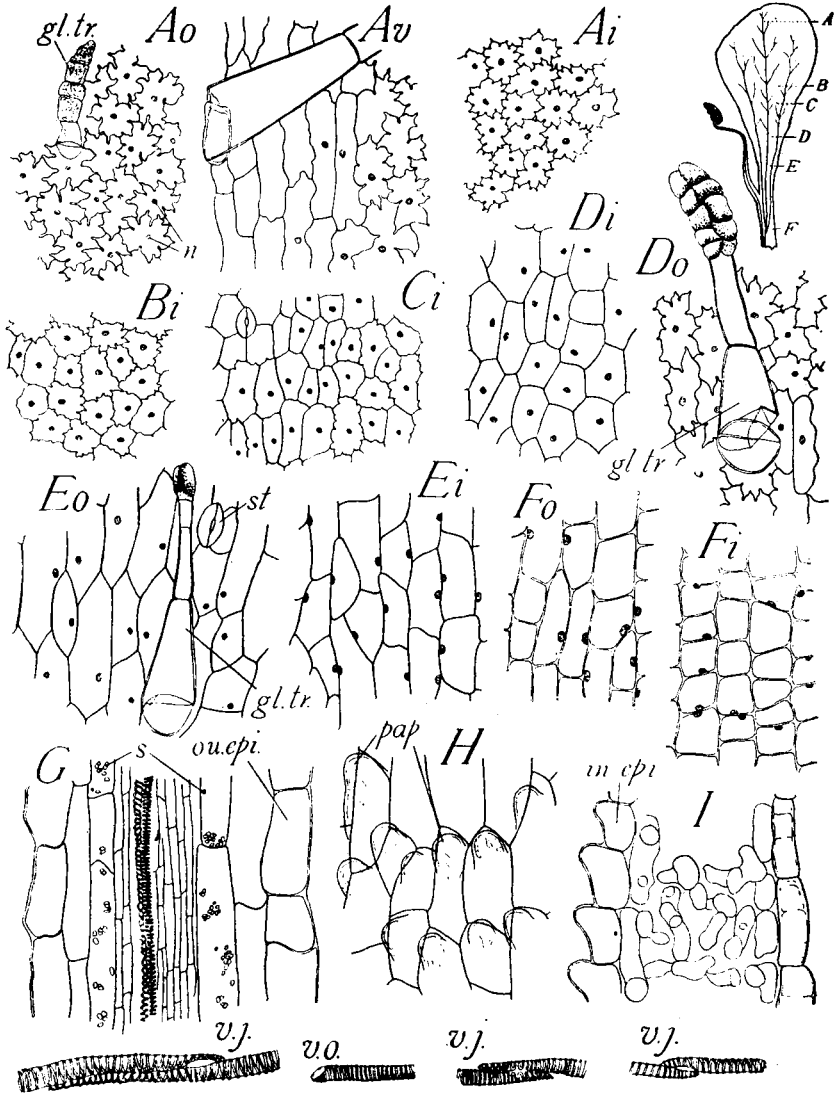


FIG. 5.—*Hyoscyamus niger* Linn., Corolla. *Ao*, outer epidermis at A (as marked on the diagram, top right); *Av*, outer epidermis at A over a vein; *Ai*, inner epidermis at A; *Bi*, inner epidermis at B; *Ci*, inner epidermis at C; *Di*, inner epidermis at D; *Do*, outer epidermis at D; *Eo*, outer epidermis at E; *Ei*, inner epidermis at E; *Fo*, outer epidermis at F; *Fi*, inner epidermis at F. *G*, longitudinal section through the basal part of the tube passing through a vein and showing starch grains. *H*, surface view of inner epidermis of the tube near position E showing papillae; *I*, longitudinal section showing a sectional view of the papillose cells represented in *H*. *gl. tr.*, glandular trichome; *in. epi.*, inner epidermis; *n.*, nucleus; *ou. epi.*, outer epidermis; *pap.*, papilla; *s.*, starch grain; *st.*, stoma; *v.o.*, end of an isolated vessel from the corolla tube showing opening; *v.j.*, vessel junction from isolated xylem elements of the tube. All  $\times 150$ .

## THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

90  $\mu$  at the base; the head measures 18 to 126 to 180  $\mu$  in length and 18 to 54 to 72  $\mu$  in diameter.

*Inner or upper epidermis:* On the lobes, the epidermal cells are similar to the corresponding ones on the outer epidermis (Figs. 5, Ai and Bi): over the veins, however, the infoldings disappear and the cells are slightly papillose. The cells on the lobes measure approximately L and T = 25 to 35 to 50  $\mu$  and R = 7 to 11 to 35  $\mu$ . On the lower part of the lobes, occasional cells measure as much as L and T = 75  $\mu$  and R = 40  $\mu$ . The cells on the tube gradually lose their infoldings (Figs. 5, Ci and Di), and at about the middle of the tube they are polygonal with only slightly curved anticlinal walls and often bear a small papilla arising from the end of the upper surface of the cell and directed towards the throat of the corolla (Figs. 5, H and I). The cells on the upper part of the tube measure L = 38 to 63 to 113  $\mu$ , T = 20 to 30 to 38  $\mu$  and R = 40 to 60  $\mu$ . Lower down, the cells are sub-rectangular, have comparatively thick lignified walls and bear pits on their anticlinal walls (Fig. 4, D). At the very base, the cells are more square in shape than rectangular, i.e., they decrease in length from the ones immediately preceding them, and measure L = 20 to 25 to 50  $\mu$  (Figs. 5, Ei and Fi). The epidermal cells on the lobes have a distinct centrally placed nucleus, whereas the rectangular ones at the base have their nuclei lying against the side walls. *Stomata* are rare or absent on the lobes but a few occur on the tube, being usually slightly depressed below the epidermal surface. A few *trichomes* are present on the lower part of the tube, but are very rare on the lobes.

*Mesophyll:* The mesophyll consists of about 3 to 10 layers of loosely arranged parenchymatous cells, the number of layers and the size of individual cells increases from apex to base, so that the petals are thinnest at the apex (Figs. 4, B, C, and D). Calcium oxalate *crystals* are rare in the lobes, but a number of isolated tetragonal prisms, truncated octahedra or irregular crystals are present in the tube, particularly in the parenchyma along the main veins. The parenchymatous cells adjacent to the main veins in the tube usually contain numerous small starch grains, either single or two-compound (Fig. 5, G). The grains do not polarise clearly, but they stain purple with solution of iodine.

*Venation:* 10 main veins arise from the base of the corolla tube; 5 enter the filaments, and each of the other alternating 5 veins enters a petal and gives rise to 2 main branches very near the base (Fig. 4, A). Thus each petal has 1 main vein and 2 main branches, all of which freely anastomose with each other, the finest veinlets running to within 3 to 15 cells of the edge of the lobes. The xylem elements of the veins of the petals consist of slender spiral vessels having circular or oval openings on the flat or tapering ends of the segments (Figs. 5, v.j. and v.o.).

### HISTOLOGY OF THE STAMENS

*Filament:* The *epidermal cells* are polygonal in surface view, elongated in the direction of the axis of the filament and are covered with a moderately thick cuticle (Figs. 6, A and B). At the base, they merge in form and shape with those of the rectangular cells of the corolla at the

adnation. The cells measure approximately  $L = 53$  to **105** to  $140 \mu$ ,  $T = 10$  to **18** to  $25 \mu$  and  $R = 21$  to **35** to  $70 \mu$ . *Stomata* are absent. The *trichomes* are mainly glandular with comparatively small heads and uniseriate stalks which are very wide at the base and taper rapidly towards the middle of the stalk; dimensions, length about  $140$  to **350** to  $560 \mu$  and diameter at base =  $40$  to **60** to  $150 \mu$ ; a few covering trichomes also occur (Figs. 6, *gl. tr.* and *tr.*). The trichomes are abundant at the base and become progressively smaller and less frequent towards the connective, in which region they are absent. The *cortex* consists of 5 to 6 rows of rounded, loosely arranged parenchyma containing numerous small scattered *crystals*, in the form of prisms or of indeterminate shape, being most abundant at the base and along the vascular strand which is centrally placed (Fig. 6, B).

*Anther*: The filament extends into a triangularly pyramidal *connective*, which has rectangular epidermal cells with a faintly striated cuticle and fairly numerous, rounded and usually open *stomata*. The cortical cells of the connective have numerous crystals, usually in the form of tetragonal prisms or of irregular shapes. The *lobes* are covered with an epidermis having a distinctly striated cuticle and consisting of isodiametric cells and cruciferous (anisocytic) *stomata* (Fig. 6, H); *trichomes* are absent. At the line of dehiscence, the outer epidermis in surface view takes the form of about two rows of small, thin-walled tabular cells measuring approximately  $L$  and  $T = 7 \mu$  and  $R = 25 \mu$  (Fig. 6, G). The epidermal cells gradually increase in size away from the stomium, becoming rounded polygonal in shape with prominent nuclei and on the lobes measure approximately  $L$  and  $T = 7$  to **20** to **50** to  $75 \mu$  and  $R = 25$  to  $63 \mu$ . The *fibrous layer* is several layers thick near the connective but gradually decreases in thickness to a single layer near the stomium where it is absent (Fig. 6, G). The walls of the pollen sacs at the stomium are held together only by the layer of small epidermal cells which form the line of dehiscence. The cells of the fibrous layer measure approximately  $L = 38$  to **50** to  $63 \mu$ ,  $T = 13$  to **20** to  $25 \mu$  and  $R = 25$  to  $38 \mu$ . They have a suberised band of thickening in the form of a spiral which appears in surface view as rods with thickened or beaded ends (Figs. 6, F and G). These spiral bands often fail to give a reaction for lignin when tested with phloroglucin and hydrochloric acid, though some fragments of the wall seen in surface view show a faint pink tinge, indicating partial lignification of the thickenings. Within the fibrous layer, remains of the tapetum are sometimes visible. The anthers dehisce at an early stage during the development of the flower.

The *pollen grains* are spherical; about  $35$  to **48** to  $56 \mu$  in diameter, with three equidistantly placed longitudinal germinal furrows, the ends of which taper towards the poles, but do not meet; in the centre of each furrow is situated a large pore about  $10$  to  $18 \mu$  in diameter; the exine bears numerous irregularly arranged minute rounded pits of slightly varying sizes<sup>9,10</sup> (Figs. 6,  $p_2$  and  $p_3$ ). In the immature grain, there are three inward folds along the position where the pores will develop (Fig. 6,  $p_1$ ). The pollen grains contain oil globules which stain yellow

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

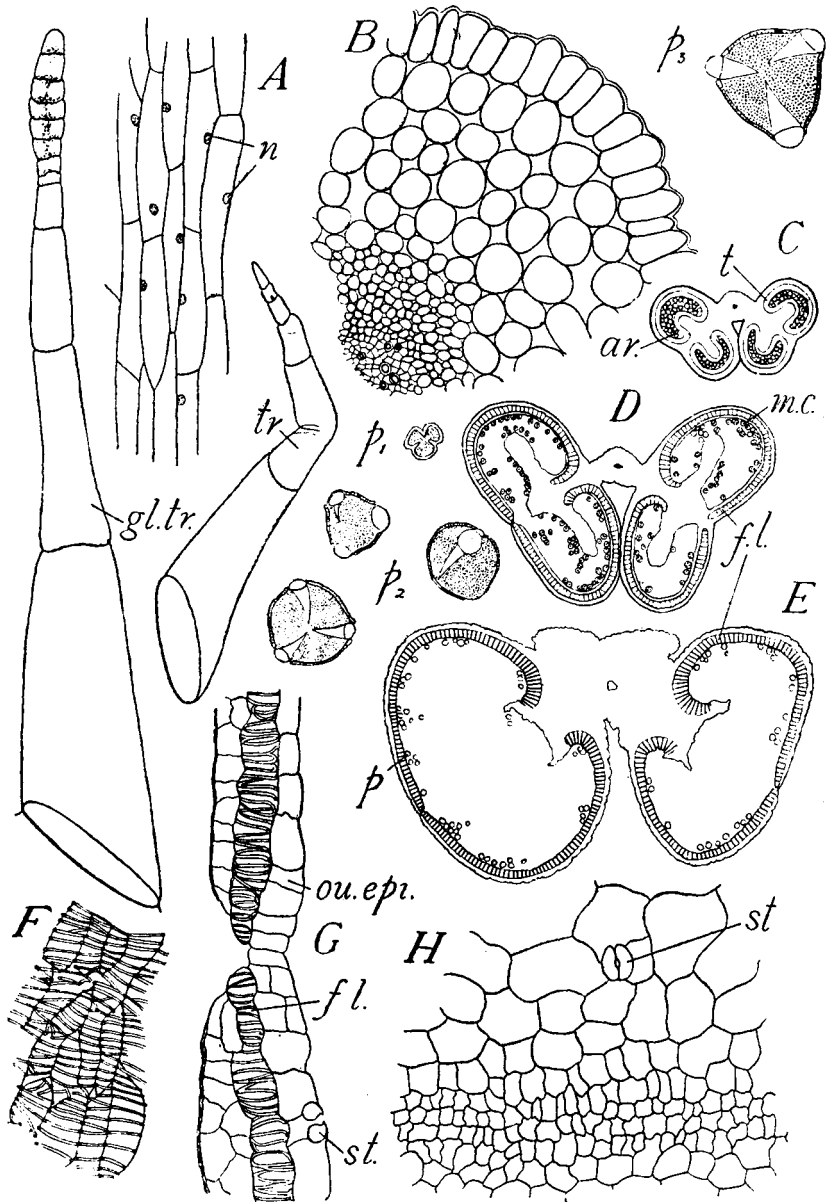


FIG. 6.—*Hyoscyamus niger*, Linn., Stamen. A, Epidermis from filament; B, Transverse section through filament; C, Transverse section through an anther showing the archesporium ( $\times 25$ ); D, Transverse section through an anther showing mother cells containing immature pollen grains ( $\times 25$ ); E, Transverse section through a mature anther containing mature pollen grains ( $\times 25$ ); F, Fibrous layer of anther in surface view; G, Transverse section of anther-wall at line of dehiscence; H, surface view of epidermis over anther at the line of dehiscence. *ar.*, archesporium; *f.l.*, fibrous layer; *gl.tr.*, glandular trichome from base of filament; *m.c.*, pollen mother cell; *n*, nuclei; *ou.epi.*, outer epidermis; *p*, mature pollen grain; *p<sub>1</sub>*, immature pollen grain; *p<sub>2</sub>*, mature pollen grains; *p<sub>3</sub>*, pollen grain further enlarged ( $\times 350$ ); *st.*, stoma; *t*, tapetum; *tr*, covering trichome from filament. All  $\times 200$  unless otherwise specified.

in solution of Sudan III; they also contain numerous minute starch grains which stain purple in iodine solution after treatment with solution of chloral hydrate for about two minutes; their diameter then measures about 1 to 5  $\mu$ <sup>11</sup>.

#### HISTOLOGY OF THE CARPELS

The *stigma* is rounded capitate, with a small oval funnel-shaped depression in the centre extended as a shallow groove reaching almost to the margin on either side and indicating its origin from two carpels (Figs. 7 A, B and E). Its surface is covered with papillæ. The papillæ contain oil globules which often float out in the mountant and stain orange yellow in solution of Sudan III. The papillose cells measure about 15 to 120 to 175  $\mu$  in length and about 18 to 28 to 36  $\mu$  in diameter at the base. In full-blown flowers, pollen grains, often with a growing pollen-tube, are adherent to the surface of the stigma. Numerous small crystals usually occur in the stigma.

The *style* is about 8 to 20 mm. long and 0.2 to 0.7 mm. in diameter. The epidermal cells in the lower part of the style, which is gradually thicker towards the base, are tabular, elongated along the axis and similar to those of the filament. The epidermal cells measure about L = 35 to 88 to 140  $\mu$ , T = 11 to 14 to 25  $\mu$  and R = 11 to 18  $\mu$ ; at the base of the style, the dimensions correspond to the lower limits. In this region, *stomata* are absent and 1 to 5 celled conical covering *trichomes*, with a rather rounded tip, occur. The *trichomes* measure about 170 to 300  $\mu$  in length and 18 to 28 to 50  $\mu$  in basal diameter. The epidermal cells on the upper part of the style have a dome-shaped outer wall (Fig. 7, C). *Trichomes* are very rare in this region, but *stomata* occur frequently and are usually raised well above the general surface of the epidermis with a large oval opening between the guard cells; they do not show any special arrangement of the subsidiary cells. Calcium oxalate *crystals* occur occasionally as isolated prisms, rosettes or clusters; starch grains are abundant in the mesophyll of the style.

In transverse section, the style shows a single row of epidermal cells covered with a finely striated cuticle; at the centre, there is an oval core of very loosely arranged small rounded cells about 3 to 6 to 9  $\mu$  in diameter, sometimes with a central irregular lacuna; on either side of the core is a small vascular bundle, the remaining tissue consists of fairly large-celled uniform parenchyma cells about 6 to 18 to 30  $\mu$  in diameter, with numerous intercellular spaces, forming a surrounding layer about 12 cells wide (Fig. 7, D).

*The Ovary*: The *outer epidermis* (Figs. H and I) consists of tabular cells with almost straight anticlinal walls; they vary somewhat in size, the dimensions being, L and T = 5 to 15 to 30  $\mu$  and R = 11 to 18  $\mu$ . *Stomata* are present and are more numerous on the lower part where they are much larger than the adjoining cells and the pores are open. The subsidiary cells do not show typical cruciferous arrangement. On occasional ovaries, a very few short covering *trichomes*, 1 to 3 cells long, occur on the outer epidermis of the upper part. The *inner epidermis*

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

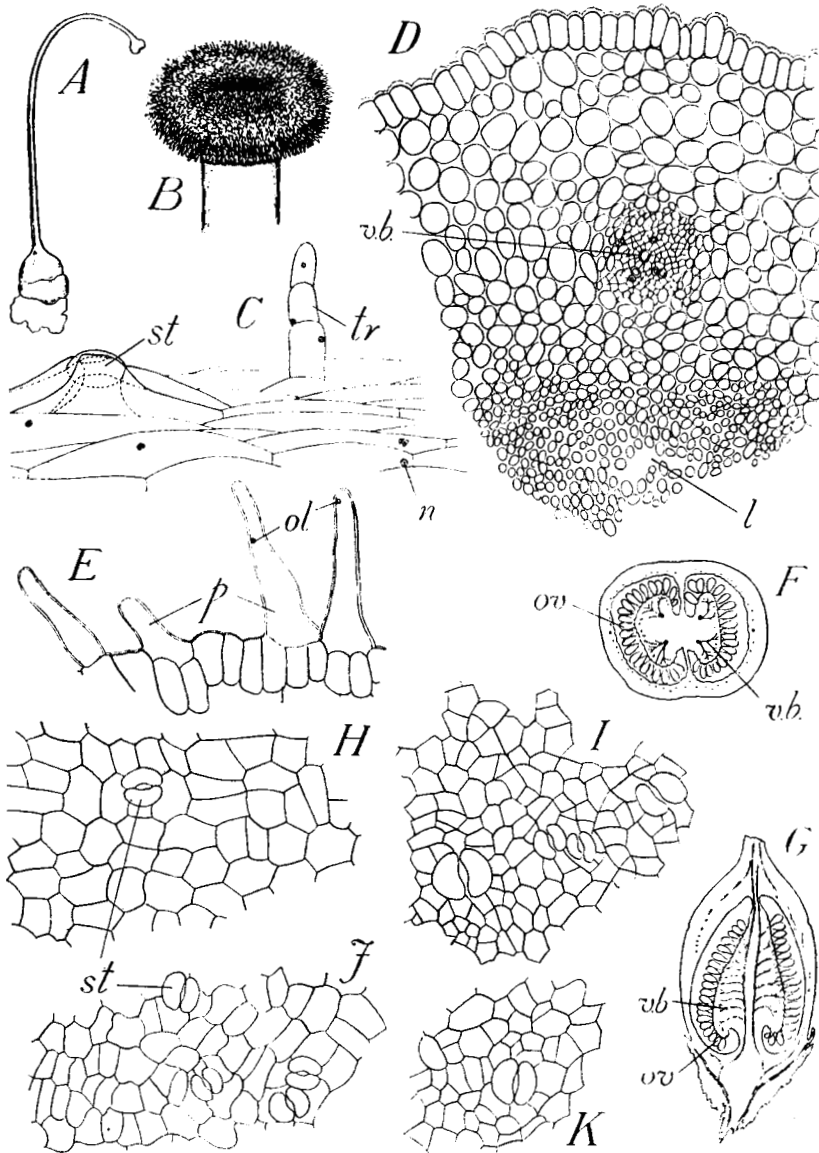


FIG. 7.—*Hyoscyamus niger*, Linn., Carpels. A, Entire gynoecium  $\times 2$ . B, Stigma  $\times 12$ . C, Epidermis of style, tangential surface view. D, Transverse section of style. E, Section through the epidermis of a stigma showing papillose cells. F and G, Diagrammatic drawings respectively, of transverse and longitudinal sections of the ovary  $\times 12$ . H, Outer epidermis over apical part of the ovary. I, Outer epidermis near base of the ovary. J, Inner epidermis of apical part of the ovary. K, Inner epidermis at the base of the ovary. *l*, lacuna; *n*, nucleus; *ol*, oil globules; *ov*, ovule; *p*, papilla; *st*, stigma; *tr*, covering trichome; *vb.*, vascular bundle. All drawings  $\times 200$  unless otherwise specified.

consists of cells similar to those of the outer epidermis, but *stomata* are more frequent (Figs. 7, J and K).

The *mesophyll* consists of closely arranged polyhedral parenchymatous cells with small intercellular spaces, many of the cells being filled with minute starch grains particularly in the upper part of the ovary; the mesophyll is traversed by slender vascular strands. *Crystals* often occur in the cells of the mesophyll, as single isolated indeterminate shaped or as groups of several large crystals.

The epidermal cells of the *septum* and the *placenta* vary between sub-rectangular to isodiametric and are often similar to those of the epidermis of the ovary wall. *Stomata* are rarely present. The ground tissue of the central part of the septum and of the placenta consists of loosely arranged isodiametric parenchymatous cells. Small microcrystals of odd shapes are abundant in the cells of the placenta and also occur frequently in the septum. As the ovary matures, the crystals acquire a definite structural form, being usually present as double pyramids, sometimes with truncated ends, cubes, right angle prisms or hemihedral crystals.

*Ovules*: Very young ovules consist of undifferentiated parenchymatous cells.

#### POWDERED FLOWERS

Henbane flowers, dried and powdered to No. 90 mesh, result in a greenish-brown powder with a typical unpleasant odour. The powder was examined after mounting it in the following: dilute glycerin, iodine solution, solution of chloral hydrate, lactophenol and in phloroglucin and hydrochloric acid. The structures which are of greatest use in the identification of the powdered flowers are arranged in order of importance as follows:

1. Pollen grains, mature grains about 40 to 50  $\mu$  in diameter having 3 pores and 3 furrows; the exine with very numerous irregularly scattered rounded pits (Figs. 8, I<sub>1</sub> and I<sub>2</sub>).

2. Fragments of the anther wall, which impart a pink tinge to solution of chloral hydrate owing to the presence of anthocyanin, and include the characteristic fibrous layer which sometimes gives a slight reaction for lignification (Figs. 8, C and D).

3. Broken pieces of trichomes, especially the large multicellular club-shaped or ovoid glandular heads with granular and yellowish contents (Figs. 8, F, G, L and P).

4. Fragments of the upper and lower epidermises of the corolla lobes with characteristic infoldings of the anticlinal walls; the fragments yielding a transient pink colour in solution of chloral hydrate (Fig. 8, A).

5. Fragments of lignified vascular strands, appearing as slender spiral elements, sometimes associated with thick walled, pitted xylem fibres from the calyx (Figs. 8, K and M).

6. Lignified rectangular cells of the lower part of the corolla tube, sometimes bearing rounded, simple pits on their anticlinal walls (Figs. 8, B and E).

THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

7. Fragments of the epidermises of the ovary composed of straight thin-walled polygonal cells; occasional stomata are present. (Fig. 8, J).

8. Fragments of the calyx lobes with wavy walled epidermal cells and cruciferous stomata. Small uniseriate covering trichomes are present on the epidermis and occasional idioblasts containing prisms or micro-sphenoidal crystals occur in the mesophyll. Certain fragments from the base of the outer epidermis of the calyx have large cicatrices left by the larger trichomes (Figs. 8, G, H, K, cr and N).

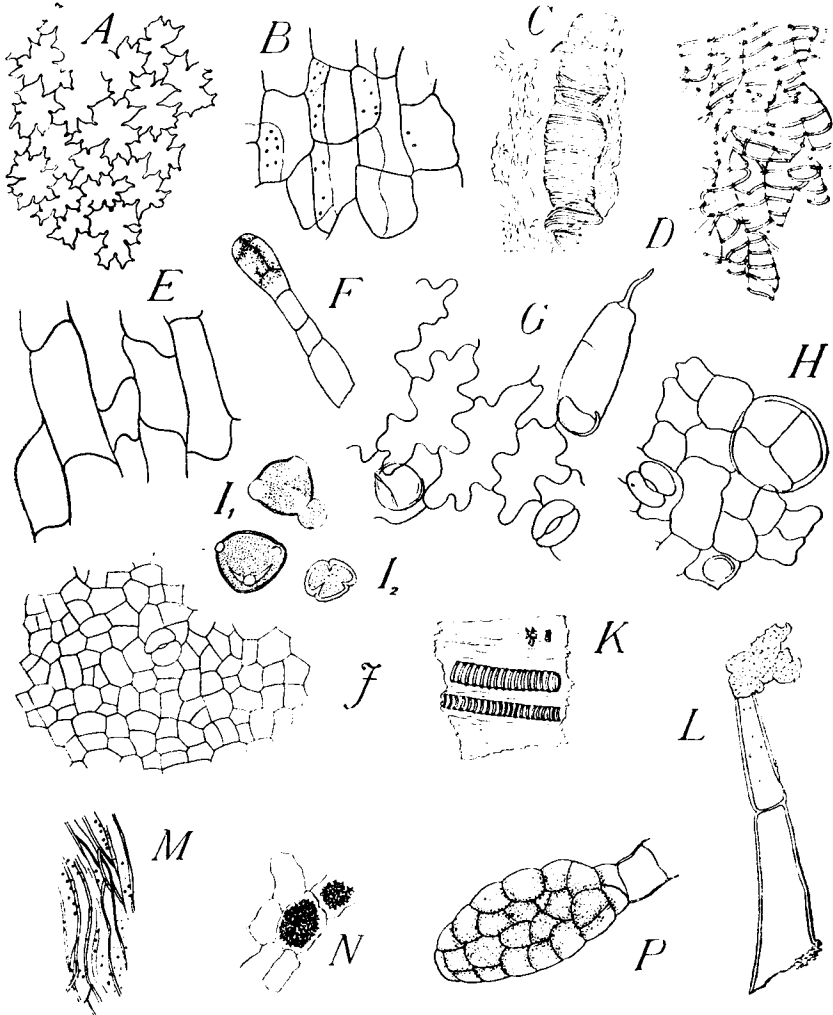


FIG. 8.—*H. niger*, Linn., Powdered Flowers. A, Epidermis of corolla lobe. B and E, Epidermis near the base of the corolla tube. C, Anther wall in sectional view. D, Fibrous layer in surface view. F, Glandular trichome. G, Epidermis from calyx lobe. H, Outer epidermis from base of calyx tube. I<sub>1</sub>, Mature pollen grains. I<sub>2</sub>, Immature pollen grain. J, Epidermis of ovary wall. K, Spiral vessels of the calyx with a few crystals. L, Broken glandular trichome. M, Group of lignified fibres from a vein of the calyx. N, Micro-sphenoidal crystal sand. P, Glandular head of a large trichome from the calyx. All  $\times 200$ .



## SUMMARY

A. A brief review of the published description of the gross morphology of the flower of *Hyoscyamus niger* Linn. has been made and a somewhat more detailed new account of the macroscopical characters is given. This is followed by a detailed illustrated description of the microscopical characters, the following being of especial diagnostic value:

1. *Calyx*: Epidermal cells varying from wavy-walled on the lobes to straight-walled at the base; cruciferous stomata numerous; glandular and covering trichomes long and numerous; abundant microsphenoidal crystals present as single crystals or as sandy masses; xylem fibres present in the midrib of the lobes.

2. *Corolla*: Epidermal cell walls with well-marked infoldings on the lobes, straight at the base; trichomes, mostly glandular, frequent on outer epidermis; stomata, rare, present on the tube only; small starch grains in cells adjacent to the spiral vessels of the xylem in the tube.

3. *Stamens*: *Filament*, stomata absent; trichomes mostly glandular, crowded towards the base but scanty elsewhere; crystals in the ground tissue. *Anther*, stomata on epidermis, fibrous layer slightly lignified, crystals in connective. *Pollen grains* spherical, with three germinal furrows and three pores; exine marked with numerous irregularly scattered pits; contain oil globules and starch grains; mature pollen grains measure about 40 to 50  $\mu$  in diameter.

4. *Carpels*: *Stigma* capitate with a funnel-shaped depression in the centre, covered with papillæ; *style*, epidermis bears stomata and occasional trichomes; crystals and starch grains in ground tissue. *Ovary*, bilocular, stomata on inner and outer epidermises of the wall; crystals present; *ovules* numerous.

5. *Powder*: The powder of the flowers of *Hyoscyamus niger* may be identified by the characters of the pollen grains, the fibrous layer of the anther walls, the large multicellular, ovoid glandular heads of the trichomes, the cells of the corolla lobes and the xylem fibres from the main veins of the calyx.

B. The chief histological features which distinguish the flower of *Hyoscyamus niger* Linn. from that of *Atropa belladonna* Linn.<sup>12</sup> are:

1. *Pollen*: The pollen grains of both flowers are very similar in size and form. The pores on the exine, however, are irregularly scattered in the pollen of *H. niger*, but are arranged in rows radiating from the poles in case of *A. belladonna*<sup>9,10</sup>.

2. *Trichomes*: Covering trichomes are absent from the calyx of *A. belladonna*, but upon that of *H. niger* they occur in considerable numbers associated with the typical glandular trichomes. The glandular trichomes of *H. niger* are clearly distinguished by their large ovoid multi-cellular glandular heads, whereas in *A. belladonna* the glandular heads are usually small, spherical and unicellular.

3. The cuticle of the calyx in *A. belladonna* is finely striated; whereas that of *H. niger* is smooth.

## THE FLOWER OF *HYOSCYAMUS NIGER* LINN.

4. The main veins of the calyx in *H. niger* have numerous lignified pitted xylem fibres, while those of *A. belladonna* are devoid of fibres.

5. The anticlinal walls of the epidermal cells of the lobes of the corolla in *H. niger* have typical deep infoldings; the corresponding cells of the outer epidermis of the corolla of *A. belladonna* have very sinuous anticlinal walls, and those of the inner epidermis have nearly straight walls and well-marked small papillæ.

C. The flowers of *Datura stramonium* are readily distinguished from those of *H. niger* by one well-known character, viz., the size and markings of the pollen grains<sup>9,10</sup>, which are about 56 to 80  $\mu$  in diameter and have a coarsely granular exine in case of *D. stramonium*. Further work on the flower of *D. stramonium* is in progress.

### ACKNOWLEDGEMENTS

We would like to express our sincere thanks to Dr. T. E. Wallis for supplying most of the specimens, for the interest he has taken in the work and for the help given in solving certain difficulties.

### REFERENCES

1. Bentley and Trimen, "Medicinal Plants," 1880, 3, 194.
2. Möll and Janssonius, "Botanical Pen Portraits," 1923, 325.
3. Berg, "Anatomischer Atlas zur Pharmazeutischen Waarenkunde," 1865, 93, plate XXXXVII.
4. Tschirch und Oesterle, "Anatomischer Atlas der Pharmakognosie und Nahrungsmittelkunde," 1900, 167, plate 39.
5. Sachs, "Text book of Botany," 2nd ed., 1882, 598.
6. Metcalfe and Chalk, "Anatomy of the Dicotyledons." Oxford University Press, 1950, 1, xv.
7. Anselmino und Gilg, "Die Bilsenkrautblätter des Handels." Arch. Pharm., 1913, 251, 367.
8. Eames and MacDaniels, "An Introduction to Plant Anatomy," 1947, 347.
9. Zander, "Beiträge zur Herkunftsbestimmung bei Honig." "Pollengestaltung und Herkunftsbestimmung bei Blütenhonig." Berlin, 1935, 279.
10. Wallis, "The Study of Pollen," Pharm. J., 1947, 158, 8, 29.
11. Lang, "Investigations of the Pollen of the Malvaceæ, with special reference to the inclusions." J.R. micr. Soc., 1937, 57, 75.
12. Wallis and Butterfield, "The flower of *Atropa belladonna* Linn." Quart. J. Pharm. Pharmacol., 1939, 12, 511.