

# INDIAN HENBANE

## PART I

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*From The Analytical Laboratory, Boots Pure Drug Co., Ltd.*

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DURING the war, and in the post-war years, a considerable quantity of henbane has been imported into this country from India. Amongst this material was some which differed in appearance and especially in microscopical characters from typical *Hyoscyamus niger*. This suggested the possibility that this henbane was derived from some other species of *hyoscyamus* and it was thought worth while to carry out some investigation of *hyoscyamus* from India ; with a view to ascertaining its botanical origin and characters. As a result, it has become apparent that there are several types of henbane growing in India and Pakistan in addition to *H. niger*. These other types have, in the past, commonly been referred to the species *H. reticulatus*, but they include plants whose habits are quite different from the typical form of this species and which, moreover, vary amongst themselves. It would appear, therefore, that there are a number of species of *hyoscyamus* growing wild in India, which might be collected as henbane. The question of the true botanical identity of these various species is still under consideration and it is hoped that more definite information will be available later.

The present paper is devoted to a description of one such species of *hyoscyamus* which grows wild in the North-West Frontier Province. This plant differs in habit from *H. niger* chiefly in the shape of its leaves, which are broadly ovate and much less indented (Fig. 1 and 2, A). It is hoped, in subsequent communications, to describe some further species of *hyoscyamus* which grow wild in India.

### MATERIAL

The following description is based on six dried specimens, numbered H1 to H6, collected in Pakistan, in the area of the North-West Frontier Province, by Professor Qazilbash, of Islamia College, Peshawar, and on plants (H7) grown under glass at Lenton Experimental Station, Nottingham, from seeds sent by Professor Qazilbash.

For comparison, seven specimens of *Hyoscyamus niger* were used. These were :—

- N1. Collected at Chelsea Physic Garden, 1937.
- N2. Cultivated at Long Melford, 1948.
- N3. Cultivated at Hitchin, 1947.
- N4. Grown at Lenton, 1948.
- N5. Cultivated in the North-West Frontier Province, 1948.
- N6. From the Department of Botany, Government of Nepal, Katmandu, 1947.
- N7. Cultivated in India, supplied by the Divisional Forest Officer, 1947.

## MICROSCOPICAL CHARACTERS

The plant is an erect, branching annual, 1 to 2 feet high. When dry, it has an odour resembling that of *Hyoscyamus niger* and a slightly bitter taste.

*Stem.* Cylindrical and smooth in the fresh state, but when dry flattened, longitudinally striated and usually showing one or more longitudinal furrows; light green, 2 to 5 mm. in diameter, slightly swollen at the nodes and clothed with white viscid hairs, up to 7 mm. long; leaf scars alternate. The transversely cut surface shows the remains of the pith with a central hollow, this is surrounded by a whitish ring of radiate xylem and a narrow, green bark.

*Leaves.* Ovate to oblong, 3 to 10 cm. long and 1.5 to 5 cm. broad, the lower two or three with petioles about a quarter the length of the leaf, the higher ones sessile, the uppermost clasping the stem; outline varying from entire to coarsely toothed, there being from 1 to 5 teeth on each side of the leaf, usually more on one side than on the other; apex acute, base acute in the lower leaves, obtuse to cordate in the upper; venation pinnate, with a broad midrib and 5 or 6 secondary veins on each side, which make wide angles with the midrib and terminate in the teeth where these are present or, if teeth are lacking, anastomose near the margin (Fig. 2, A).

The surface is covered with long white trichomes and has a viscid feel, especially when fresh. The dried leaves are thin and brittle, usually crumpled and broken; they are yellowish to greyish green in colour, the uppermost sometimes with a purple tinge.

*Flowers.* Typically solanaceous, the lower solitary in the axils of the leaves, the upper in a one-sided spike rolled back before flowering; about 20 mm. long with a pedicel 2 to 3 mm. long. Calyx urceolate, 10 to 15 mm. long and 4 to 8 mm. wide, having 5 lobes, each with an apical spine and 10 main veins; green, very hairy and persistent. Corolla funnel-shaped, 15 to 20 mm. long and 15 to 20 mm. in diameter at the mouth, the 5 lobes rounded and slightly unequal, very thin, pale buff with very prominent purple veins. Androecium of 5 free stamens, 2 long, about 12 mm. and 3 short, 8 to 10 mm., attached to the base of the corolla. Filaments very hairy in the lower half. Anthers 2 mm. long, black and dehiscing longitudinally. Ovary superior, and bilocular, consisting of 2 united carpels, smooth, cream-coloured, globular, about 2 mm. in diameter; it contains numerous ovules, showing axile placentation. Style filiform; stigma bilobed. Floral formula  $K(5).C(5).A5.G(2)$ .

*Fruit.* An ovoid-oblong pyxis, 12 to 15 mm. long, green, surrounded by the calyx (Fig. 2, C), which becomes larger in size, thicker and more coriaceous in texture after fertilisation, with 5 broad, shortly pointed lobes protruding above the fruit, which contains numerous seeds.

*Seeds.* Reniform and flattened, 1 to 1.5 mm. in diameter and 0.5 mm. thick; immature seeds green, mature seeds light brown. Testa finely reticulate, having 12 to 16 reticulations across the flat surface of the seed; the walls of the reticulations corrugated and the surfaces of the

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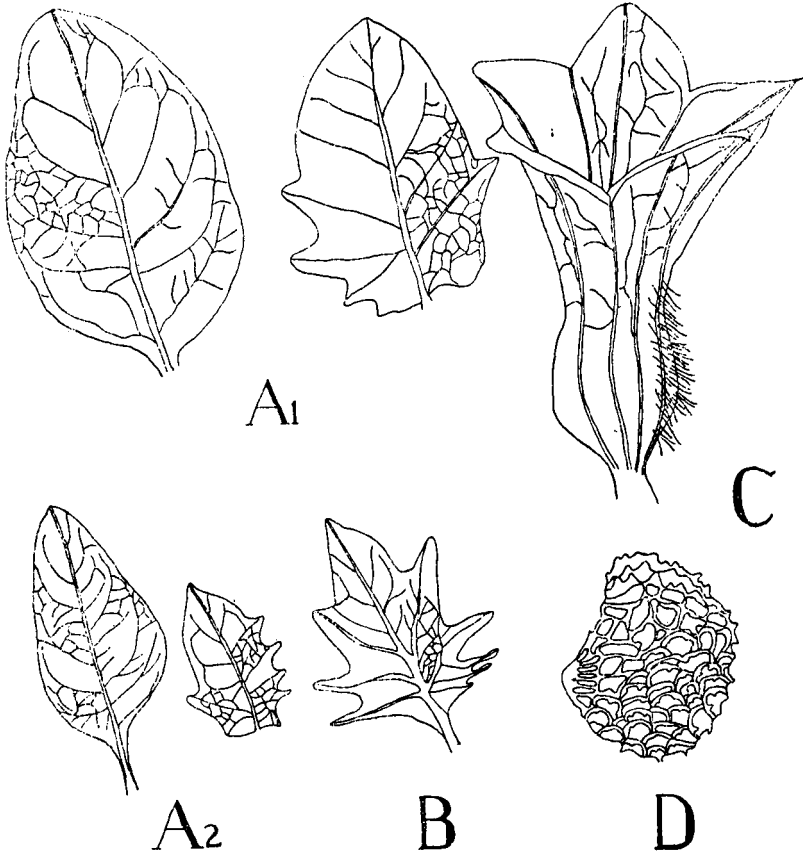


FIG. 2. A.1. Whole leaves, upper surface, natural size. A.2. Whole leaves, lower surface, natural size. B. *Hyoscyamus niger* leaf, natural size. C. Fruit  $\times 4$ . D. Seed  $\times 20$ .

depressions rugose (Fig. 2, D). The seed has a single scar of attachment to the placenta on one side. The weight of 100 seeds is 0.06 to 0.07 grammes.

### MICROSCOPICAL CHARACTERS

**STEM. Epidermis.** Longitudinal rows of tetra- to hexagonal, tabular cells, 75 to 400 $\mu$  long, 70 to 155 $\mu$  wide and 25 to 55 $\mu$  high. Outer walls 5 to 10 $\mu$  thick, stratified and showing faint cuticular striations, parallel to the long axis of the stem; inner walls also thickened; anticlinal walls straight, thin, with occasional simple pits. Stomata rare, similar to those of the leaf, but lying at the same level as the epidermal cells. Trichomes similar to those of the leaf. Crystals absent.

**Cortex.** Consist of one outermost layer of ordinary parenchyma cells, containing chloroplasts; about 5 layers of collenchyma, averaging 45 $\mu$  radially, 60 $\mu$  tangentially, and 200 to 500 $\mu$  longitudinally, occasional cells very elongated, resembling fibres; about 4 layers of colourless parenchyma, showing intercellular spaces.

The endodermis consist of 2 layers of cylindrical cells with the axis directed longitudinally, containing starch grains 1 to 5 microns in diameter, mostly simple but occasionally 2 to 4 compound. All the cells of the cortex have cellulose walls.

*Pericycle.* Consists of 1 or 2 layers of common parenchyma with, on the outside of the primary phloem, small groups of fibres, measuring 10 to 20 $\mu$  radially, 25 to 50 $\mu$  tangentially, and up to 5000 $\mu$  longitudinally, with only slightly lignified walls. Fibres are lacking in the young stem.

*Phloem.* Primary phloem endarch, in bundles made up of groups of sieve tubes and companion cells, embedded in phloem parenchyma. The secondary phloem forms a continuous ring. Sieve tubes 12 to 16 $\mu$  in diameter, transverse walls oblique, sieve plates and callus evident. Companion cells 3 to 4 $\mu$  in diameter. All cells with cellulose walls.

*Cambium.* Consists of 1 or 2 layers of very thin-walled cells, tetragonal in transverse section. Average dimensions, radial 4 $\mu$ , tangential 12 $\mu$ , longitudinal 60 $\mu$ .

*Xylem.* Primary xylem endarch, in groups opposite the primary phloem; the secondary xylem forming a continuous ring. Consists of annular, spiral, reticulate and pitted vessels, 15 to 75 $\mu$  in diameter; wood parenchyma cells, usually 5-sided with pitted walls, averaging 15  $\mu$  radially and 20 $\mu$  tangentially and wood fibres, with oblique slit-shaped pits, about 20 $\mu$  in diameter. Medullary rays, uni- to triseriate, consisting of tangentially elongated pitted parenchyma cells. All elements of the xylem have lignified walls; intercellular spaces absent.

*Pith.* Consists of large, thin-walled parenchyma cells with cellulose walls and showing numerous intercellular spaces, which usually appear triangular in transverse section. At the periphery, a ring of phloem bundles, similar in structure to the normal phloem and having small groups of fibres, resembling the pericyclic fibres, on the inner side.

*LEAF. Upper epidermis.* Epidermal cells of the interneural region 100 to 200 $\mu$  long by 50 to 100 $\mu$  wide by 20 to 40 $\mu$  high, with sinuate anticlinal walls and a thin cuticle. Epidermal cells of the main veins straight-walled and elongated in the direction of the vein, 150 to 400 $\mu$  long by 25 to 50 $\mu$  wide by 20 to 40 $\mu$  high, (Fig. 3, B, ep. 1) the cells bearing the hairs are higher and wider than the normal epidermal cells and have rounded edges. Stomata 35 to 65/sq. mm., averaging 40 by 22 $\mu$ , lying slightly above the level of the epidermal cells and surrounded by 3 to 5 subsidiary cells, one of which is markedly smaller than the others. Stomata absent over the midrib and the primary veins. Covering trichomes numerous, conical, uniseriate, of 2 to 4 cells, 75 to 290 $\mu$  long (Fig. 3, D, c). Glandular trichomes fairly numerous, especially on the veins, 450 to 750 $\mu$  long, with a uniseriate stalk of 2 to 6 cells, showing cuticular reticulations and a multicellular, glandular head (Fig. 3, D, gl). On the veins, in addition to the above types, are some very large trichomes, up to 7000 $\mu$  long and containing up to 12 cells; the basal cells being 500 to 1400 $\mu$  long by 40 to 160 $\mu$  wide; the apical cell sometimes forming a rudimentary gland (Fig. 3, E).

*Lower epidermis.* (Fig. 3, A and B, ep. 2). Very similar to the upper,

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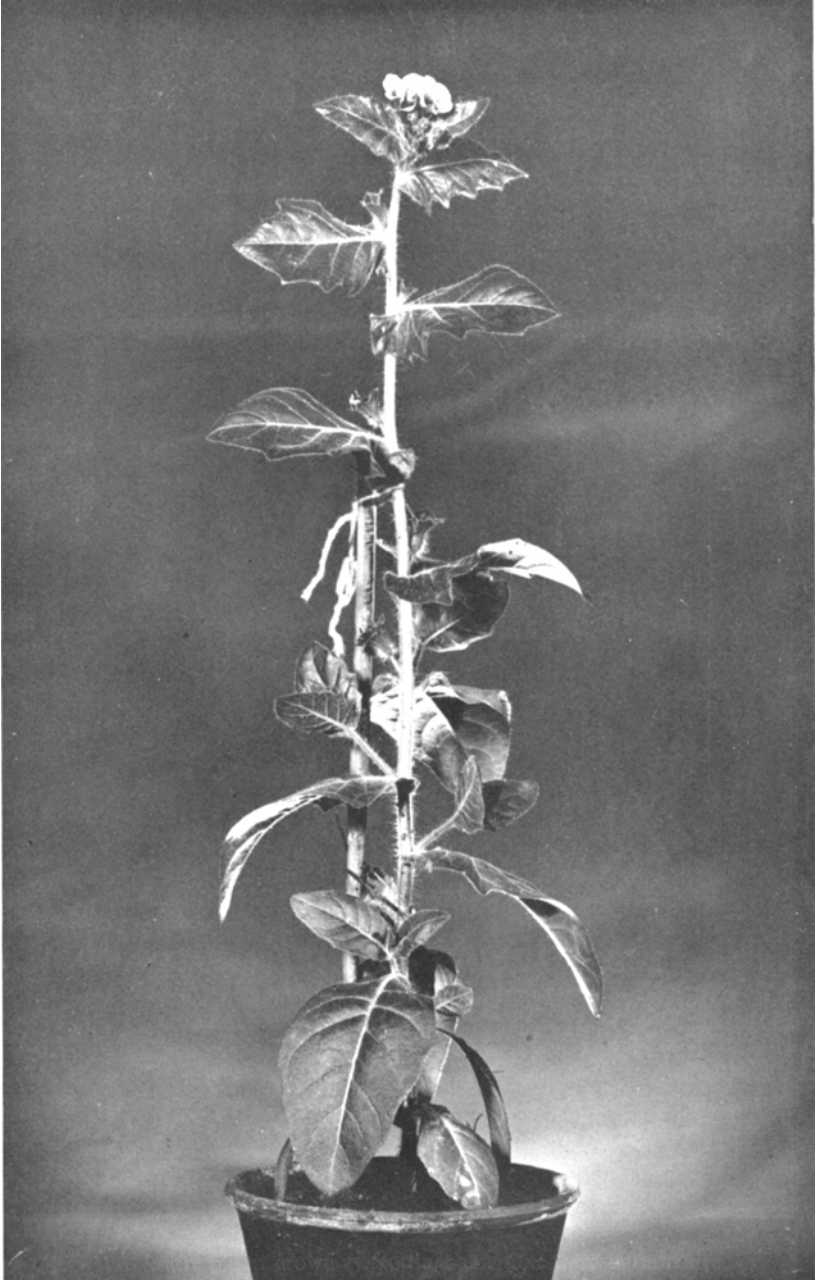


FIG. 1. Entire plant, grown at Nottingham. One-third natural size.



but the stomata are more numerous, 40 to 112/sq. mm. and slightly larger, 45 by  $30\mu$ . The trichomes are also more numerous.

*Mesophyll.* On the upper side of the leaf, one row of palisade cells, containing chloroplasts; cells averaging  $85\mu$  high by  $20\mu$  wide (Fig. 3, B, p); chloroplasts 2 to  $4\mu$  in diameter. There are large intercellular spaces, especially under the stomata.

The spongy mesophyll consists of 3 or 4 layers of more or less stellate cells, 24 to  $30\mu$  in diameter, with large intercellular spaces (Fig. 3, B, sp). Idioblasts in the upper layer contain calcium oxalate crystals of three kinds :—

(a) Tetragonal prisms (Fig. 3, F, pr.).

(b) Cluster crystals. These are commonly composed of a small number of prisms. A type consisting of single large prism with a number of smaller ones attached is of frequent occurrence (Fig. 3, F, cl.).

(c) Microsphenoidal crystals.

The prisms and clusters are 4 to  $48\mu$  long by 3 to  $26\mu$  wide.

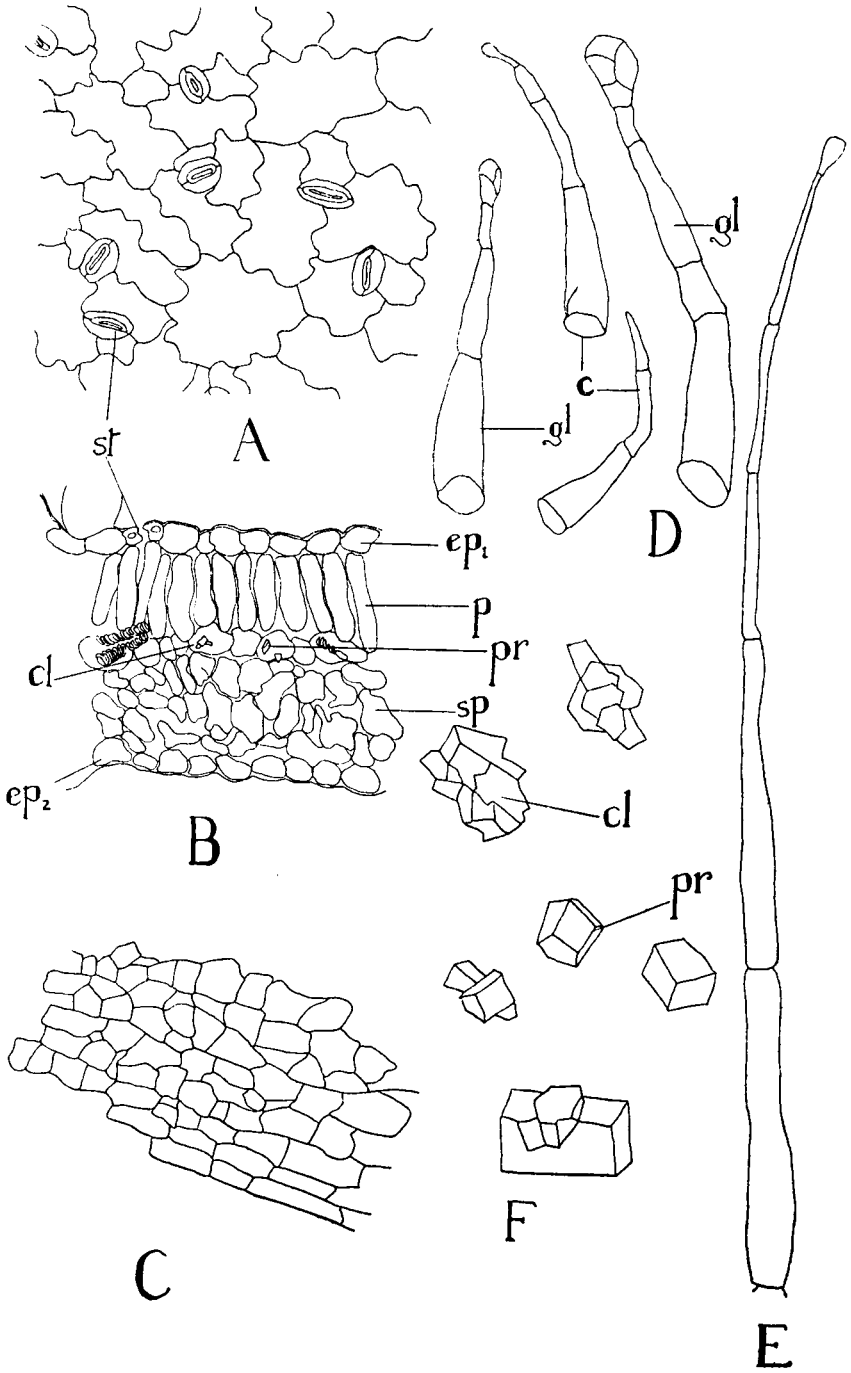
*Midrib.* There is 1 layer of collenchyma on the upper surface and 1 to 3 layers on the lower; cells about  $30\mu$  in diameter and up to  $500\mu$  long. Within the collenchyma is colourless parenchyma, about 10 layers on the upper and 4 on the under side, with large intercellular spaces; the cells sometimes containing crystals of calcium oxalate. Cells average  $60\mu$  in diameter by 200 to  $400\mu$  long. Endodermis of 1 or 2 layers of cells with slightly thickened cellulose walls, containing starch grains and chloroplasts.

*Meristele.* In the form of an arc, made up of 8 to 10 simple, collateral vascular bundles separated by uni- or biseriate medullary rays. Xylem of 7 to 8 layers consisting of annular, spiral and reticulate vessels, 15 to  $24\mu$  in diameter, embedded in xylem parenchyma; all elements lignified. Phloem consisting of sieve tubes and companion cells, embedded in phloem parenchyma. Perimedullary phloem, in groups corresponding roughly in number and position with the vascular bundles, and in form similar to the bundle phloem, is also present. The pericycle consists of 3 or 4 rows of somewhat thickened, fibre-like cells, 10 to  $25\mu$  in diameter and 200 to  $300\mu$  long, with cellulose walls.

*FLOWER. Calyx. Upper epidermis.* The basal cells are rectangular, with straight anticlinal walls, 15 to  $25\mu$  long, 15 to  $25\mu$  wide and 15 to  $25\mu$  high. Upwards the cells become larger, 30 to  $65\mu$  long, 15 to  $30\mu$  wide and 15 to  $25\mu$  high, the walls become more sinuate and the shape irregular, except over the veins, where the rectangular shape is preserved. Towards the top the cells are again smaller. The epidermis has a cuticle about  $4\mu$  thick which, over the main veins, sometimes shows cuticular striations, running parallel with the direction of the vein. The stomata increase in number towards the top of the calyx, where the number per sq. mm. is 80. Each stoma is surrounded by 3 subsidiary cells. The trichomes are very numerous and similar to those of the leaf.

*Lower epidermis.* Similar to the upper (Fig. 3, C).

*Mesophyll.* Consists of 4 to 6 layers of thin-walled parenchyma cells,





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containing chloroplasts; cells more or less stellate in form, averaging  $25\mu$  in diameter, with very large intercellular spaces. Idioblasts filled with microsphenoidal crystals of calcium oxalate fairly numerous towards the base of the calyx only; prisms and cluster crystals absent.

*Meristeles.* These are similar to the meristeles of the leaf.

*Corolla. Upper epidermis.* At the base, the epidermal cells are elongated and rectangular in shape, 35 to  $90\mu$  long, by 15 to  $30\mu$  wide by about  $20\mu$  high; the anticlinal walls are slightly wavy and the outer walls have a fairly thin, striated cuticle. Towards the apex, the cells become smaller and the anticlinal walls sinuate. Stomata very rare; trichomes absent. *Lower epidermis.* Cells similar in shape to those of the upper epidermis, but slightly larger. Trichomes present, of two kinds:—(a) Short, conical, covering trichomes, 1 to 4 cells long. (b) Glandular trichomes, almost confined to the veins in the lower part of the corolla; they have a 1- or 2-celled stalk and a well-developed multicellular glandular head.

*Mesophyll.* Consists of 4 or 5 layers of spongy parenchyma. Cells stellate, with large intercellular spaces.

*Stamen. Filament.* Epidermal cells elongated-rectangular with straight walls; no stomata. Trichomes of 3 kinds are present:—(a) With a uniseriate stalk and a multicellular glandular head. (b) Covering trichomes, similar to those of the leaf. (c) Capitate trichomes, having a long apical cell. The mesophyll consists of about 5 layers of thin-walled parenchyma cells, with a central vascular strand containing lignified spiral vessels.

*Anther.* Epidermis of irregular cells with a few stomata. Mesophyll of ordinary parenchyma cells with fairly large intercellular spaces. The meristeles consist entirely of xylem, which contains lignified spiral vessels. There is a fibrous layer of 2 to 3 layers of cells, near the connective.

*Pollen.* The pollen grains have 3 pores and an irregularly pitted exine, diameter  $45$  to  $55\mu$ ; diameter of pores 10 to  $15\mu$ .

*Pistil. Ovary.* The epidermal cells of both surfaces are tetragonal, stomata rare. The mesophyll consists of about 10 layers of closely packed parenchyma cells, without intercellular spaces. The meristeles consist wholly of xylem, made up of spiral vessels, the walls of which are incompletely lignified.

*Septum and Placentæ.* Epidermis similar to that of the ovary wall; mesophyll of spongy parenchyma with large intercellular spaces.

**FRUIT.** *Pericarp. Outer epidermis.* More or less tetragonal, tabular cells  $55$  to  $95\mu$  long,  $15$  to  $30\mu$  wide and  $15$  to  $22\mu$  high; anticlinal walls thin and slightly wavy, outer walls with a very thin cuticle. Trichomes absent.

FIG. 3. A. Lower epidermis of leaf, surface view. B. Transverse section of interneural lamina of leaf. C. Lower epidermis of calyx, near base, surface view. D. Glandular and covering trichomes from the leaf. E. Very long trichome from midrib of leaf. F. Calcium oxalate crystals. c, covering trichomes; cl, cluster crystals; ep<sub>1</sub>, upper epidermis; ep<sub>2</sub>, lower epidermis; gl, glandular trichomes; p, palisade layer; pr, prismatic crystals; sp, spongy mesophyll; st, stomata. F  $\times 300$ , remainder  $\times 150$ .

*Inner epidermis.* Tabular cells, 75 to 130 $\mu$  long, 20 to 40 $\mu$  wide and 20 to 26 $\mu$  high, anticlinal walls thin and wavy.

*Mesophyll.* Thin-walled, stellate cells, 13 to 26 $\mu$  in diameter and 40 to 75 $\mu$  long, with large intercellular spaces. Contains numerous vascular strands, each consisting of 1 to 3 spiral vessels, 4 to 12 $\mu$  in diameter, with lignified walls. The parenchyma cells are filled with starch grains, polyhedral to subspherical in shape, 2 to 10 $\mu$  in diameter, mostly simple, but including occasional compound grains of 2 to 4 components.

*Septum and Placentæ.* Epidermis similar to the inner epidermis of the pericarp; mesophyll of thin-walled, stellate parenchyma, 30 to 60 $\mu$  in diameter and 75 to 200 $\mu$  long, containing starch similar to that in the pericarp.

QUANTITATIVE DATA

*Palisade ratio.* The palisade ratio of the material under examination is from 4.25 to 5.5. This range is exactly the same as that found for *H. niger*.

*Stomatal index.* The range of stomatal index of the upper surface was from 19.2 to 24.0 and of the lower surface from 18.7 to 26.0. These figures compare with values of 21.0 to 22.5 for the upper surface of *H. niger* and 22.1 to 24.8 for the lower surface given by Rowson.<sup>1</sup>

*Vein-islet number.* The vein-islet numbers found for the species investigated ranged from 4.5 to 15.5; the range for *H. niger* was 6 to 16.

*Calcium oxalate crystals.* The prisms and cluster crystals in our unnamed species were, in general, larger than those of *H. niger* and this feature provides the best means of distinguishing the two species when broken or in the form of powder. In Table I are shown the ranges of sizes of the crystals in all the specimens examined and also the percentages of crystals in each sample which exceeded 25 $\mu$  in length.

TABLE I  
DIMENSIONS OF CALCIUM OXALATE CRYSTALS

Specimen	Size		Number less than 25 $\mu$ long	Number greater than 25 $\mu$ long	Percentage greater than 25 $\mu$ long
	Minimum $\mu$	Maximum $\mu$			
<i>Unnamed Species</i> :—					
H.1... ..	6	42	390	120	23.6
H.2... ..	6	40	308	104	25.4
H.3... ..	4	40	352	146	24.4
H.4... ..	5	40	498	196	28.3
H.5... ..	6	45	317	142	31.0
H.6... ..	4	45	384	138	26.0
H.7... ..	4	48	360	220	38.0
All specimens ...	4	48	2609	1066	29.0
<i>H. niger</i> :—					
N.1... ..	5	35	364	37	9.2
N.2... ..	4	32	591	49	7.5
N.3... ..	4	28	549	37	6.3
N.4... ..	4	26	460	5	1.1
N.5... ..	6	32	480	20	4.0
N.6... ..	5	26	410	18	4.2
N.7... ..	5	30	374	15	3.8
All specimens ...	4	35	3228	181	5.3

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These figures were obtained by the examination of powders, prepared from representative samples of the specimens. It was observed that, in both species, the size of the crystals varied markedly from leaf to leaf, one leaf containing large crystals and another smaller ones while, from some leaves, crystals were entirely absent. It is thus clear that no reliance, from this point of view, can be placed on the examination of single leaves, but, by examining representative samples of powders it was found that, while the crystals of *H. niger* never exceeded  $35\mu$  in length, and except in one specimen, did not exceed  $32\mu$ , all specimens of the Indian material contained crystals up to  $40\mu$  long and some up to  $48\mu$ . Moreover, the percentage of crystals exceeding  $25\mu$  in *H. niger* was never greater than 10 and averaged only about 5, while in the Indian material it ranged from 23.6 to 38.0 per cent.

*Alkaloid contents.* The content of total alkaloids, calculated as hyoscyamine, of the 6 samples, H1 to H6, grown in India, are shown in Table II. These were determined by the method of the British Pharmacopœia for *Hyoscyamus*.

TABLE II  
ALKALOID CONTENTS

Specimen	Total alkaloids, as Hyoscyamine	Specimen	Total alkaloids, as Hyoscyamine
	per cent.		per cent.
H.1 ... ..	0.038	H.4 ... ..	0.034
H.2 ... ..	0.038	H.5 ... ..	0.046
H.3 ... ..	0.031	H.6 ... ..	0.055

*Ash values.* In Table III are shown the results of determinations of total and acid-insoluble ash, by the methods of the B.P., on the material under investigation and the amounts of ash soluble in dilute hydrochloric acid, obtained by difference.

The new material thus has a soluble ash ranging from 15.7 to 18.3 per cent. This quantity is distinctly higher than is normally found in

TABLE III  
ASH VALUES

Specimen	Total Ash	Acid-insoluble Ash	Acid-soluble Ash
	per cent.	per cent.	per cent.
<i>Unnamed Species :—</i>			
H.1 ... ..	21.5	4.9	16.6
H.2 ... ..	21.5	4.8	16.7
H.3 ... ..	22.3	4.6	17.7
H.4 ... ..	22.7	4.4	18.3
H.5 ... ..	23.5	6.1	17.4
H.6 ... ..	21.7	6.0	15.7
H.7 ... ..	20.2	3.2	17.0
<i>H. niger :—</i>			
N.1 ... ..	10.0	3.0	7.0
N.2 ... ..	13.7	1.7	12.0
N.3 ... ..	15.3	1.3	14.0
N.4 ... ..	16.0	4.6	11.4
N.5 ... ..	19.8	3.6	16.2
N.6 ... ..	21.7	3.3	18.4
N.7 ... ..	25.1	6.0	19.1

*H. niger* grown in England. The 3 samples of *H. niger* obtained from India, however, had soluble ash up to or above this range.

## GERMINATION

Hyoscyamus seed is known to be difficult and erratic in germination. For this reason a germination test was carried out on the Indian seed. Three methods of preliminary treatment were tried :—(A) The seed was placed in the refrigerator, at approximately 5°C., overnight ; (B) The seed was soaked in a solution of hydrogen peroxide, approximately 2 per cent. w/v, for 18 to 24 hours. This method of treatment was suggested for belladonna seed by Sievers<sup>2</sup> ; (C) The seed was soaked in concentrated sulphuric acid for 2½ minutes and then washed with water. This method is recommended by Newcomb and Haynes<sup>3</sup>.

The seeds so treated, together with untreated seed (D) were sown in pans on 30th July, 1948. Fifty seeds were put in each pan and 2 pans were sown with each type of seed. John Innes Seed Compost was used and the pans placed in the greenhouse. Counts were made after 10 and 17 days.

The results are recorded in Table IV.

TABLE IV  
GERMINATION OF INDIAN HENBANE SEED

Pan	1st Count	2nd Count	Average of Two Pans 2nd Count	Germination  per cent.
A. 1	2	2	2.5	5
A. 2	1	3		
B. 1	35	40	26.0	52
B. 2	9	12		
C. 1	1	2	1.5	3
C. 2	1	1		
D. 1	3	5	4.0	8
D. 2	3	3		

The results show that the treatment with hydrogen peroxide had a marked effect in stimulating germination. The other two treatments, that with sulphuric acid and refrigeration actually produced inferior germination to that obtained with the untreated seed. As regards the sulphuric acid treatment, this confirms the experience of Sievers<sup>2</sup> with belladonna seed. He states that treatment with sulphuric acid was of little use. It is probable that the period of refrigeration used in this experiment was too short. Melville and Metcalfe<sup>4</sup> found refrigeration successful in stimulating the germination of belladonna seed, but they used periods of 7 or 14 days. It is hoped to try their method with hyoscyamus seed this year.

The plants reached the flowering stage in 8 weeks after sowing and were then gathered and either dried or preserved in alcohol for examination.

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### SUMMARY

1. A type of *hyoscyamus* growing wild in India is described. The species to which this plant should be referred has not yet been determined.

2. This plant may be distinguished from *H. niger*, when unground, by the shape of the leaves, which are ovate to oblong and much less indented. The powder is distinguishable because the calcium oxalate crystals present are larger than those in *H. niger*; the presence of any calcium oxalate crystals exceeding  $35\mu$  long, or of more than 10 per cent. exceeding  $25\mu$  may be taken as indicating something other than true *Hyoscyamus niger*.

3. The total alkaloid contents of the specimens examined were, except in one case, less than the minimum required by the British Pharmacopœia for *Hyoscyamus*.

4. The acid-soluble ash was greater than that usually found in *H. niger* grown in England, but about equal to that found in *H. niger* from India.

5. A report of a germination test on the seeds is given. It was found that soaking the seeds for 18 to 24 hours in 2 per cent. w/v solution of hydrogen peroxide before sowing gave the best results.

Our thanks are due to Professor Qazilbash, of Islamia College, Peshawar and to Mr. K. C. Chatterjee, of Boots Pure Drug Company (India), Ltd., for the provision of material; to Mr. A. W. Billitt, of Lenton Experimental Station, Nottingham, for carrying out the germination test and for growing the plants; to Dr. R. Melville, of the Royal Botanic Gardens, Kew, for information; and to Mr. H. O. Meek, for helpful criticism. We are indebted to the Directors of Boots Pure Drug Company, for permission to publish this paper.

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### DISCUSSION

MISS B. JOHNSON read a summary of the paper.

DR. T. E. WALLIS (London) said that the authors seemed to indicate that this plant was different from *Hyoscyamus niger* or the other known species of *Hyoscyamus*, but he hoped they would be very careful in future work on the same material in deciding that it really was something different. Experience with plants in general showed to what an enormous extent details of structure varied in the same plant. The most extraordinary differences in the leaves of plants known to be of the same species might be found. He thought the shape of the calcium oxalate crystals was more important than their size. It was known that the size of crystals in henbane and similar plants varied enormously. Unless a careful review was made of their size, one might arrive at an inaccurate result. This plant closely resembled annual henbane, and it might better be compared with henbane generally than with *Hyoscyamus niger*.

DR. J. M. ROWSON (London) said that he had been interested in the

remarks on the action of hydrogen peroxide on the germination of seeds. He had himself had some experience of the erratic nature of the germination of *Hyoscyamus* seeds. He also was sceptical as to whether this was a different species of *Hyoscyamus*. He had grown annual henbane for the last ten years, with the same strain of seed and it was amazing to note the variation which occurred. He could produce leaves identical with those figured in the paper, broadly ovate, or with just one marginal tooth. He did not think there was any difference in that respect, nor was there much difference in the crystal sizes and total ash. The low alkaloidal content was not of great significance. The alkaloidal content of *Hyoscyamus* varied greatly with the environment in which it was cultivated, and it was possible that here they had poor cultivation. If the authors had details of the alkaloidal content of the Nottingham-grown material, it would be interesting to see how that compared with the Indian samples.

DR. W. MITCHELL (London) asked if there had been any attempt to characterise the alkaloids. It would be interesting to know whether they differed from those of normal *Hyoscyamus*.

MR. A. R. G. CHAMINGS (Horsham) said that, in the cultivation of belladonna in Leicestershire, he had found that successive refrigeration of the seed was much better than a single refrigeration.

DR. C. MELVILLE (Manchester) said that the use of calcium oxalate crystal determinations for identification purposes was interesting, and this could be extended. The illustration of the trichomes from the leaf gave the impression that the head of the gland was relatively simple, composed of not more than eight or ten cells. In *Hyoscyamus niger* the gland often contained upwards of twenty cells, with frequently two cells at the apex, one of which extended or projected beyond its companion; so that if the glands on this *Hyoscyamus* were relatively simple, it might prove a characteristic for differentiating it from *Hyoscyamus niger*.

DR. G. E. FOSTER (Dartford) asked whether the authors had investigated the volatile alkaloidal content of the drug.

MISS JOHNSON said that they would give further consideration to the suggestion that the plant was possibly a variation of annual henbane. The amount of material had been so small that it had been impossible to do anything more than the ordinary alkaloidal determination and the volatile alkaloids had not been determined. Regarding germination, they had stored their seeds in the refrigerator overnight at 5°C. and as stated, that had given very poor results. Since the paper had been written, they had tried longer periods of refrigeration. They would now try successive periods. Unfortunately, they had not yet had any reports on the effect of longer refrigeration.

MR. FORSDIKE said they had consulted authorities in this country and in India, and were satisfied that their material represented a species different from *Hyoscyamus niger*; it was hoped before next year to give it a definite botanical name. They had found in commercial material, during the war in particular, a henbane which differed in a number of ways from the typical *Hyoscyamus niger*, and they were sure that more than one species of *Hyoscyamus* was represented.



VEGETABLE PURGATIVES—PART I

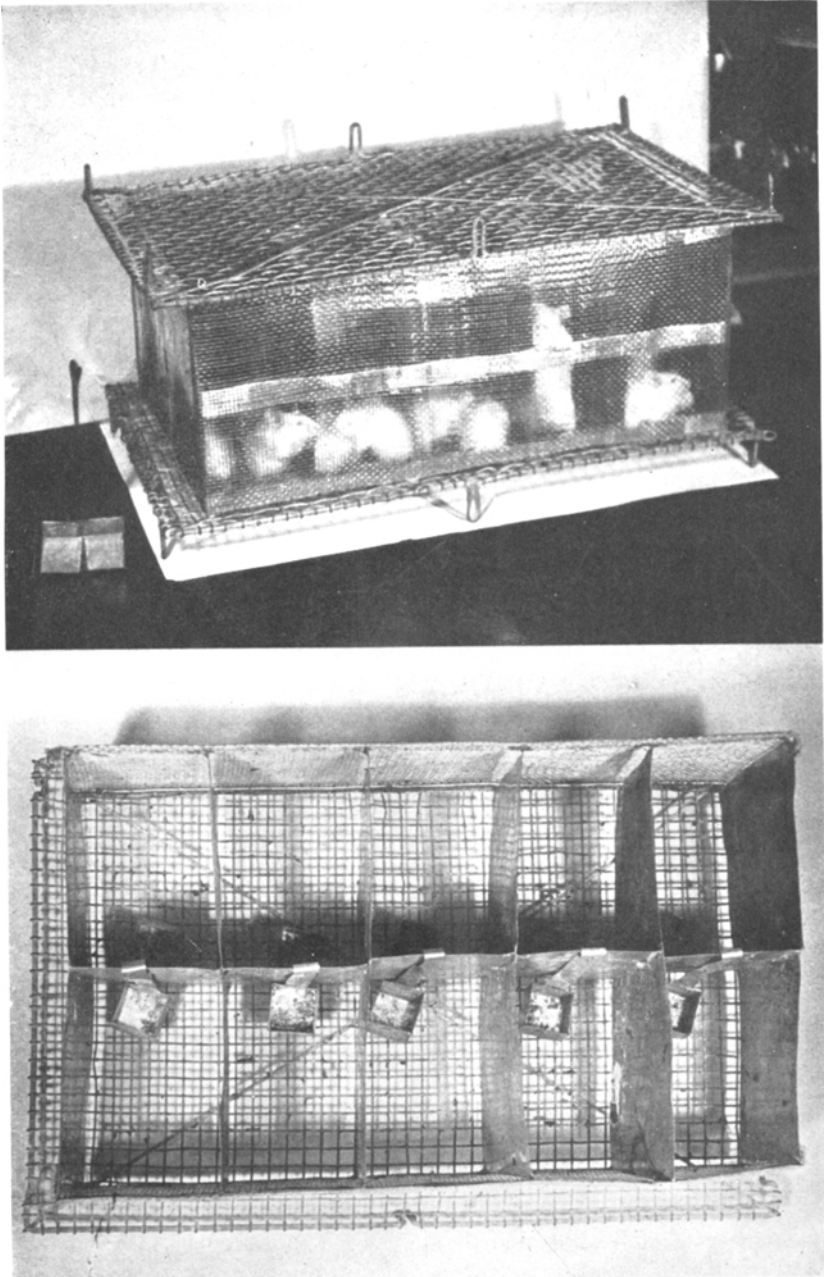


FIG. 1. Photographs of the cage. Upper: Side-view of the cage with a pair of food containers on the left. Lower: Top-view of the cage with the top grid removed.