REVIEW ARTICLE

THE PHARMACOGNOSY OF *ATROPA BELLADONNA* LINN.

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Introduction

ATROPA BELLADONNA Linn., family Solanaceae, is a perennial herb growing in calcareous soil to a height of about 2 metres: it is indigenous to Britain and most countries of Central and Southern Europe. A broad rootstock produces several aerial shoots which may branch and which bear numerous leaves; the leaves are broadly ovate, entire, petiolate, borne alternately in the lower part of the stem but occurring in pairs of unequal size in the upper part; flowers occur singly on short drooping pedicels in the axils of the pairs of leaves in the upper part of the stem, often associated with a few small leaves of a suppressed shoot. Eichler[1] has described and figured the upper part of each stem as a monochasial cymose inflorescence with adnation of the subtending leaf through one complete internode, the second leaf at each node and the dwarf leafy shoot representing a suppressed dichasial system. The flower has a campanulate purple corolla up to 3·5 cm. long, K(5) (C5) A5 G(2), stamens epipetalous; fruit a black fleshy berry up to 2 cm. in diameter with persistent calyx and containing numerous sub-reniform seeds. A number of tapering cylindrical roots up to 50 cms. long are attached to the rootstock.

It seems probable that belladonna, which is indigenous to Greece and Italy, was known in classic times and was used therapeutically. Unfortunately the botanical descriptions, recorded by Theophrastus, Dioscorides and Pliny for the materials *Mandragora, Strychnos* and *Solanum* respectively, are insufficient to enable them to be identified with belladonna, and some characters described by these authors are certainly not those of belladonna[2,3,4]. The first definite descriptions of belladonna date from the early 16th century; it was mentioned in the *Grand Herbier* printed in Paris about 1504, was described by Tragus[5] in 1532 as *Solanum hortense nigrum* and was both figured and described in 1542 by Fuchs[6] as *Solanum somniferum, S. somnificum* or *Dollkraut*. A photograph of the drawing by Fuchs is reproduced as Figure I.

Linnaeus[7], in 1753, named both the genus ATROPA and the species *belladonna*, but 4 years previously, in his *Materia Medica*[8], he had recorded the following monograph:—

89 ATROPA *Hort. Cliff* 57, *Hort. Upsal.* 45.
Solanum maniacum multis *Bauh. Hist.* 3. 611.
LOC: Pannonia, Austria, Anglia, Perennis, cicur.
PHARM: BELLADONNAE Baccae, Folia, ▽.
QUAL: venenata. *Insueta, praestans, caute.*
VIS: phantastica, paralytica, narcotica, anodyna.
USUS: Dysenteria, Rubor, Cachoëtes, Tumor mammarum, Fistula.
COMP:

(▽ is the Linnean symbol for water).

The leaves or flowering tops, also the root and rootstock, of belladonna are used for medicinal purposes. The leaf was introduced into the London Pharmacopœia of 1809 and the root was introduced into British medicine by Squire about 1860.

It is the purpose of this review to summarise the more recent work which has been carried out upon the pharmacognosy and physiology of belladonna. A well established corpus of knowledge of the morphology, anatomy, histology and chemistry of the drug, both leaf and root, may be found in the textbooks, to which the reader is referred.

CULTIVATION

A certain amount of belladonna herb and root is collected from wild plants, but the greater quantities are grown commercially. Gerrard[9] examined materials from wild species growing on limestone and from commercially grown samples and concluded that the wild plants were superior in alkaloidal content. This conclusion was challenged by Schmidt[10], by Carr and Reynolds[11] and by Carr [12], amongst others, who have found the tops and roots from cultivated plants to be richer in alkaloids than the material from the wild stand. Such a sub-division of all materials into " wild " or " cultivated " without further definition of the factors governing the growth of either group is too arbitrary to be of critical value. The amount of alkaloid present in the dried drug depends, amongst other things, upon the strain of plant from which it has been produced, the soil in which it has grown, including the addition of manure to the soil, the climatic conditions during growth, the age of the material when collected and the conditions under which drying has been carried out. When the plant is cultivated a number of these factors may be controlled and the optimum conditions for growth selected, hence we shall consider each of these factors in detail.

Germination. Many samples of belladonna seed are slow and erratic in germination. This is to some extent due to the occurrence in fully matured berries of a small proportion of light seeds which float on water and are non-viable[13], but the major cause of erratic behaviour is a physiological or mechanical dormancy which is not associated with the size or colour of the seed but is, to some extent, correlated with the separation of the seeds from the berries. Thus seeds separated from the ripe berries, washed free from pulp and dried, had a higher germination percentage than those which were allowed to dry within the fruit before separation[14]. Several chemical and physical methods have been proposed to break down this dormancy; Sievers[13] found that soaking the seeds in a 60 per cent. solution of commercial hydrogen peroxide for 18 to 24 hours gave the best results, freezing the seeds, also scarifying the testa, accelerated the germination, but soaking the seeds in strong sulphuric acid for different periods of time was of little value. The stimulus of initial refrigeration was supported by L'Vov and Iakovleva[15] (seeds stratified in moist sand in a cellar throughout the winter), by R. Melville and Metcalfe[16] (seeds refrigerated for 3, 7 and 14 days) and by Heit[17] (seeds pre-chilled at 3° to 5°C. for 25 days). Melville and Metcalfe found that

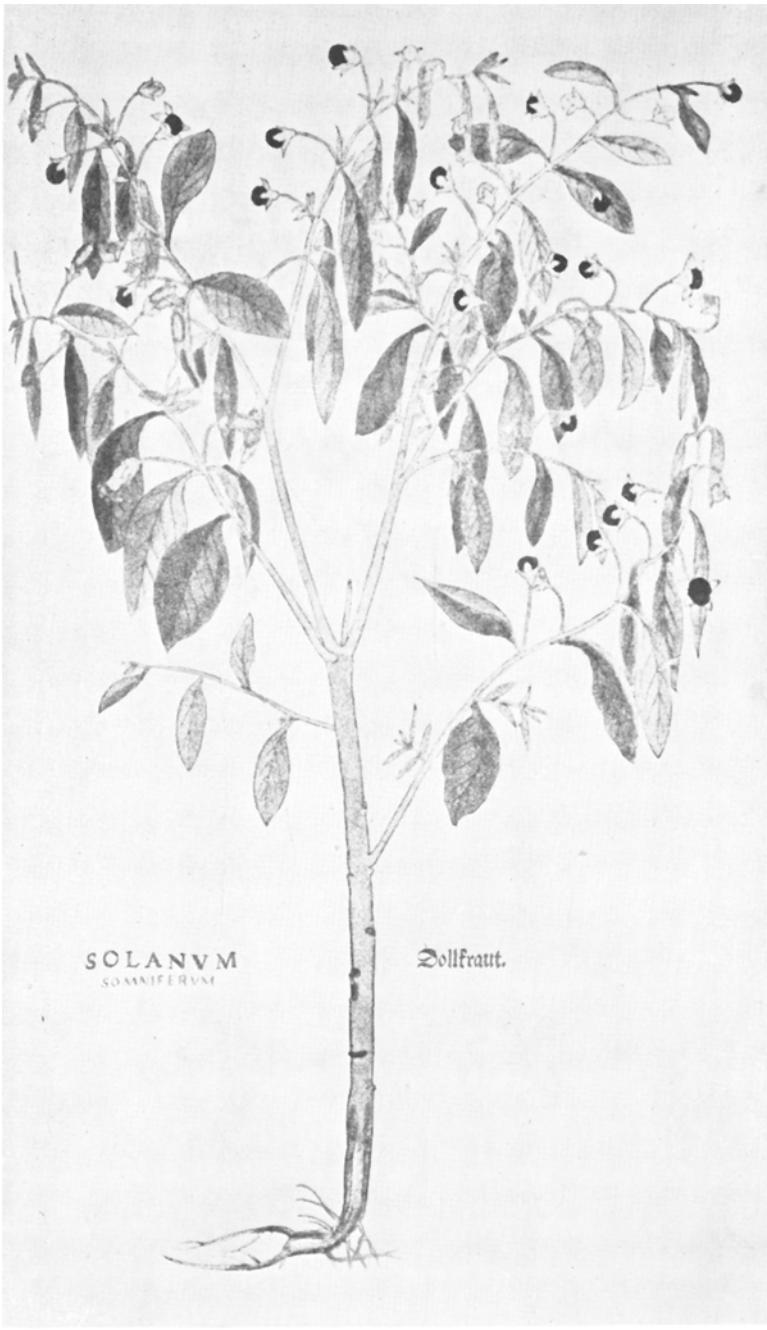

FIG. 1.—*Atropa belladonna* Linn., Reproduction of figure from Fuchs, "*De Historia Stirpium*," 1542.

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the germination of their controls increased greatly as the length of the experiment was increased, this they suggested was due to the diurnal fluctuation in temperature of the greenhouse in which the seeds were grown, Heit also found the use of fluctuating temperatures a suitable method of breaking dormancy when seeds were in the presence of a 0·2 per cent. solution of potassium nitrate. Scarification of the testa resulted in fungal attack, but a number of seeds were observed to germinate[16]. Stillings and Laurie[18] obtained a 50 per cent. germination by immersing seeds in 70 per cent. sulphuric acid for 1 minute, soaking in water and starting in an environment of high temperature and high humidity. A stimulus to germination and to growth using a mixture of aneurine hydrochloride and ascorbic acid has also been reported[19].

Plant Selection. A number of investigations have been made to select strains of plants which contain a high proportion of alkaloids and which retain this character in their progeny. Sievers[20] found that the individuality of the plant exerted a greater influence upon the ultimate yield of the drug and upon the amount of alkaloid therein than did variations in soil, manuring, weather and other factors. He crossed selected individuals, examined the F_1 and F_2 generations grown in different habitats, and concluded that the alkaloidal character of the maternal parent was inherited[21]. Miller and Reid[22] also Arny[23] claimed to have produced strains of plants of high alkaloidal content by selection and inbreeding. James[24,25,26,27,28,29] maintained some 40 strains in cultivation and produced hybrids between some of the races. A number of these were examined over a period of 5 seasons, but no one strain appeared to be consistently outstanding in plant vigour or in alkaloidal production: although 3 strains showed themselves capable of giving good growth and alkaloidal production in cultivation. James concluded that there was so much variation between different parts of the same plant and between the same parts at different ages as to render difficult the establishment of a factor for constant alkaloidal content in a particular strain of plant. No final conclusions may be drawn from these results as to the occurrence of distinct genetic races possessing a high alkaloidal content, it is certain that the environment exerts a marked influence upon alkaloidal yield of the plants and more detailed and carefully designed experiments will be necessary to elucidate the matter.

Manures and Soils. In analysing the results obtained by manurial treatments, three distinct objectives must be considered; they are (1) the weight of the plant material produced, (2) the assay, or alkaloidal content, of that material and (3) the absolute weight of alkaloid produced by the plant. To consider the assay value alone may be to obtain a false impression of the value of the treatment under investigation. Thus decrease in the dry weight of a leaf containing an unchanged weight of alkaloid will result in an increased assay figure, but to interpret such a result as an increase in alkaloid production is wrong. In consequence many modern results are expressed in terms of total weight of alkaloid rather than as a percentage of the dry weight.

(1) *Weight of Plant Material.* A number of workers have shown

that the application of manures, especially those containing nitrogen, resulted in increased growth and weight of belladonna plants. Thus Ransom and Henderson[30] obtained the following weights of green tops per acre: control (shade) 4 tons, control (sun) 7½ tons, sodium nitrate (1 cwt. per acre) 8¾ tons, mixed fertiliser (17 cwts. per acre) 17¾ tons. Stillings and Laurie[18] obtained an increase in crop yield by the use of high concentrations of balanced fertilisers containing nitrogen, potassium and phosphorus, whilst Brewer and Laurie[31] found that high levels of nitrogen manuring were necessary to produce heavy crops, but low levels of potasssium and phosphorus were satisfactory. Similar results have been obtained by Dafert[32] and by Dafert and Himmelbaur[33], and different organic or inorganic sources of nitrogen appear equally suitable. James[34] has shown that ammonium sulphate 1 cwt. per acre increased the weight of tops, but that heavier applications of the fertiliser decreased the yield. The following table by James indicates the effects of different nitrogenous fertilisers.

TABLE I

ATROPA BELLADONNA.—EFFECTS OF NITROGENOUS FERTILISERS ON DRY WEIGHT (JAMES)

Treatment	Dry weight per plant (g.)		
	Leaf	Stem	Root
Control	9·8	4·9	23·1
Nitro-chalk...	23·2	11·9	21·7
Ammonium sulphate	23·6	10·4	22·3
Sodium nitrate	20·9	11·9	35·1
Dried blood	23·2	10·7	25·0

By withdrawing essential elements from balanced sand cultures James has shown that potassium, calcium and phosphorus exert a slight positive influence on the crop yield, calcium withdrawal caused poor root formation and potassium withdrawal resulted in poor leaf development.

(2) *Alkaloidal Assay.* Contradictory evidence exists as to the ability of nitrogenous or other fertilisers to increase the percentage alkaloidal content of belladonna. Carr and Reynolds[11] found that all manurial treatments (kainit, basic slag, farmyard manure, sodium nitrate or superphosphates) produced lower assays than the controls. Ransom and Henderson[30] obtained similar results using different commercial fertilisers and Carr[12] found no change in assay of tops when farmyard manure or calcium cyanamide were used as sources of nitrogen, but nitrates (2 cwt. per acre) produced a lower assay. On the other hand Chevalier[35] produced leaves with 0·76 per cent. of alkaloid (control 0·32 per cent.) using farmyard manure and nitrates: Boshart[36] also Torricelli[37] have shown that nitrogenous fertilisers increase the amount of alkaloid present. Brewer and Laurie[31] applied nitrogenous fertilisers to the plants in the vegetative stage and noted an increase in alkaloidal content and De Conno[38] found that nitrogenous fertilisers, especially ammonium nitrate, increased the total alkaloidal content of the root. Cromwell[39] found an

increase in alkaloidal content in plants treated with ammonium sulphate but no increase when calcium or potassium nitrates were employed. Using pot cultures James[27,28,29] found increases in alkaloidal content of plants treated with different nitrogenous fertilisers; similar but less pronounced results were obtained in field experiments, these are shown by Tables II and III.

TABLE II

ATROPA BELLADONNA—EFFECTS OF NITROGENOUS FERTILISERS ON ALKALOIDAL CONTENTS

POT CULTURES (JAMES)

Treatment	Assay (per cent.)			Alkaloids per plant (mg.)			
	Leaf	Stem	Root	Leaf	Stem	Root	Whole plant
Control	0·21	0·21	0·31	20·8	10·3	71·6	102·7
Nitro-chalk	0·29	0·24	0·45	67·7	28·3	94·6	190·6
Ammonium sulphate	0·30	0·27	0·42	70·8	28·1	83·8	192·7
Sodium nitrate ...	0·40	0·28	0·39	83·6	33·3	136·9	253·8
Dried blood ...	0·42	0·24	0·53	97·5	25·6	132·4	255·5

TABLE III

ATROPA BELLADONNA—EFFECTS OF NITROGENOUS FERTILISERS ON ALKALOIDAL CONTENTS OF TOPS

POT CULTURES AND FIELD PLOTS (JAMES)

	Control	Ammonium sulphate	Sodium nitrate
Dry weight per plant (g.) :—			
Pot culture	9·8	23·6	20·9
Field plot	104·3	109·1	137·3
Assay (per cent.) :—			
Pot culture	0·21	0·30	0·40
Field plot	0·63	0·71	0·59
Alkaloids per plant (mg.) :—			
Pot culture	20·8	70·8	83·6
Field plot	650·8	773·1	811·4

Added potash salts produced a slight decrease in alkaloidal content or had no effect (Boshart[36], Carr[12], Chevalier[35], James[34]). Phosphatic manures produced a slight increase in alkaloids (James, Carr) or had no effect (Chevalier), and liming produced a slight increase in alkaloids (James).

(3) *Total Alkaloids per plant.* Tables I, II and III show that nitrogenous fertilisers produce considerable increases in dry weight per plant, some increase in assay and hence considerably increased weight of alkaloid per plant. James has also shown that a slight increase in total weight of alkaloid has resulted from liming or from phosphatic manuring but no change resulted from potassic manuring.

(4) *Soils.* A number of conflicting results described above may be due to differences in soils, whilst Table III shows a marked contrast between the results of pot cultures and field experiments. Carr and Reynolds[11] indicated that soil variations produce belladonna plants of different activities. Warin[42] found that the alkaloidal contents of extracts of belladonna

prepared from plants grown on chalky soil were greater than from plants on sandy soil or on argillaceous soil. Torricelli[37] reported that dried tops from plants grown in damp clay loam contained 0·3 per cent. of alkaloids, but when grown in light sandy soil they contained 1·0 per cent. Stillings and Laurie[18] have shown that a soil *p*H of 5·5 to 6·5 is optimum for alkaloid production whilst Sukhorukov[43] also stressed the influence of soil *p*H upon the accumulation of alkaloid in the plant.

We may thus conclude that a light calcareous soil with abundant balanced nitrogenous manuring during plant growth are essential for the production of a good belladonna crop possessing high alkaloidal activity.

Climate and Season. Marked variation occurs in the alkaloidal contents of belladonna crops grown under identical cultural conditions in different years[11,12,25,26,27]. Average temperature, sunlight and rainfall may contribute to these variations. A number of workers have shown that plants grown in full sunlight are richer in alkaloid and more luxuriant in growth than those grown in the shade, a total increase in yield of alkaloid per acre up to eightfold being recorded[30,44,45,46]. Carr[12] compared assay figures of commercial crops with meteorological records during the growing seasons for six years and found the lowest percentage assays occurred in years that were either very wet or very dull. Burmann[47] compared the assays of the harvested crop with the average diurnal temperature (4 observations per day) throughout growth and found a positive correlation between the values over a period of 5 years; he concluded that the cloudiness of rainy weather influenced the potency rather than the rainfall itself[48]. More recently Runge[49] has suggested that the alkaloidal content of a sample is dependent on climatic conditions throughout growth, being higher after warm sunny weather. This suggestion summarises the situation; insufficient statistics are available to determine the detailed individual significance of light, temperature and rainfall.

The two following experiments are of interest, but are not readily correlated with the foregoing observations. During three growing seasons Carr[12] shaded certain plants with white, green and red muslins and found the average assays of tops to be: control 0·51 per cent., under white muslin 0·51 per cent., red 0·68 per cent., green 0·77 per cent. Ripert[50] covered certain root crowns with cartons during March and allowed the shoots to grow in the dark for 84 days, the blanched leaves and stems were much richer in alkaloid (0·9 per cent.) than those of controls (0·5 per cent.), whilst the roots of blanched plants (0·21 per cent.) contained less alkaloid than the controls (0·24 per cent.). Exposure of the blanched tops to daylight for 9 days resulted in some decrease in alkaloidal content (0·75 per cent.).

Collection and Drying. Much evidence exists to show that the highest assay is found in tops at the time of flowering[31,36,24,25,26,27,28,51], although a number of workers have found little difference in the assay of leaves collected from May to September[12,42,49]. The analyses of Runge[49] for alkaloids in all aerial parts harvested at different times were:—April 30th 0·63 per cent., July 2nd 0·64 per cent., early August 0·71 per cent., September 26th 0·61 per cent. Commercial growers harvest three crops

annually (May, August and early October) from plants in their second and subsequent years of growth; thus three cuttings of actively growing leaves and young flowering tops are obtained. There appears to be little variation in alkaloidal content of roots throughout the year or over a period of several years[12,52]. Rapid drying at 50° to 60°C. in a free current of air or at 30°C. *in vacuo* prevents loss of alkaloid by enzyme activity, whilst Flück has shown that the enzymes responsible for alkaloid degeneration may be destroyed by heating at 100°C. for 15 minutes before drying is commenced with improved alkaloidal content of the product[53,54]. No differences in assay have been found between sun-dried or shade-dried leaves, but shade drying is preferred since such leaves retain their green colour[55,56]. Brewer and Laurie[31] dried entire plants and found that during the process there was a migration of alkaloids from the root to the foliage, and when only the aerial parts were dried the alkaloid initially present in the stem passed into the leaves.

Structural Characters

Aerial Parts. The anatomical and histological characters of the leaf, stem, flower, immature fruit and immature seed of *Atropa belladonna* are described briefly in the monograph of the British Pharmacopœia 1948 for Belladonna Herb. Formerly the leaves only were official and details of their macroscopical and microscopical characters are found in the textbooks of pharmacognosy or in the anatomical atlases[57,58,59,60,61,62,63]. The histological characters of the stem are described by Moll and Janssonius[64], but no detailed drawings are given; the striated cuticle, cruciferous stomata and protective trichomes of the epidermis, also the idioblasts of sandy crystals of calcium oxalate found in the cortex, phloem and pith are similar to those of the leaf; phloem, cambium and xylem form a continuous cylinder, the pitted vessels of the xylem being 50μ in diameter; perimedullary groups of phloem are present; small groups of fibres are present in the pericycle and also adjacent to the perimedullary groups of phloem. A description of the histology of belladonna flower is given by Moll and Janssonius and the structures, including the immature fruit and seed, are figured and described by Wallis and Butterfield[65]. Figure 2, taken from that paper, shows the characters of the powdered flowers, the chief diagnostic characters of which are the fibrous layer of the anther wall, subspherical tricolpate pollen grains about 40μ in diameter, papillose inner epidermal cells from the upper part of the corolla, thickened epidermal cells from the lower part of the corolla and pigment present in a number of the corolla cells.

Subterranean Organs. The morphology, anatomy and histology of belladonna root and rhizome have been described in pharmacognostical literature and the characters are summarised in the British Pharmacopœia 1948 for the root and rootstock which are official. C. Melville [66] has prepared detailed descriptions and sketches of the histological characters of the root, rootstock, stem bases and stolon. The root has a well-marked cork and phelloderm; wide, parenchymatous secondary phloem; large parenchymatous xylem with radially arranged scattered groups of

xylem vessels, tracheids and fibres and with scattered groups of interxylary phloem; the central primary xylem is diarch. The parenchymatous cells are filled with starch grains and idioblasts of sandy crystals of calcium oxalate occur; in older roots a concentric zonation of the xylem may occur. The rootstock may be up to 15 cm. in diameter; the broad xylem is pronouncedly thickened as a dense woody cylinder with lignified xylem parenchyma, groups of vessels, often with groups of interxylary phloem on the inner side, and an abundance of fibres forming the greater

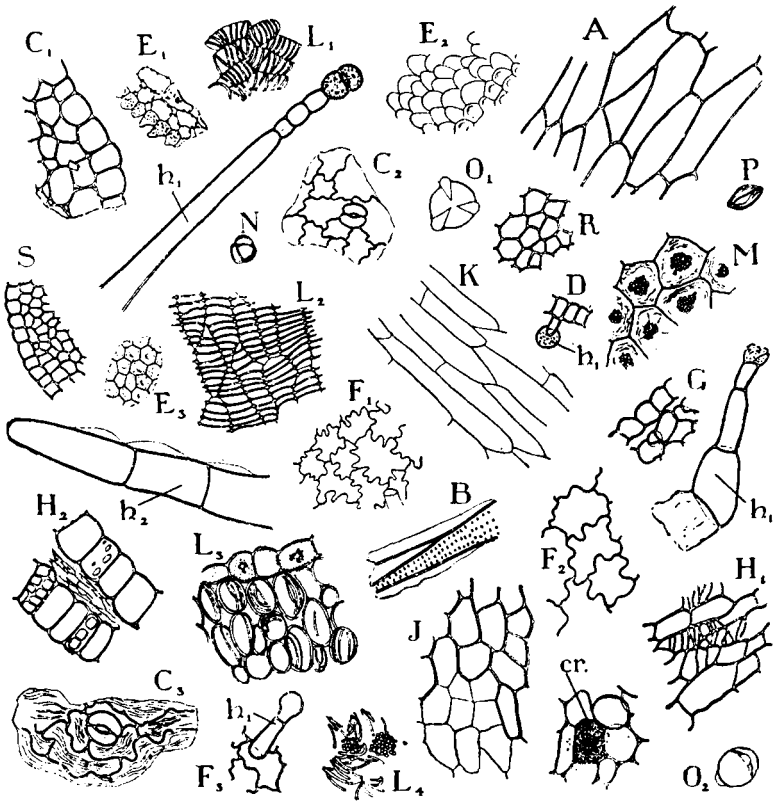

FIG. 2. *Atropa Belladonna* Linn., powdered flower. A, Epidermis of pedicel. B, Vessel from pedicel. C_1, Section of epidermis and mesophyll of calyx. C_2, Epidermis of calyx. C_3, Epidermis of calyx showing cuticular striations. D, Section of epidermis of calyx. E_1, Section of papillose epidermis of corolla. E_2 and E_3, Papillose epidermal cells of corolla. F_1, F_2 and F_3, Outer epidermis from upper parts of the corolla. G, Section of non-papillose epidermis of inner side of corolla. H_1, Thickened epidermal cells from base of corolla. H_2, Section of epidermis from base of corolla. J, Transitional region between unthickened and thickened cells near the base of the corolla. K, Epidermis from filament. L_1 and L_2, Fibrous layer of anther wall in surface view. L_3, Longitudinal section through wall of anther-lobe. L_4, Broken spirals from fibrous layer and chloroplasts from the epidermis of the anther. M, Epidermis of anther lobes. N, Young pollen grain. O_1, Pollen grain in polar view. O_2, Pollen grain from the side. P, Abortive pollen grain. R, Epidermis from ovary wall. S, Fragment of ovule. *cr.*, idioblast containing microsphenoidal crystals of calcium oxalate. h_1, glandular trichomes from calyx, pedicel and outer surface of corolla; h_2, covering trichome from inner surface of corolla or base of filament. All × 140. (Wallis and Butterfield.)

part of the tissue; concentric zones of unlignified xylem parenchyma are also found in the older rootstocks; a perimedullary phloem is present and also a central pith which may be lacunar; groups of pericyclic fibres are, at times, present. The vessel elements of the rootstock are shorter, and those of the stem bases are longer and narrower, than those

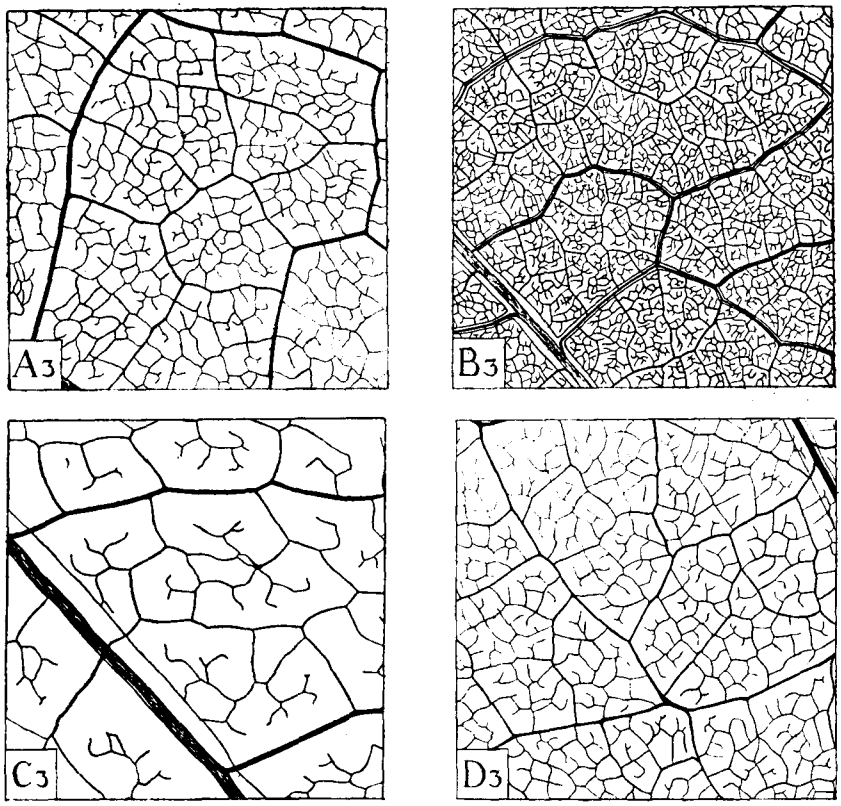

FIG. 3. Vein islets in cleared leaves; A3, *Atropa belladonna;* B3, *Ailanthus glandulosa;* C3, *Phytolacca decandra;* D3, *Solanum nigrum.* All × 8. (Forsdike.)

of the root; the vessels of the stolon are similar to those of the root. Xylem fibres are more abundant in both rootstock and stem bases than in the root.

Numerical Values. These methods have been developed in recent years for the characterisation of certain drugs and also, at times, to distinguish between a number of drugs possessing similar characters. The following values have been recorded for belladonna: —

(1) *Palisade Ratio* of leaf: —

6 to 10 (Wallis and Forsdike[67]),
5·6 (Bogarosh[69]),
6 to 10 (Boswijk[71]),
6·5 to 7·25 (Feinstein and Slama[68]),
1·6 to **6·0** to 10·4 (George[70]),
4·5 to **6·9** to 9·2 (Youngken and Hassan[72]).

(2) *Stomatal Number* per square mm. of leaf epidermis:—

Upper Surface	*Lower Surface*	
60	150	(Moll and Janssonius[64])
7 to 18	77 to 177	(Rowson[73]),
0 to 50	75 to 140	(Boswijk[71]).

These values are very variable within the species and are of little diagnostic use.

(3) *Stomatal Index* for lower surface of leaf is 19 to 24 (Rowson[73,74]).

(4) *Vein Islet Number.* Forsdike[75] has used the appearance of these islets in cleared leaves to distinguish between belladonna, ailanthus, *Phytolacca decandra* and *Solanum nigrum.* Figure 3 shows the leaves of these four species at the same magnification.

(5) *Cork Cells* per square mm. of root surface (when in 22/44 powder) is 400 to 600 (C. Melville[76]).

(6) *Vessel Index* (the percentage of vessels wider than 135μ in the root when in 22/44 powder) is 0 to **0·5** to 2·35 (C. Melville[76]).

Boswijk[71] recorded the number of idioblasts of calcium oxalate in 1 square mm. of leaf as 9 to 40 but considered the figure too variable to be of diagnostic importance.

Belladonna Alkaloids and their Biogenesis

The Alkaloidal Mixture. Good samples of dried belladonna herb contain 0·25 to 0·9 per cent., the dried root 0·3 to 1·0 per cent., of total alkaloids, of which *l*-hyoscyamine is the main component, occurring along with small amounts of *l*-hyoscine and of optically inactive atropine (*dl*-hyoscyamine). Earlier workers [77,78,79] found that racemic atropine rather than *l*-hyoscyamine was the dominant alkaloid in some material and concluded that samples varied considerably in the proportional contents of these two alkaloids. More recent critical methods for the quantitative separation of *l*-hyoscyamine, atropine and *l*-hyoscine have been developed[80,81,82,83] and they have shown that the alkaloidal mixture from belladonna root may contain 0 to 26 per cent. of atropine, and from the tops 0 to 40 per cent. of atropine. There is strong evidence to suggest that in the actively growing plant atropine is absent but as the plant becomes less vigorous some of the *l*-hyoscyamine is racemised to atropine. Kuhn and Schäffer found two maxima of total alkaloid production and of hyoscyamine content in all parts of the plant, the first occurring at the time of flowering, the second occurring towards the end of the growing season. The roots were found to show the greatest dominance of hyoscyamine at these two maxima, but in winter the proportion of hyoscyamine in the total alkaloidal mixture from the roots fell to 33 per cent. Racemisation also occurs if the drying process is protracted and in order to ensure the presence of the more active *l*-hyoscyamine the actively growing tops should be carefully and quickly dried immediately after collection. The hyoscine content of the total alkaloidal mixture has been found to be remarkably constant between 5 and 11 per cent.[83]. Mesnard[84] has examined the racemisation of *l*-hyoscyamine in galenical preparations

and considered the rate to be much slower than would be anticipated from a knowledge of the *p*H of the solution; this retardation he attributed to the presence of resins in the extractive matter.

Genetical Control. Under the heading of Plant Selection we have already seen that the great variations in alkaloidal content due to climate, soil and age of collection of plants have precluded a critical analysis of the possible existence of a gene for high alkaloid production in belladonna. The remarkable constancy of the proportion of hyoscine to total alkaloid is of interest in this connection for it may indicate an inherent character of the plant which maintains such a balanced production.

A study of the influence of colchicine on belladonna seeds or stem apices has shown that tetraploid plants or branch chimeras may be produced. Such plants are characterised by having in each cell nucleus twice the number of chromosomes present in the normal diploid plant ($2n = 72$); in consequence of the increased nuclear volume the pollen grains, stomata and other cells are also larger in size. The physiological balance of such plants is changed and both Szomolanyi[85] and Rowson[86] have reported increased potential for alkaloid production. A maximum of 153 per cent. increase in alkaloidal content of a 2nd year tetraploid plant has been recorded with an average increase of 93 per cent.[86]. From these results it has been concluded that the ceiling of production of alkaloid is determined by the nuclear complement but that the factors of environment determine the achievement of this production[87].

Distribution. Histochemical examination of living material has shown that the alkaloids occur only in living cells and are most abundant in metabolically active tissues, e.g. young cortical and pith cells, endodermis, bundle parenchyma and phellogen. Alkaloids do not occur in vessels, fibres, sieve tubes, collenchyma or the pith of older stems but are found in the xylem storage parenchyma of mature roots: they appear to be stored in the vacuole of each cell[27,28,88]. In the berries alkaloids have been found in the fruit wall, placenta and seed coat. The removal of the testa does not prevent germination and James found alkaloids formed by the actively metabolising cells of the young roots and stem apices in seedlings when about 3 weeks old[89,90].

A typical analysis[25,37,49,91] of the percentage alkaloidal distribution in the dried aerial growth of belladonna is:—leaf 0·9, young stems 0·5, old woody stem 0·1 to 0·4, unripe fruit 0·8, ripe fruit 0·6, seed 0·4. The incorporation of reasonable quantities of stem in belladonna tops is thus permissible without adversely affecting the total alkaloidal content of the official drug.

Locus of Synthesis and Translocation. For many years it has been tacitly assumed that the leaf was the site of synthesis of alkaloids and that there was no large-scale transportation of alkaloid throughout the plant. Kuntz[92] showed that the percentage of alkaloid gradually increased in root and seed throughout the growing season, it steadily decreased in leaf and stem, but there was no marked downward migra-

tion of alkaloids towards the root at the end of the growing period. Sukharukov[43] found very little evidence of alkaloid diffusion throughout the growth of the plant.

Recent investigations by means of reciprocal grafts have shown that for a number of species of the Solanaceae, including *Atropa belladonna*, the region of alkaloid production in the plant is the root[27,28,40,87,93,94]. When belladonna stems are grafted onto tomato roots as stock the aerial parts, after growth, are free from mydriatic alkaloids, but in reciprocal grafts the aerial parts of tomato grown upon belladonna roots contain abundant mydriatic alkaloids. In grafts between belladonna and tobacco the aerial parts of belladonna contained nicotine, and only traces of atropine, when granted on tobacco roots. Grafts between belladonna and potato have also been produced, and in every instance the nature of the root has determined the presence or absence of alkaloids in the aerial parts, thus indicating that the alkaloids themselves or their immediate precursors are synthesised in the roots. Stem exudations from the cut xylem of actively growing belladonna plants have been shown to contain hyoscyamine in the ascending transpiration stream, and this indicates that the alkaloids themselves are synthesised in the root, the method of translocation to the leaves being in the transpiration stream of the xylem. The finding by Stillings and Laurie[18] of the migration of alkaloids from root and stems into the leaf during the drying of entire plants, to which reference has been made previously (p. 207), supports this fact, although the authors make no comment thereon. It also explains the observation of Rosenkranz[95], amongst others, that in the leaf the alkaloids appear to reside near the veins and midrib. The entire absence of alkaloids from the sieve tubes supports the conclusion that there is no translocation of alkaloids in the downwards direction from leaf to root.

Biogenesis. Histochemical investigation, sand culture or manurial experiments have endeavoured to show a parallelism between the production of proteins and of alkaloids in the living plant[27,28,29,39,40,96,97]. Soluble nitrogenous bodies such as the amines may to some extent be diverted from protein synthesis to alkaloid synthesis, alternatively these fractions used in alkaloid production may be derived from protein breakdown. Cromwell concluded that the alkaloids of belladonna were built up from the products of protein breakdown and carbohydrate intermediaries. Robinson[98,99] synthesised tropinone from succindialdehyde, methylamine and acetone-dicarboxylic acid; he considered that the succindialdehyde and the methylamine may arise in the living tissue from the interaction of formaldehyde and ornithine, and acetone-dicarboxylic acid from citric acid. Cromwell made stump injections of 18 different nitrogenous substances into belladonna plants and found significant increases in alkaloidal contents when using putrescine; he also isolated putrescine from fresh belladonna plants and concluded that this substance was an intermediate in the synthesis of hyoscyamine, being derived by deamination from arginine. An enzyme capable of oxidising putrescine, with the production of ammonia and an aldehyde, has been isolated from belladonna

root and Cromwell hypothesised the production of succindialdehyde, required for the Robinson synthesis, as:

Direct synthesis
↘
arginine→ornithine→putrescine→succindialdehyde.
Protein breakdown ↗

Cromwell also hypothesised a synthesis of scopine and hence of hyoscine and of the volatile base N-methyl-pyrroline.

James[97] fed isolated leaves with solutions of different amino acids and found small but significant increases in weight of alkaloid when *l*-arginine and *l*-ornithine were used, and concluded that some *in situ* synthesis of alkaloid occurred in the leaf. The enzyme arginase, capable of converting *l*-arginine into *l*-ornithine and urea, also free ornithine have been isolated from belladonna. Since *l*-arginine is a universal constituent of plant proteins, and since alkaloid production can occur simultaneously with protein breakdown, James concluded that *l*-arginine and *l*-ornithine can act as precursors of the tropane alkaloids.

Dawson[100] has discussed the work outlined above, and considered Robinson's "model" synthesis to be improbable in nature; James' evidence for synthesis in the leaf he rejected as too slender in comparison with that from graft experiments, and hence the conclusions drawn from isolated leaves are invalidated. The presence of the arginase enzyme system and the isolation of certain amino acids from belladonna were only considered as very remote evidence of their relationship to alkaloidal synthesis since they are found in the tops of belladonna where no synthesis occurs, moreover they are normally geared to protein synthesis and are found in many plant species which do not produce alkaloids.

VARIETIES

A number of strains of belladonna have been noted by James[25,26], differing in plant habit, in the development of the suppressed axillary shoots, colour of corolla, and in the size and shape of mature fruit, but full descriptions of these strains have not been published as yet. A yellow flowered and yellow berried variety of the species is also well known[26,86,101].

The claims made for Bulgarian belladonna root in the treatment of Parkinsonism resulted in a full investigation of the anatomy and chemistry of the root which was shown to be identical with that of *Atropa belladonna* grown in England[102,103,104,105].

Indian Belladonna. The taxonomy of this drug has been a problem for many years; *Atropa belladonna* is known to grow in India and to be used commercially[106,107,108]. In 1917 and 1918 Holmes drew attention to the occurrence of a distinct commercial drug derived from wild plants of *Atropa lutescens*, formerly regarded as a variety of *A. belladonna*[109,110]. Wartime shortages of European belladonna during the last decade led to renewed interest in this drug[111,112] and monographs in the 5th and 7th Addenda to the British Pharmacopœia 1932, and in the British Pharmacopœia 1948 defined Belladonna Herb and Belladonna Root as derived from either the European or the Indian plants.

The " Index Kewensis " equates *A. acuminata* Royle and *A. lutescens* Jacquemont to *A. belladonna.* R. Melville[113] has shown the species of Royle and Jacquemont to be identical, the name *A. acuminata* taking precedence. Melville has indicated the differences between that species and *A. belladonna* and considers these sufficient to regard the Indian material as a separate species. A detailed investigation of the anatomy of the subterranean organs of *A. acuminata* has been carried out by C. Melville[114]. The numerical values in Table IV indicate the differences between the Indian and European drugs:—

TABLE IV

Value	*A. belladonna*	*A. acuminata*	Author
Vessel Index (Root No. 22/44 Powder)	0 to 2·35	11·2 to 14·3	Melville[76]
Cork Cells Number (Root No. 22/44 Powder) ...	400 to 600	265 to 371	,,
Palisade Ratio (Leaf)	1·6 to 10·4	2·2 to 13·5	George[70]
Stomatal Index (Leaf, lower surface)	19 to 24	16 to 18	Rowson[73,74]

CONCLUSIONS

A discussion of recent advances in our knowledge of the pharmacognosy of belladonna has been presented under the four main headings of Cultivation, Structural characters, Alkaloids and their biogenesis, Varieties. The conclusions which may be drawn from the evidence available have been recorded along with the discussions in each section of the work and the following results arising from modern research work have been presented:—

(1) The breaking of dormancy in seeds by chilling, fluctuations in temperature or acid treatment.

(2) The slight effect of calcium, potassium and phosphorus upon yield of plant matter and of alkaloid in comparison with the marked effect of nitrogenous manuring of the growing plant.

(3) The importance of collecting actively growing aerial parts and the rapid drying of leaf, stems and roots in order to obtain maximum content of *l*-hyoscyamine in the crude drugs.

(4) Anatomical characters of the flower, immature fruit, stem, rootstock, root and stolon and the use of numerical values to characterise belladonna leaf and root.

(5) The influence of chromosome number of the nucleus upon the potentiality of the plant for alkaloid production.

(6) Alkaloids are produced by the plant in the root only and move to the aerial parts in the transpiration stream. There is no downward translocation of alkaloids in the phloem.

(7) The use of putrescine and arginine by the plant as intermediates in the biosynthesis of hyoscyamine has been suggested but the evidence is not conclusive.

(8) The taxonomy, numerical characters of leaf or root, and the anatomy of the subterranean organs of Indian Belladonna have been discussed.

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