REVIEW ARTICLE

ALKALOID FORMATION IN PLANTS*

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The Accumulation of Alkaloids in Plants

In speaking of the relations between alkaloids and the plants that form them, it is convenient to begin with the distribution of the alkaloids within the plants' tissues. This is something that can be described with a good degree of accuracy and certainty, virtues which are not shared by all branches of our inquiry. The pioneer work was done by Errera and his school[1–6], working at Brussels over the 20 years from 1886 to 1906; and they did the job so thoroughly that little that is really new has been added since. Their aim was to show where alkaloids accumulate in plants, not limiting themselves to a single kind of alkaloid nor a single species of plant. They therefore needed a reagent of low specificity, reacting generally with alkaloids; and which should also have the additional property of readily entering cells and reacting with the alkaloids *in situ*. After many trials, they decided that the best for their purpose was Bouchardat's reagent, i.e., iodine (1 per cent.) in potassium iodide solution (1 per cent.). To show that their precipitations were alkaloidal and to prevent any confusion with the quite different glycogen and protein reactions, they showed that the reactions were absent from the same tissues previously treated with ethanolic tartaric acid solution (5 per cent.) to remove the alkaloids. More specific reagents, such as methylal-sulphuric acid for morphine, were used as appropriate for confirmation. They examined a very wide range of alkaloid-bearing plants, covering alkaloids of such diverse types as nicotine, the tropane series, morphine and its allies, colchicine, the glycosidal alkaloid solanine, and many groups such as those of the legumes, orchids and Amaryllidaceæ, which at that time were little known and imperfectly characterised.

One of the plants most searchingly examined in this way was *Atropa belladonna*, and it has since been examined by other workers including those in my own laboratory[8,9]. No serious discrepancies have come to light. Moreover, the same general results apply to all the numerous plants examined by the Brussels school, and Errera was able to sum up by drawing attention to 4 main tendencies[10].

Alkaloids tend to accumulate in:—

(1) *Very active tissues*, such as meristems of stem and root apices, including the apices of lateral roots while still embedded in the parent pericycle. Young potato sprouts are particularly rich in solanine and should not be eaten. Particularly interesting is the accumulation in wound cambia arising below the cut surfaces.

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Molle, of the Brussels school, showed these accumulations in the regenerating cells below the actually damaged ones of *Clivea miniata* leaves.

(2) *Epidermal and hypodermal tissues*, often including the hairs of the epidermis. It has been suggested that the relatively volatile nicotine may be lost in this way from the surface of *Nicotiana* plants.

(3) *Vascular sheaths*, pericycle and endodermis, but not the conducting tissues themselves[1].

(4) *Latex vessels* (where present) as in all parts of the opium poppy plant, but especially in the capsules[2].

At the other end of the scale, dead cells within the living plant rarely contain much alkaloid. *Cinchona* barks may hold as much as 10 per cent. of total alkaloids; but they occur strictly within the living parenchyma, not in the dead cork and fibres[11]. An exception is provided by *Datura* seeds. After fertilisation the perisperm contains alkaloids and, during the development of the seed, the perisperm cells are exhausted and dry to a thin membrane of crushed cells. These dead cells do contain much of their original alkaloid content, which is readily leached out with water[12]. *Berberis* alkaloids accumulate in the wood of old stems, partly in the walls of dead cells, but still mostly in living cells adjacent to the vascular bundles[13,14].

Within the living cell itself the alkaloids are almost always accumulated in the vacuole as water-soluble salts of the usual vegetable acids, or sometimes as salts of special acids, such as cinchotannic acid in *Cinchona*. The salts remain in solution and are not precipitated even in *Cinchona*. The limitation of the alkaloids to the cell contents can be made more visible by plasmolysing the cells before applying the alkaloid reagent. Wildeman's demonstration[5] with the epidermal cells of orchid petals seems to have been amongst the earliest. Further confirmation comes from the simple observation that, if tissue sections are cut so thin that the individual cells are opened, the alkaloids are lost from them. Any treatment, such as etherisation, that destroys the semipermeability of the protoplast allows the vacuole contents to escape, and the alkaloids are lost with them. If, instead of immersing a root tip directly in Bouchardat's reagent, it is first treated with a drop of ether and the ether afterwards removed, the alkaloid streams out from the root tips and forms a cloud of precipitate in the surrounding iodine[8].

Chaze[15] made careful observations on radicles of germinating *Nicotiana* seeds. He found the meristematic cells to contain aleurone, i.e., storage protein, grains which broke down as germination proceeded. As the proteins disappeared, they were replaced by minute fluid droplets which took up neutral red, i.e., behaved as vacuoles. These slowly increased and ran together, finally forming the large central vacuole. From the earliest stages of liquefaction, the vacuoles gave alkaloid reactions with iodine. The vacuole is the only part of the cell in which alkaloid accumulation can normally be demonstrated. With the exceptions already mentioned, the impregnation of cell walls by alkaloids, found in some crude

drugs, is a *post mortem* happening permitted by the breakdown of the protoplasmic membranes.

The life history of alkaloid accumulation in cells can also be followed by histochemical methods up to a point. That is, major changes of concentration can be followed, but the methods cannot be made quantitative within a cell, so minor changes are doubtful. Many alkaloid-bearing cells, such as those of *Cinchona* leaf primordia, are devoid of alkaloids in their very early stages[16]; then, during vacuolation, alkaloids accumulate rapidly to a maximum. This may be maintained throughout the further life of the cell, as in the bark parenchyma of *Cinchona*, or there may be a slight falling away. This is characteristic of peripheral pith cells. Cells towards the centre of the pith usually lose their alkaloids entirely as they reach maturity. It is still uncertain whether the loss is due to translocation towards the peripheral tissues of the stem, as the early investigators supposed, or to actual breakdown *in situ*.

Similar ontogenetic sequences occur in plant organs as a whole, and can be established by quantitative macroscopic analysis from a fairly early stage. Leaves are the most convenient and most frequently investigated parts. The time curve reflects that of individual cells. In the basal leaves of belladonna plants investigated through their growing season from April to June, the absolute amount of total alkaloids in the leaf at first increased; but from a fairly early stage began to decline, either on account of transport out of the leaf, or on account of decomposition. It is worth noting that the total alkaloid as a percentage of dry weight decreased from the earliest analyses obtained, emphasising the relative slowness of alkaloid synthesis[17,18].

The accumulation of alkaloids during the life history of the plant as a whole shows several interesting variations. The seeds of alkaloid-forming plants may themselves have no, little, or much, alkaloid in their tissues. *Nicotiana* and opium poppy seeds contain no nicotine nor morphine respectively. *Atropa*, *Datura* and *Erythroxylon coca* seeds have only scanty alkaloids, usually located in peripheral tissues. *Strychnos* and some lupins have larger quantities located in the storage tissues. There is a characteristic behaviour during germination for each of these three classes[18].

As representative of the first, barley will serve. There is no hordenine in the grain. Quantities equivalent to about 0·5 per cent. dry weight appear in the radicle during the first few days of germination[19]. They increase during the first 7 to 10 days and disappear rather rapidly between 14 and 18 days[20]. No hordenine appears again during the life-history of the plant. It is notable that it is lost while the seedling is in vigorous juvenile growth, not when it has become senescent. It is formed during a period of temporary excess of mobilised reserves; and one might suppose that, at this time, more of its amino-acid precursor is formed than can be accommodated in the protein pattern. The germination of *Nicotiana* seedlings shows a similar start; but the alkaloid goes on increasing during the life of the plant.

The second type of behaviour is typified by *Atropa* and *Datura*[21,22]. The small amount of alkaloid in the seed itself disappears. The first

appearance of alkaloids in the germling is again in the radicle and at a very early stage. I have found alkaloids in *Datura* radicles when they were only 2 mm. long. Nevertheless, these alkaloids seemed to be formed *de novo* and not to be merely translocated from the seed's original supply; because the peripheral tissues containing the preformed alkaloids can be removed without detriment to the new formations in the radicle. Again it is noticeable, as before, that the very youngest stages of the radicle, before it is 2 mm. long, are alkaloid-free[8].

The third and more complex story is illustrated by the seeds and seedlings of *Strychnos*, which have alkaloids both in the endosperms and in the embryos[23]. During germination, the alkaloids in the endosperm are broken down under the influence of the embryo; those of detached endosperms kept under the same conditions remain unaltered[24]. This is the way in which the reserve starch behaves in cereal endosperms. Alkaloids in the embryo are also broken down at first and later begin to accumulate again. Sucrose behaves like this in the embryos of barley. These alkaloids, and the more fully investigated alkaloids of lupin seedlings[25], behave like metabolically labile reserves; but their significance in this way should not be exaggerated, because they account at best for so small a proportion of the nitrogen and other materials of the seed.

The behaviour of the alkaloids during the later development of the plant is well illustrated by that of nicotine in tobacco[26]. It is interesting to note how closely nicotine accumulation runs with that of protein in the period of rapid growth, though of course on a smaller scale. Later, nicotine tends to be lost more than protein, and both show secondary increases when the growth of lateral shoots is encouraged. Nevertheless, too much significance must not be read into these parallels, because the accumulation of inorganic salts shows just the same behaviour. They are all consequences of the plant's general growth rather than closely related to one another.

It may be as well to emphasise at this point that everything so far said has applied to the accumulation of alkaloids rather than to their formation. We cannot, for example, assume that the cells in which alkaloids can be shown to accumulate are necessarily the cells in which they were synthesised; but this rather trite observation has not always had the attention it demands.

Solanaceous Grafts

The Solanaceæ are conspicuous for the ease with which they will form interspecific grafts, and these have played a considerable part in recent alkaloid studies; in fact, ever since Strasburger (1885) grafted thornapple cuttings on to potato stocks and had the tubers examined for alkaloids[27]. The chemists who did the analyses for him reported traces of atropine in the tubers.

The alkaloid-forming species in the family are very numerous and from this standpoint they fall into three classes; those that form

(*a*) *solanine and its allies*, i.e., the species of *Solanum* and *Lycopersicum*, including potatoes and tomatoes;

(*b*) *the tropane alkaloids*, particularly the species of *Atropa*, *Datura* and *Duboisia*;
(*c*) *nicotine and its allies*, mainly *Nicotiana*.

Strasburger's original experiment used a scion of type (*b*) grafted on to a stock of type (*a*). All the possible combinations of *a*/*b*, *b*/*c* and *a*/*c*, and their reciprocals, have now been performed with a great variety of species and the resulting grafts analysed in their various parts for tropane and nicotine alkaloids. The solanine-formers have simply been regarded as "alkaloid-free", so far as these other two types of alkaloid are concerned. The general result has been that the alkaloid, characteristic of the stock species, has been found in both stock and scion in about the usual concentrations; and the alkaloid characteristic of the scion species has been virtually absent from both the stock and the scion itself[18]. To this there seem to be some exceptions, and Mothes and Romeike have recently claimed that, when belladonna is grafted on tomato, the results differ with the variety of tomato used[28]. With some tomato strains no tropane alkaloids are demonstrable in the belladonna scions; but with others detectable quantities are formed. If this is confirmed it will be an important result.

Recently, it has become possible to perform an elegant variation on the grafting technique. In all the above an alkaloid "unnatural" for one or the other species is concerned. Evans and Partridge published a method for the separate estimation of hyoscine and hyoscyamine in mixtures[29]. The method depends upon a simple partition chromato-

TABLE I

ALKALOID ANALYSES OF *Atropa belladonna* AND *Datura innoxia*
Normal plants

Species	Sample	Total alkaloids	Hyoscine	Hyoscyamine	Ratio
		mg./g. dry weight			
Atropa belladonna ..	leaves	2·25	0·19	2·20	0·09
Datura innoxia.. ..	leaves	0·93	0·79	0·15	4·6
	stems	3·25	2·52	0·54	4·6
	tap roots	0·82	0·81	0·05	17·4
	fine roots	2·00	1·69	0·34	5·0
	leaves	1·80	1·65	0·14	11·4
	tap roots	1·75	1·65	0·10	16·9

graphy and can readily be adapted to the natural mixtures occurring in plants of the type (*b*) above. James and Thewlis[30] have examined the two species *Atropa belladonna* and *Datura innoxia* (frequently spoken of as *D. metel*) in this way. It turns out that the ratio of the amounts of hyoscine/hyoscyamine are very different in the two plants and surprisingly constant for each species. Table I shows the original series of results obtained, and these have since been confirmed. The mean value of the ratio for a considerable number of belladonna analyses was 0·12. The leaves, stems and fibrous roots of the *Datura* species show an excess of hyoscine, and much less hyoscyamine, with the result that the ratio rises about 50-fold. In tap roots it rises even higher, due to the almost

total disappearance of hyoscyamine, and this is the only considerable variation of the ratio noted within either species.

Using these two species it is possible to create a graft of the type *b/b* in which no alkaloids foreign to either component are introduced. The results are a confirmation of the older ones. The results in Table II show that, whichever way round the graft is made, the ratio characteristic of the stock appears in the scion as well as in the stock itself. The constancy of the ratio in belladonna is very striking; the somewhat greater variation of the ratio in *Datura* results from the error in estimating the very small amounts to which the hyoscyamine is reduced. Evans and Partridge, in a recent letter to *Nature*[31], say that they have themselves obtained similar results and have succeeded in analysing the hyoscyamine fraction further, revealing the presence of small quantities of meteloidine, in *Datura innoxia.*

TABLE II

ALKALOIDS IN RECIPROCAL GRAFTS OF *Atropa belladonna* AND *Datura innoxia*

Graft	Sample	Total alkaloids	Hyoscine	Hyoscyamine	Ratio
		mg./g. dry weight			
A. belladonna on *D. innoxia*	scion	1·07	0·91	0·08	11·4
	stock	1·20	0·90	0·12	7·6
D. innoxia on *A. belladonna*	scion	1·75	0·16	1·60	0·10
	scion	1·81	0·15	1·60	0·09
	stock	1·10	0·13	1·03	0·12

The distribution of the alkaloid in the scions receiving them has received some attention. The concentration in the leaves may frequently reach a level commensurate with the normal concentration in the leaves of the stock species. It is possible to grow the grafts to maturity and obtain a crop of fruit from the scions. When tomatoes are grown upon belladonna or thornapple stocks, great interest attaches to the question whether the fruit will contain alkaloids also, though remaining—as they do—to all appearances normal. There has been considerable diversity of opinion about the answer. Continental workers have frequently reported the identification of mydriatic alkaloids in such fruits[32,33,34]. Our own experience has been consistently negative, as in the results of Table III. Mothes[38] has reported that, of 3 varieties of tomato raised

TABLE III

ALKALOIDS IN TOMATO ON BELLADONNA GRAFT

		mg./g. dry weight
Tomato scion	leaves	8·05
	fruit 1–2 cm.	0·00
	2–3 cm.	0·00
	>3 cm.	0·00
	ripe	0·00
	pedicels	1·00
	stem	1·90
Graft union	—	2·25
Belladonna stock	stem	0·90
	roots	2·85

on *Datura* stocks, one had no alkaloid in the fruit, one had a little, and the third relatively large quantities. Miss Steenstra, in Amsterdam, similarly reported that some varieties of tomato accumulate alkaloids while others do not. Mrs. Wilson, recently at Oxford and now at Reading, arranged exchanges of seed with Miss Steenstra, and has now examined 10 different varieties of tomato grown as scions on belladonna stocks, and has been unable to demonstrate tropane alkaloids in any of them. She has also compared analytical methods with Miss Steenstra. It has become the practice in some southern states in America to grow tomatoes commercially upon *Datura* stocks. This is done because tomato roots suffer heavy infections to which *Datura* is immune. Although traces of alkaloids can be detected in the fruits by large-scale reductions of material, no harmful results seem to have afflicted the population.

Translocation of Alkaloids

The alkaloids exist in the plant exclusively as water-soluble salts. They are therefore likely to be moved from one part of it to another. There are two main directions of translocation: (*a*) towards the leaves and (*b*) away from them; whether the movement is physically upwards or downwards is relatively unimportant. Movement of solutes into the leaves mainly depends on the movement of the transpiration stream, the water of which is evaporated from the internal leaf surfaces, leaving the solutes stranded. Movement away from the leaves is mainly by way of the phlœm towards points of consumption of metabolites, such as the stem, root and flowering apices.

Movement of alkaloids in the transpiration stream passing from roots to leaves is an established fact. It is rendered probable by the graft results already discussed, and confirmation comes from the analysis of the sap seen to extrude from the stumps of decapitated tobacco plants. Dawson has shown this to contain 0·2 mg. of nicotine per ml.[36]

Movement in the phlœm presents a prettier problem. There is general agreement that alkaloids cannot be demonstrated within sieve tubes by histochemical methods, although neighbouring cells, such as phlœm parenchyma and bundle sheaths, may contain considerable quantities. This may only mean that movement keeps the concentrations in the sieve tubes too low for detection. There are, on the other hand, numerous results which are consistent with the idea of a phlœm transport. It was shown earlier, for example, that mature belladonna leaves lose alkaloids while attached to the plant; whereas detached leaves of a comparable age do not lose alkaloids until a general breakdown sets in.

The usual device of stem-ringing is not available for examining translocation among the Solanaceæ, because they have internal phlœm; and with the aim of getting round this difficulty Mrs. Wilson has performed a number of variations of the grafting method.

Her first device was the rooting of alkaloid-free scions, as illustrated in Figure 1. Belladonna was side-grafted on to *Physalis* or tomato and grown on to a convenient size. Then a downward-pointing flap

was cut from one side of the young belladonna stem and surrounded by damp moss in a split pot. Rooting took place after about a fortnight and the graft was grown on for 2 or 3 months. Without the roots it would have remained alkaloid-free in all its parts. Analysis of a belladonna/tomato graft with roots on the scion revealed 1·84 mg. of alkaloid in the scion roots themselves, 0·35 mg. in the scion shoot, a doubtful trace in the stem below the scion roots, and 0·51 mg. in the tomato stump and roots. It can only be supposed that the alkaloids found in the tomato stock had travelled down the belladonna stem between the two root systems. One would not expect to find appreciable quantities of alkaloids in the stem itself, since they do not accumulate in the basal parts of the stems of normal belladonna plants.

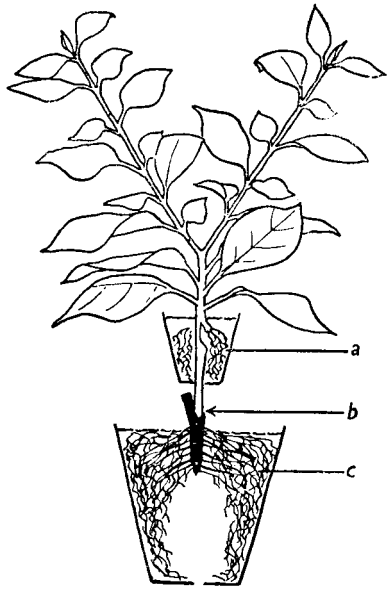


FIG. 1. Diagram of an *Atropa belladonna*/tomato "flap-rooted" graft.

a. Scion roots.
b. Side graft union.
c. Tomato stock.

After Wilson[37].

Approach grafts also gave corresponding results. A belladonna and tomato plant grown together in a single pot were side-grafted together and grown on without further manipulation. At harvest the tomato roots were found to contain 0·94 mg., or nearly a third, of the belladonna alkaloids in the graft. Similarly, grafting a rooted belladonna plant to an alkaloid-free belladonna scion carried by a tomato stock caused the accumulation of belladonna alkaloids in the stem and roots of the tomato stock. As in the first manipulation, the alkaloid must have travelled down through a considerable length of belladonna stem—initially free from alkaloids—to reach the tomato roots.

An interesting variation of this technique was obtained by grafting belladonna and potato together. Here the potato plant formed young tubers as well as roots below the graft union. At the time of harvest the new potatoes were to all appearances normal; but a pair taken for analysis contained 0·74 mg. of belladonna alkaloids. The occurrence of tropane alkaloids in potatoes raised on *Datura* stocks has been observed by Mothes[38,39].

Bridge-grafts provided another method. In these a downwardly-directed flap, an inch or more long, was raised from the side of a normal belladonna stem. A converse upwardly-directed flap was cut in an alkaloid-free belladonna scion, and the two grafted together. After 17½ weeks' growth, belladonna alkaloids were recovered from the tomato

stock carrying the scion to the extent of 23·71 mg. These experiments have been frequently repeated with consistent results, and seem to make it clear that belladonna alkaloids do travel along belladonna, and other, stems in a direction opposite to that of the transpiration stream and, therefore, probably in the phlœm. It was shown that they travel into fine roots and young tubers, i.e., into tissues which are rapidly growing and metabolising. The one unexpected exception seems to be the young fruits, and it is still uncertain whether the alkaloids that are normally abundant in belladonna berries are conveyed there from the roots or are synthesised *in situ*.

The Site of Alkaloid Synthesis

All parts of a plant are involved in the earlier stages of alkaloid synthesis, but, if attention is limited to the final and most characteristic steps, it is possible that the site is more limited. The results of the grafting experiments described above are usually taken to indicate that synthesis is predominantly in the root. Confirmation comes from the observation of alkaloid synthesis in tissue cultures of detached tobacco[40] and *Datura* roots[41], and probably from the early appearance of alkaloids in root meristems of barley, tobacco and opium poppy when all other parts, including the seed itself, are alkaloid-free, and from the rapid appearance of alkaloids in alkaloid-free scions, or even cut leaves of scions, when they are induced to root.

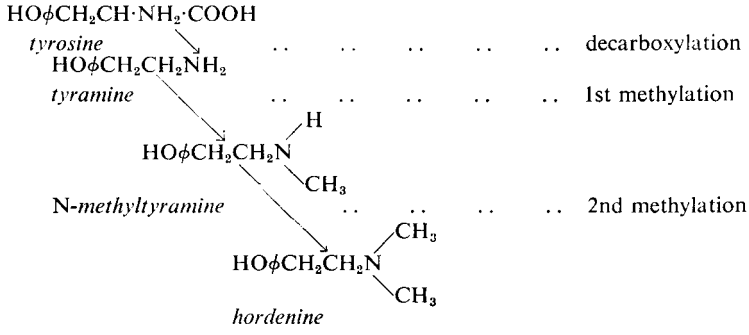
A number of exceptions has been listed. Detached *Cinchona succirubra* leaves are reported to increase their quinine when kept in the dark; *Nicotiana glauca* forms anabasine either as stock or scion[43]; and *Nicotiana glutinosa* scions form nornicotine, apparently by demethylating nicotine received from the roots[43,44]. In reciprocal grafts of *Datura tatula* and *Datura ferox*, the former continues to yield its normal hyoscyamine when a scion. *Datura ferox*, as a scion, contains its normal meteloidine. Both contain hyoscine, and it has been suggested that hyoscyamine and meteloidine are either being formed *de novo* or by reduction of hyoscine in their respective shoots[45]. It is difficult to assess the significance of these results at present, because the mixture of alkaloids in the stocks has not yet been analysed, and because the ratio of hyoscine/hyoscyamine, or hyoscine/meteloidine, is very different in the scion from the value given for the normal top.

Attempts to form nicotine and tropane alkaloids in cut leaves and shoots have led to confused results, and the most usual and vigorous site of synthesis certainly seems to be the young root, probably in vacuolating cells. The evidence from grafts, that alkaloids are not normally formed in shoots is not quite conclusive on account, for example, of the possibility of a suppressing influence of a foreign stock (cf. also Mothes[38]).

Mechanism of Alkaloid Synthesis

It is a convention, credible at present, that alkaloid synthesis may be taken as starting with an appropriate amino-acid, such as ornithine leading to tropane alkaloids, or proline to the nicotine series. Numerous

attempts have been made to obtain evidence for the occurrence of such series in plant tissues, so far with very little success, the main obstacle being, apparently, the extreme sluggishness of the reactions which it is wished to investigate. As a type, the relatively simple formation of the alkaloidal amine hordenine may be briefly discussed. Its isolation and characterisation were accomplished by Léger[19] in 1906, and the following mechanism of formation from tyrosine was suggested by Raoul[46] in 1937.



Formation of hordenine is restricted to young barley roots during the first 3 weeks. It increases more or less steadily to a maximum during the first fortnight, and then more rapidly disappears. Evidence of the reaction-path comes from identification of the substances concerned and from feeding experiments. It has been shown that free tyrosine is present in 3-day-old barley seedlings, though it may be present in the seed only combined in its proteins[47]. Tyramine and *N*-methyltyramine have been identified by separation on paper chromatograms. They are present only in small amounts and could be recognised from about the 10th to the 14th day[20]. *N*-methyltyramine has been isolated in larger quantities by V. S. Butt at Oxford. After separation on a cellulose column it was obtained as its hydrochloride giving an undepressed melting point at 148° C. 15 mg. was obtained from the germination of 1 kg. of Spratt-Archer barley. *N*-methyltyramine has also been isolated by Kirkwood and Marion[45] from a strain of barley that does not produce hordenine.

Two major difficulties are encountered in feeding experiments; hordenine is formed only at a time when the endosperm is providing abundant amino-acids, and the surface of barley seedlings is normally infested with micro-organisms that may affect external nutrient additions. This is particularly important in the initial decarboxylation, since some bacteria appear to be particularly active in decarboxylating amino-acids.

To get round the first difficulty, Kirkwood and Marion[49] fed labelled tyramine to barley seedlings and subsequently identified similarly labelled *N*-methyltyramine and hordenine chromatographically. The intensity of the activity was higher in the *N*-methyltyramine than in the hordenine. Labelled tyrosine (*dl*-tyrosine-2-C^{14}) gave similar results[50], and the evidence was taken to support the existence of the series of reactions

proposed. Since no labelled tyramine was found, it was presumed that its existence was transient. No evidence of the absence of contaminating micro-organisms was provided.

To overcome both difficulties a series of experiments has been made in the Oxford laboratories by Mrs. S. V. Barber, using excised barley embryos in sterile culture. The embryos were deprived of their endosperms, which were replaced as a source of nutrients by White's medium, without the glycine usually included. They were then germinated, suspended in the medium by vigorous æration in tubes containing 15 embryos each. About 1 tube in 10 became infected after several days and was discarded. After 9 days the embryos had roots about 1 inch long and, grown in this way, were entirely devoid of hordenine or its suggested precursors. When an endosperm extract was added to the White's medium, hordenine was formed. This method, therefore, provided a suitable means of testing possible intermediates. Addition of tyramine and *N*-methyltyramine did not result in any formation of hordenine detectable on chromatograms, even though the bases could be shown to enter the embryonic tissues by the staining due to their partial oxidation to melanins.

The failure to form hordenine might be due to lack of methyl donors. The experiments were, therefore, continued with additions of formate, betaïne, choline or methionine as well as the base. Betaïne proved toxic at the concentration used (15 mg./l.). Formate + tyramine produced no synthesis of hordenine, but choline and methionine when added with tyramine both caused the formation of small amounts of *N*-methyltyramine and hordenine. Methionine with added N-methyltyramine also gave hordenine. The efficiency of methionine as a methyl donor in plant tissues has also recently been emphasised by its action in *Dicentra* seedlings during the synthesis of protopine[56]. There is therefore good evidence that hordenine may be formed in barley by two successive methylations of tyramine, but the evidence that the tyramine comes from tyrosine is much less conclusive. Prolonged attempts to identify a tyrosine decarboxylase in barley gave consistently negative results. A vigorous glutamic decarboxylase was, however, isolated, the produce of whose action was γ-amino-butyric acid[51]. It is, therefore, conceivable that hordenine may originate from glutamic acid rather than from tyrosine, and that addition of the phenolic group may come after the decarboxylation instead of before. Whichever is the order of events, the origin of the phenol is at present equally uncertain.

Consequences of Alkaloid Formation

As mentioned earlier, the alkaloids in the reserve tissues of seedlings may disappear and their carbon and nitrogen probably be returned to metabolic circulation. The amount of material concerned in these changes is, however, very small, usually less than 1 or 2 per cent. of the nitrogen, and probably much less of the carbon. When alkaloids accumulate in any considerable quantity it is usually in tissues such as the bark of *Cinchona* or the old stems of *Berberis*, from which they are

not lost. The alkaloids cannot be regarded as having much significance as metabolic reserves.

Attempts to show that they exercise a protective function have also met with little success. While it may be that alkaloids are toxic to many predators if taken in sufficient dosages, it appears to be very rarely that this happens in practice. For example, aphids parasitise *Nicotiana* to the great detriment of the plants; they are not poisoned by the nicotine in the plant saps, but can be conveniently destroyed with nicotine sprays. Alkaloid toxicity is specific and often too limited to protect against species dangerous to the plant. The alkaloids of *Atropa belladonna* are highly toxic to the human species, which is not an eater of belladonna, but are innocuous in quite large doses to farm animals, rabbits, birds, aphids, caterpillars and flea-beetles. The last two are common and dangerous pests of belladonna crops. Plant parasites, such as mistletoe on *Duboisia* and dodder on *Conium* and *Delphinium*, are not controlled by the alkaloids of the host, though the alkaloid may pass in considerable quantity into the parasite. Fungal and bacterial attacks are not averted either. Any protective results of alkaloid formation must be slight and erratic. So far no convincing evidence has been produced that alkaloids serve any significant rôle in the plants that produce them, and alkaloid-free scions grown on stocks that form no alkaloids show no abnormalities that might be attributed to their absence.

The Causes of Alkaloid Production

Alkaloid-forming plants, probably about 10 per cent. of the known flora, may be regarded as those in which an additional metabolic reaction-chain has been evolved. The taxonomic distribution of related groups of alkaloids in related species is consistent with this suggestion. Qualitative changes in the alkaloids formed by a particular species, or even changes in their relative quantities, are very difficult to bring about physiologically. The primary control lies with the gene complex. It is known that single genes may directly control the existence or operation of individual enzymes. In crosses of *Nicotiana tabacum* with *N. glauca* the principal alkaloids of the F_1 generation were anabasine and nornicotine, characteristic of *N. glauca*. The formation of nornicotine could readily be explained on the assumption that *N. glauca* possesses a gene controlling the demethylation of nicotine to nornicotine, which is absent from *N. tabacum*, in which the main alkaloid is nicotine itself[44,54].

Any new enzyme formed owing to the mutation of a gene might bring a whole chain of new reactions into being. It is not necessary to suppose that each additional step would require a further mutation. Adaptive enzymes may arise as a response to the presence of a new compound capable of acting as substrate. Such enzymes, stable only in the presence of their substrates, are already known to exist in the higher plants. Pre-existing enzymes of low specificity might react with the new products and modify them further, and some reactions may even be spontaneous, requiring no catalysis. By such processes, discussed in rather more detail elsewhere[55], it is possible to suppose that the great wealth of alkaloids

(not to mention pigments, tannins, glycosides and other plant products) have come into being. Whether they will have any significance for the existence of the plant that forms them is entirely secondary. The alkaloids are evolutionary try-outs. They may be called waste, more or less in the sense that experimental models and chance by-products are waste. They are created by a blind designer, who scores far more failures than successes, but the failures are just as necessary and as inevitable a part of the evolutionary process as the triumphs.

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