REVIEW ARTICLE

PHYSIOLOGY OF ALKALOIDS*

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We have little idea of the total number of alkaloids or their distribution in the plant kingdom. Quite apart from the well-known sources of alkaloids found within systematic natural orders, traces of the selfsame alkaloids may be found widely scattered throughout unrelated generat Their presence and amount is largely governed by the ability of the plan. to effect biosynthesis and also its ability to tolerate these products.

We know today that nicotine,occurs not only in the genus *Nicotiana* but that it is found, in addition, in Equisetaceae, Lycopodiaceae, Crassulaceae, Papilionaceae, Cannabinaceae, Chenopodiaceae, Asclepiadaceae and Compositae. In the family Solanaceae we find it also in the genus *Duboisia*, *Solanum* and *Atropa*, but mostly only in traces. If nicotine is introduced into *Atropa* or *Lycopersicum*, for instance, by suitable grafting on a *Nicotiana* root stock, the leaves of the scion when exposed to strong light show bleaching of the chloroplasts. Such leaves may become completely white, and live as parasites on the stock, which nourishes the scion with its own leaves. *Atropa sp.* can form nicotine, but cannot withstand high concentrations of this substance[1].

Although most scions of different species upon *Nicotiana* stock are damaged by the nicotine formed in the root and ascending to the shoot, there are remarkable exceptions. Thus the composite *Zinnia* grows well. This we explained by the finding that *Zinnia* itself synthesizes nicotine, and that its nicotine-content is not less than that of low-alkaloid tobaccos[2]. This means that during evolution a resistance must have developed parallel with the development of other new characteristics. This resistance is likely to depend upon a complex of factors, and to effect a marked increase in the content of alkaloid by an artificial mutation, caused say by X-rays, is not easy if the factor of resistance is not already developed.[3]

One of the main causes for the limitation of the occurrence of alkaloids could be due to incompatibility. Extremely high alkaloid concentrations are very rare in living tissue. Careful and extensive selection work has shown that aberrant types with remarkably high alkaloid content do occur occasionally but are localised. Obviously high alkaloidal content is associated with a decreased vitality[3–5].

An instructive example is provided by ergot of rye. We know of strains that contain more than 1 per cent of alkaloid in the sclerotium, but for

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artificial culture on rye-fields only strains which contain 0·3 to 0·5 per cent of alkaloid are used. Probably the high-alkaloid strains have less vitality, which means a much reduced yield. This suggests that the optimum alkaloidal content is genetically fixed and shows only limited variation. This is also true for the alkaloid composition of ergot. It has been possible to select strains that contain a preponderance of alkaloid, which is valuable for industrial production[5,7]. We have found such a strain to keep its exclusive character, no matter whether cultivated on rye or on wild grasses[8]. Although the number of ergot strains in nature is enormous, we ourselves have isolated thousands, it is interesting that some are geographically localised.

I think that the selection of stable races, which are quantitatively and qualitatively valuable, is the necessary presupposition for the permanent use of medicinal plants in therapeutics and for the production of distinct plant substances. If by quality-breeding the step from the wild to the cultivated plant could be speeded, it would take only a short time for the drug market to have sufficient materials of reliable source and at low cost.

Alkaloid Synthesis

Site of Formation

The development of the alkaloid character is not only a genetic problem but also a problem of physiology. Unfortunately the number of well-investigated alkaloidal plants is very small, and limited to plants with alkaloids which are predominantly or exclusively formed in the root, and accumulation of which in the organs of the shoot is the result of a translocation. This is true for the synthesis of nicotine in some tobacco species, and of hyoscyamine-hyoscine in *Datura*, *Atropa* and *Hyoscyamus*.

Several institutes have assisted in this discovery. The most important proofs are those that follow. Excised roots in sterile culture are able to form alkaloids and can excrete large quantities into the surrounding liquid medium. It may be that this cannot be altered, as the prevention of the flow of alkaloids from the root to the shoot would effect a high accumulation of alkaloids in the root, and probably this would mean a self-toxication of the root-tissue. Isolated leaves, however, form no alkaloid or only a little. But when they are rooted, alkaloid appears. If a scion of an alkaloid-free plant is grafted on the root of *Nicotiana*, *Atropa*, *Datura* and others, the alkaloids characteristic of the stock appear in a short time. Investigation of the bleeding-sap of such graftings showed that the alkaloids migrated from the root to the shoot. If, on the other hand, a scion of *Nicotiana*, *Atropa* or other alkaloidal plant is grafted to an alkaloid-free root (e.g., *Cyphomandra*), the scion remains low in or devoid of alkaloid. Summaries of the history of these investigations have been published by Dawson[9], Iljin[10], James [11] and Mothes[12].

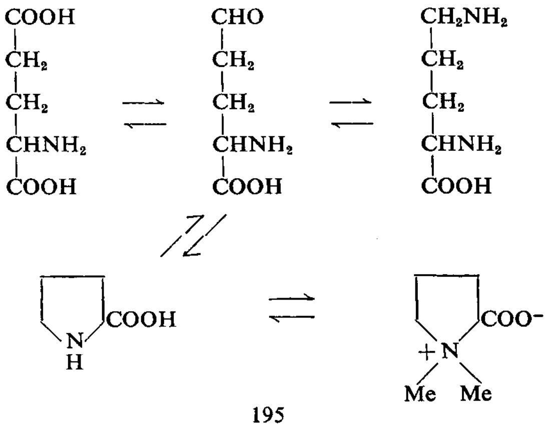
There are other alkaloids which are formed predominantly in the shoot (solanidines)[13–15], and alkaloids which cannot be formed in the isolated root (lupine-alkaloids)[16–18]. Probably the number of alkaloids synthesised in aerial parts is much greater than those in the roots. This is indicated by the investigations of Cromwell on *Conium*[19], of Shibata on *Ephedra*[20] and of Leete on *Catha*[26]. The arbitrary division of the alkaloids according to whether they are formed in root or shoot is not possible, as, for example, when the synthesis of nicotine had been discovered to take place in the tobacco root we had already called the root the "Hauptbildungsstätte" (chief place of formation) with some reservation[21], because we had found that young leaves could also synthesize nicotine[22]. The formation in the shoot of alkaloids normally synthesised in the root has since been confirmed by various investigators[22–25].

In plants where the root is the "Hauptbildungsstätte" of the alkaloids the ability of the shoot to form alkaloid seems to be associated with the influx of certain precursors from the root. We found[24,27], that the hyoscyamine-synthesis in *Atropa* scions on various tomato strains was not always the same. This might indicate that a leaf or a shoot during its development loses its capacity to perform by itself all the reactions necessary for the complete synthesis of an alkaloid. A fully developed leaf behaves like a mutant, a gene of which has become ineffective for the alkaloid synthesis.

Influence of Development and Ageing

The influences of development and ageing on the metabolism have been rarely investigated. I am sure that some negative results found in the investigation of the synthesis of alkaloids using isotopes were due to insufficient attention being directed towards these points of the physiology of development. There are numerous publications reporting experiments in which a probable precursor in its labelled state was fed to plants, and where the lack of success led to the conclusion that the substance investigated could not be the precursor. In these experiments, however, whether any synthesis occurred during the experimental time was not observed.

Thus it was found that seedlings of *Medicago sativa* were incapable of forming stachydrine from [2–^{14}C]-ornithine[28,29], though the synthesis of proline from ornithine has been proved using several organisms. Recently it was shown that these seedlings can convert ornithine to glutamic acid, and that, in the presence of pyridoxal, both ornithine and glutamic acid are converted to proline. The methylation of proline to stachydrine is possible when the plant is fed folic acid together with methionine[30].



Obviously the young plants require the precursors in an amount sufficient for the deamination and activation of amino acids as well as for the methylation of the N-atom.

In the growing plant a similar differentiation of the chemical performance takes place in the root. For instance, nicotine is produced only in any quantity in the growing tip of the tobacco root. In the older parts there is little or no synthesis at all, although they are still growing in thickness, and cell division takes place. All the factors promoting cell elongation and branching of the root also promote alkaloid synthesis. But it is not yet known to which cells of the root-tips and to which processes the alkaloid synthesis is bound. A remarkable effect is observed when the number of growing root-tips is experimentally increased, which can easily be done by repeatedly cutting the roots. Thus a threefold increase in the absolute nicotine production can be produced over 3 months[31].

TABLE I

LEAF-CUTTINGS OF *Nicotiana rustica*, 136 DAYS OLD. CONTROL: ROOTS NOT CUT. EXPERIMENT: ROOTS CUT THREE TIMES AFTER 45, 84 and 105 DAYS. ALL THE RESULTS FOR THE CUT ROOTS ARE INCLUDED IN THE TOTAL ANALYSIS. RESULTS RELATED TO 10 LEAF-CUTTINGS

	Analysis after days	Fresh weight g.	Dry weight g.	Total N mg.	Protein N mg.	Soluble N mg.	Nicotine N mg.
Control							
Lamina	136	118	13·2	753	256	497	115
Midribs and stalks	136	60	6·9	320	138	182	41
Roots	136	237	14·2	638	312	326	32
Total		415	34·3	1711	706	1005	188
Experiment							
Lamina	136	110	11·7	750	200	550	245
Midribs and stalks	136	54	6·6	318	138	180	63
Roots 1st cut ..	45	34	1·9	89	43	46	2
,, 2nd cut ..	84	53	3·7	155	70	85	9
,, 3rd cut ..	105	45	3·0	130	55	75	10
End of experiment	136	76	6·5	307	137	170	30
Total		372	33·4	1749	643	1106	359

Nitrogen Uptake

It should be noticed that the excess production of alkaloid is not related to an excess absorption of nitrogen: the control plants with intact root systems take up just as much nitrogen from the medium, but they form much larger quantities of amides, especially glutamine, and accumulate them in the leaves as the plants with cut roots accumulate the nicotine. Up to date it has not been possible to effect a synthesis of nicotine or hyoscyamine in excised roots which have been deprived of their tips. Moreover, the roots must be growing, as roots without tip-growth cannot perform this synthesis.

Low concentration of auxin promotes the root growth in terms of length and dry weight. Such roots form much nicotine. Higher concentrations inhibit the growth in length and the formation of dry weight; they also inhibit the nicotine synthesis, but not to the same extent as the protein synthesis. On the contrary, these low-alkaloidal roots are high in protein, and they respire intensively[32,33]. (Tables II and III.) Thus it is possible that the elongation of the cells of the root-tips, but not their division, is closely related to the alkaloid synthesis.

TABLE II

NICOTINE PRODUCTION, GROWTH AND RESPIRATION ACTIVITY OF EXCISED ROOT SEGMENTS OF *Nicotiana tabacum* VAR. TURKISH, DURING CULTURE FOR THE PERIODS INDICATED (SOLT)[32]

Segment type and treatment	Initial			1 week			2 weeks	
	Nicotine µg.	Dryweight mg.	QO_2	Nicotine µg.	Dryweight mg.	QO_2	Nicotine µg.	Dryweight mg.
Mature segments ..	286	18·8	4·7	284	17·8	1·3		
Tip segments	65	3·0	12·4	773	30·0		4437	130·0
Tip segments with 10^{-8} M indole acetic acid ..				134	15·4	5·9	147	29·0

TABLE III

Nicotiana rustica. DEVELOPMENT OF ROOTED LEAF-CUTTINGS IN A MEDIUM OF ZINZADZE CONTAINING VARIOUS CONCENTRATIONS OF INDOLE ACETIC ACID (IAA). DURATION: 37 DAYS[33]

No.	IAA concentration mg./l	Organ	In 10 leaf-cuttings: Dry weight g.	In 10 leaf-cuttings: Protein N mg.	In 10 leaf-cuttings: Nicotine N mg.	In per cent dry weight: Protein N	In per cent dry weight: Nicotine N
0	Control	Leaves	2·3	84	6	3·65	0·026
1	0	Leaves	19·3	439	126	2·27	0·652
		Roots	16·6	270	49	1·62	0·295
		Total	35·9	709	175		
2	1	Leaves	20·7	500	142	2·41	0·689
		Roots	20·6	406	48	1·97	0·230
		Total	41·3	906	190		
3	3	Leaves	15·4	389	60	2·53	0·390
		Roots	14·7	332	21	2·25	0·144
		Total	30·1	721	81		
4	10	Leaves	12·7	355	32	2·80	0·249
		Roots	9·2	322	3	3·50	0·030
		Total	21·9	677	35		

Some years ago we studied this problem of the relation between nitrogen absorption and alkaloid synthesis, because nitrogen assimilation in many plants can be closely related to the formation of very specific root-substances, which represent primary nitrogen assimilates and translocation forms of nitrogen. Thus we found that in the roots of Boraginaceae, Platanaceae, Aceraceae and many Papilionaceae, allantoin and allantoic acid[34–36] are formed, and probably citrulline in the roots of Betulaceae[37] and acetylornithine in the roots of Fumariaceae[38]. These four substances occur in the roots and the bleeding-sap of the various species of these families in large amounts.

To study experimentally the relations between nitrogen absorption and nitrogen assimilation on the one hand and alkaloid synthesis on the other we grafted *Atropa* upon *Lycopersicum*. In the root of *Lycopersicum* no hyoscyamine was formed. We did not feed these graftings as usual

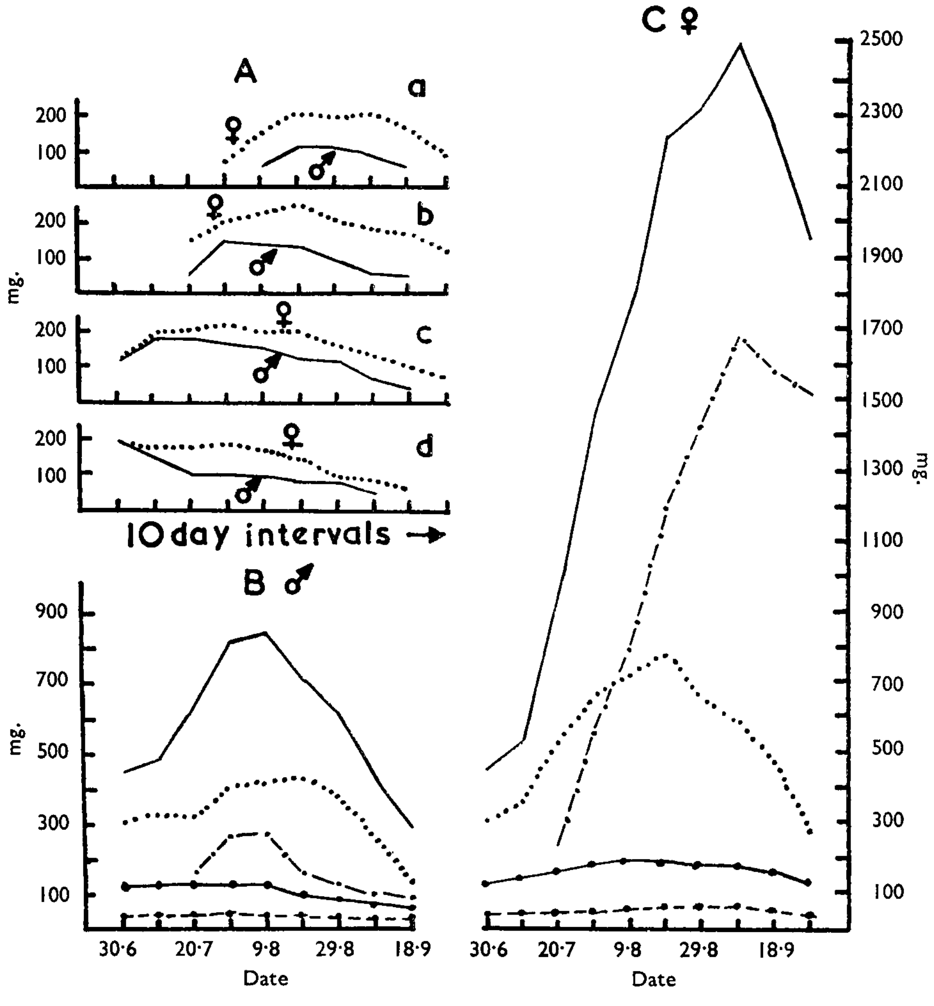


FIG. 1. *Cannabis sativa*. The development of total nitrogen in male (B) and female (C) individuals, mg. total nitrogen in one plant.

A. Leaves at different insertions, (*a*) 150–210 cm. high. (*b*) 100–150 cm. high. (*c*) 50–100 cm. high. (*d*) 0–50 cm. high.
B. Male plant. ——— Whole plant. . . . . . . Leaves ● — ●. Flowers and side shoots ●—●. Stalk and stem —●— Root.
C. Female plant, same key as male plant.

by the roots but only by spraying the leaves with solutions of ammonium nitrate, thus shifting the primary nitrogen assimilation to the leaves. We did not observe a noticeable alkaloid synthesis[39,40].

But if we fed intact plants of *Atropa*, *Datura* and *Nicotiana* by spraying the leaves, without adding nitrogen to the soil, alkaloid was produced in large quantities. *Atropa*, *Datura* and *Nicotiana* roots are, therefore, able to synthesize alkaloid, receiving the necessary nitrogen from the leaves. The decisive factor for the alkaloid production was not the process of nitrogen assimilation, but that of root growth.

Influence of the Shoot and Flowering

The growth of roots and therefore their activity in alkaloid synthesis is decisively influenced by the shoot. This is particularly so with the hapaxanthic plants, which have a terminal inflorescence, when the development of flowers often effects a permanent suppression of the roots. This suppression not only results in a check of growth but also of nitrogen-absorption. This phenomenon can be observed particularly well with male individuals of hemp (*Cannabis indica*) (Fig. 1). When the male flowers develop, the absolute nitrogen content of the plants begins to fall. The roots excrete nitrogen into the medium. This inhibition of the roots may be effected partly by an acute carbohydrate deficiency because the flowers absorb the stream of assimilates. Obviously there are still other causes involved, probably those of a hormonal character[41].

The conditions are similar, though less extreme, with *Nicotiana* species, especially with those strains which during and after flowering do not branch.

All the factors inhibiting the formation of flowers promote alkaloid synthesis. For a long time agriculturalists have used this fact when topping and pruning the plants[42,43]. Hofstra[44] and Reuter[45] have clearly shown that the excess of alkaloid in such plants is not caused by concentration of the normal content of alkaloid into a smaller shoot-volume, but that there is excess production. We have noted that the leaves of an *Atropa* scion on tobacco root are bleached by the nicotine from the tobacco root. If the *Atropa* scion flowers it does not bleach. We found that under the influence of the flowering *Atropa* the nicotine content of the tobacco root decreases[1]. This effect of flowering can be demonstrated under more physiological conditions with plants photoperiodically sensitive. Among others we used *Nicotiana sylvestris*, a long-day plant. Under short-day conditions it remains in the rosette-stage and continuously produces new leaves, and its root system grows without inhibition. Under long-day conditions the plant shoots, forms flowers and ceases to produce leaves. Its root growth is inhibited from this stage. Such a plant contains only about one-fourth of the amount of alkaloid compared to the plant under short-day conditions[31]. (Table IV.)

There are still other differences in the alkaloidal content. The flowering plant under long-day conditions contains predominantly nornicotine, the plant of the same age under short-day conditions contains predominantly nicotine. (Table V.)

This process of demethylation usually does not take place in the root. There we find approximately the same ratio of nicotine to nornicotine independent of the day length. Possibly the nornicotine of the root is not formed from nicotine, but has a primary character. It is also found in young roots, in the culture of excised roots[46], and in the bleeding-sap[44,45,47] of various *Nicotiana* species. The nornicotine of the shoot, however, is undoubtedly formed in the greatest amount secondarily from nicotine[48,49]. In certain tobacco strains there is practically no demethylation, in others it occurs only during the drying of the leaves or during fermentation. These strains represent an important group of low-nicotine tobaccos. They are, however, not originally low in nicotine. Other

TABLE IV

PHOTOPERIODICAL INFLUENCE UPON ALKALOID SYNTHESIS IN *Nicotiana sylvestris*. (Mothes and others[31])

Date	Experiment	State of development	In one plant: g. dry weight, root	shoot	total	mg. nicotine, root	shoot	total	Nicotine in per cent dry weight, root	shoot	total
29.2.56	beginning	rosette	0·21	1·00	1·21	1·8	6·2	8·0	0·86	0·67	0·66
13.3.56	long day	leaves expanded	1·46	6·15	7·61	13·1	33·6	46·2	0·90	0·55	0·61
	short day	rosette	1·72	4·11	5·83	14·8	21·5	36·3	0·85	0·52	0·60
22.3.56	long day	elongation of shoot	5·0	14·5	19·5	31·3	36·2	67·5	0·62	0·25	0·35
	short day	rosette	5·8	10·0	15·8	80·5	45·6	126·1	1·39	0·46	0·80
3.4.56	long day	first flowers	9·5	24·1	33·6	63·5	44·7	108·2	0·67	0·19	0·32
	short day	rosette	13·1	10·8	23·9	141·3	58·7	200·0	1·07	0·54	0·84
16.4.56	long day	full blossom	7·8	27·0	34·8	62·4	26·2	88·6	0·80	0·10	0·25
	short day	rosette	17·8	12·3	30·1	264·3	94·4	358·7	1·48	0·37	1·20

TABLE V

PHOTOPERIODICAL INFLUENCE UPON SYNTHESIS OF NORNICOTINE IN *Nicotiana sylvestris*. (Mothes and others[31]). (Nicotine = 100.)

Date	Experiment	Root: Nicotine	Nornicotine	Anabasine	Shoot: Nicotine	Nornicotine	Anabasine
3·4	long day	100	1·5	1	100	1	trace
	short day	100	1·5	1	100	1	trace
16·4	long day	100	2·5	2	100	10–15	trace
	short day	100	2	1·5	100	4	trace
24·5	long day	100	4	3			
	green leaves				100	25	trace
	yellow leaves				nearly all nornicotine		
	total				100	500	trace
	short day	100	3	2			
	green leaves				100	3	trace
	yellow leaves				nearly all nornicotine		
	total				100	30	trace

strains and species demethylate in ageing leaves, for example, our *Nicotiana sylvestris*, or they immediately demethylate in the shoot the nicotine ascending from the root, e.g., most strains of *Nicotiana glutinosa*[31,50]. Thus the difference between long-day and short-day plants of *Nicotiana sylvestris* is only indirectly caused by flowering. Since flowering plants have old leaves only, they demethylate relatively more strongly than plants which do not flower and which continuously form new leaves. In both cases the old yellow leaves contain only nornicotine.

The process of demethylation is not reversible, even if CH_3-donators are given to the leaves[51]. Probably the demethylation is achieved by an oxidative degradation of the methyl group and not by transmethylation[52,53]. Thus old and young, growing and full-grown organs, differ in their capacity to add methyl groups at the N-atom. Probably there is a lack of sufficient activity in the corresponding enzyme systems, in which folic acid plays a special rôle. These changes in an ageing organ are of a general importance. I refer to the investigations of Cromwell and Rennie[54], who found that leaves of *Beta vulgaris* are able to oxidize choline and betaine aldehyde to betaine, but that only to a small extent can they methylate glycine, even if methionine is fed.

Since this process of methylation also plays a decisive rôle in the synthesis of the more important substances, for example, the purines, lack of activity in synthesizing alkaloids and betaines in full-grown or ageing organs reflects a state of general impotence.

TABLE VI

Nicotiana sylvestris, MG. OF SCOPOLETINE IN PHOTOPERIODICALLY TREATED PLANTS. (Kala[57].)

Experiment	Stage	Root		Stem		Leaves		Flowers	
		per plant	per cent dry weight	per plant	per cent dry weight	per plant	per cent dry weight	per plant	per cent dry weight
Short day	rosette	0·36	1·3	—	—	0·05	1·2	—	—
Long day	flowering	1·2	29·0	0·08	2·0	0·40	6·0	0·6	4·0

But it would be wrong to draw general conclusions from the few cases investigated up till now. Thus we have studied the biosynthesis of cumarines, which can also be synthesised in the root. Reciprocal graftings of legumes and the culture of excised roots have shown this distinctly[55,56]. But to what extent they can be formed in the shoot is not yet certain. Their synthesis is not restrained by flowering but, on the contrary, it is promoted[57]. (Table VI.)

Influence of State of Development

Thus, besides the inherited characteristics, the chemical activity of the plant plays an important rôle which changes quantitatively or even qualitatively during the course of development. It is also the cause of some apparent contradictions in the publications of several authors.

I refer to the investigations of our laboratory concerning the formation of hyoscine in *Datura*. Usually in the roots of *Atropa*, *Datura* and *Hyoscyamus*, hyoscyamine and hyoscine are formed side by side. It is not yet certain whether the epoxide is formed primarily or from the hyoscyamine. Young plants (few weeks old) contain usually more hyoscine than hyoscyamine[58–60], but in the shoot there are great differences: some species contain almost exclusively hyoscyamine, others hyoscine. Romeike[61] found that in *Datura ferox* the hyoscyamine on ascending into the leaves is oxidised to hyoscine. Leete, Marion and Spenser[62] fed *Datura* with [α-^{14}C]-ornithine and obtained radioactive hyoscyamine; hyoscine, however, was not radioactive. They concluded that the two alkaloids had different precursors. It seems to be more probable that during the time of experiment no hyoscine has been formed from hyoscyamine. In principle it is a similar problem to that encountered in the synthesis of stachydrine.

There are other examples. Trautner[63] reports that in *Duboisia myoporoides* of a Northern Australian origin hyoscine prevails throughout life, whereas in those of a Southern Australian origin the formation of hyoscyamine begins after 4 to 6 months and almost completely disappears in older plants. This is of commercial importance.

The influence of the ageing of organs and of the state of development of the plant is frequently dismissed but the relationship is not always clear. In barley the methylated derivatives of tyramine arise from tyramine and tyrosine in the roots of young plants only[64,65]. According to the investigations of my collaborator Rabitzsch, in seedlings, tyramine, methyltyramine, hordenine and candicine are successively formed. After about 30 days these substances disappear.

Areschkina[66] reports that in the growing parts of *Senecio platyphyllis*, platyphylline-*N*-oxide primarily occurs. After flowering it disappears from the aerial parts and platyphylline accumulates in the rhizome.

Smirnova turkestana, in May, contains only smirnovine; in August, only sphaerophysine[67].

The results of Sander[14] are very interesting. He showed that the steroid alkaloids are not exclusively, but primarily, formed in the shoot, young organs being especially active. After flowering, the alkaloid content decreases, but *Nicotiana* is in contrast to this because the developing fruits are a preferred site of tomatine degradation.

As the degradation or loss of alkaloids in older organs has often been described (*Nicotiana*, *Atropa*, *Datura*, *Lupinus*, *Papaver*, etc.), I shall not discuss it. In many instances the time of flowering is the time of peak of the alkaloid content.

Besides its genetical constitution, the state of development of a plant or an organ can have great influence on its alkaloid content. This becomes more evident when we study the effect of external factors on the qualitative and quantitative development of the alkaloidal character. There is an immense literature on this subject, but a literature full of contradictions.

To help eliminate the effects of development and especially of flowering, we recently worked with isolated leaves of various alkaloid plants, which were rooted by a short auxin treatment. Such leaf-cuttings do not form shoots and flowers, and show a completely uniform growth of roots and lamina for several months. In the growth of a leaf, cell division plays a minor part. The intercostal parts grow only by cell enlargement. The growing leaves provide excellent conditions for the growth of roots, since no buds, no flowers or fruits compete with them. Carbohydrates, auxins and other leaf-substances are at the disposal of the roots in an optimal way, and a richly branched active root system is formed.

The chemical performance of this root system can be elucidated by the analysis of the lamina. We can distinguish between two groups of substances: proteins, amino acids, purines, and nucleic acids, which follow the growth in an almost stoicheiometric way, and alkaloids, amides and nitrates, the content of which is related not to the activity of the lamina but to the root system. These substances are secondarily accumulated in the lamina, and can be found there in enormous quantities. A leaf which under normal conditions contains 1 per cent nicotine may increase its

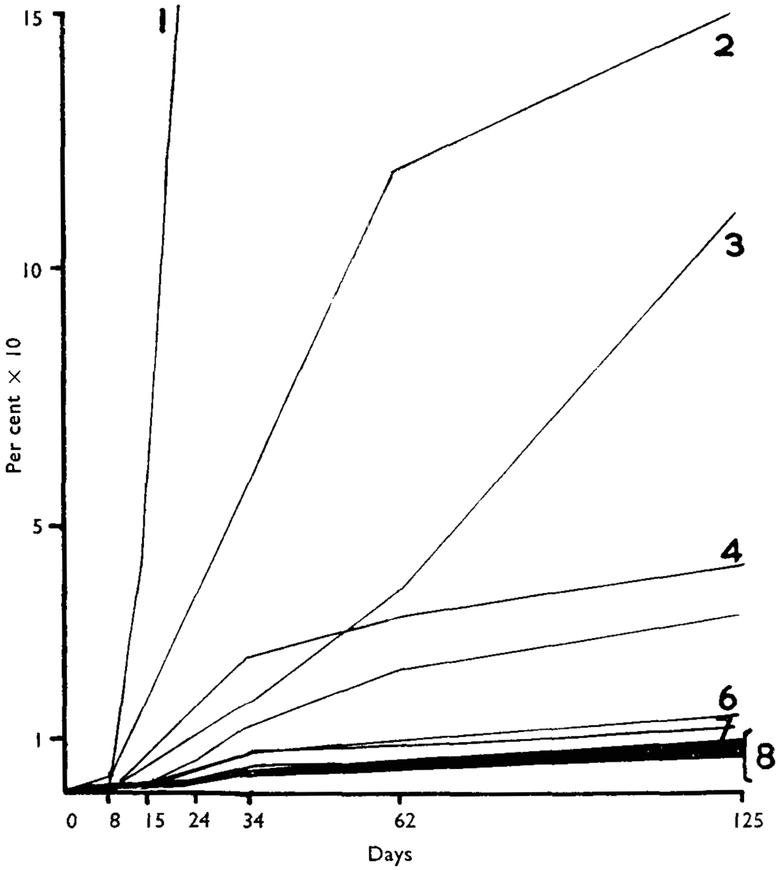


FIG. 2. *Nicotiana rustica.* Rooted leaf-cuttings. Half of the lamina was used as a control and the amount of total nitrogen was arbitrarily taken as 100. The amounts of the other half after different periods of cultivation is related to this control. (See ref. 68).

1. Nitrate-N. 2. Amide-N. 3. Nicotinic-N. 4. Ammoniacal-N. 5. N in solution. 6. Total-N. 7. Amino-N. 8. Fresh weight, dry weight, protein-N, purine-N.

content up to 20 per cent. Three or four months after the beginning of the experiment, the protein- and purine-nitrogen increases with the dry weight some 10 times, the nicotine-nitrogen 100 times, the amide-nitrogen 150 times, and the nitrate-nitrogen 1500 times (Fig. 2). The accumulation proceeds very uniformly for the first 3 to 4 months[68], enabling a close study to be made on the effect of the external conditions.

External Conditions

Temperature

An external condition which is most difficult to regulate when alkaloid plants are cultivated in the field is the temperature of the soil. Its effect on the growth and the activity of roots has been little studied. We have made some preliminary experiments in which roots were exposed to different temperatures (11–12°, 20–21°, 30–31°) while keeping the shoots under uniform conditions. We worked with plants that had been deprived of all their leaves and buds except one leaf. As compared with leaf-cuttings, such plants have a stem, and this allows the leaf to be isolated thermally from the soil. But deductions must make allowances for the fact that variation of temperature may also effect a change in the oxygen supply.

These experiments were made with *Nicotiana rustica*, and they showed that the most intensive protein synthesis in the root took place at low temperatures, but that the most nicotine was formed at medium temperatures. A soil temperature of 30° inhibited growth, nitrogen-uptake, protein and nicotine synthesis[33]. This may not be the same for other species, but that protein and nicotine synthesis have different optimum temperatures seems to be of importance.

Light

The effect of light is much better studied than the effect of temperature, but unfortunately the two are mostly not differentiated from one another. In regions with a high insolation, the alkaloid content of the plants does not usually exceed that of the northern regions, and is not only related to modifications but also to genetical fixations. There are in Bulgaria *Atropa* strains with an unusually high content of alkaloid, which is conditioned genetically[69]. The same is true of ergot strains in warm dry Southern Europe. How such strains arise, and why they can survive in these climates, is as yet unanswered.

Examples such as these are often described, but whether they are a matter of genetical fixation or of a temporary modification by the surroundings has not been sufficiently investigated.

We made some experiments, again with non-flowering leaf cuttings, where only the light factor was varied. In an earlier publication[68] we showed with *Phaseolus* and *Symphytum* that with high light-intensity the root nitrogen uptake and nitrogen assimilation is limited by the nitrogen concentration. However, if the leaves are shaded, light is the limiting factor.

The confining effect of the light factor is well elucidated by the synthesis of nicotine. This influence of light can manifest itself very differently. A presupposition for the growth of the roots and therefore for the nicotine formation is that the roots are well supplied with carbohydrates; that means that the light intensity is sufficiently high. A very high intensity promotes the whole metabolism strongly for some days or weeks but soon causes remarkable damage, which becomes apparent as a strong depression of photosynthesis, a decrease of soluble sugars in the root, a complete check of the nitrogen-absorption, a stoppage of root growth, and therefore a complete check of nicotine synthesis[70]. This light effect needs a more detailed analysis. Probably the lack of efflux of carbohydrates effects an inhibition of photosynthesis and the absorbed light, conducted to a "wrong substrate", damages the chloroplast apparatus photodynamically. Our collaborator Parthier showed in unpublished experiments that even low light intensities have a detrimental effect if they are offered continuously. Enormous quantities of coarse-grained starch accumulate in the chloroplasts and gradually burst the plastids, which are apparently injured mechanically in an irreversible way. But one might as well take into consideration a photo-oxidative destruction of auxin. We can suppose that such processes also play an important part in an intact ageing plant.

It is possible that the detrimental effects of the light factor become manifest only in isolated leaves. Generally the alkaloid content follows the light intensity. The efficiency of the different spectral regions has not been studied sufficiently. It has frequently been reported, however, that the synthesis of the steroid alkaloids is promoted by ultra-violet light[71–72]. It may be that the same is true for the alkaloids of the sclerotia of *Claviceps purpurea*. If the rye plants, which nourish the sclerotia, are grown under glass, or if the ears are enveloped with Cellophane, the alkaloid content is much lower than that obtained with normal light[73]. Whether decreasing the light necessary for assimilation brings about a parallel decrease in alkaloid content must be investigated, for we know that the awn and glume of the ears play an important rôle in nourishing the grains[74–76]. The physiology of the ears of cereals is in need of thorough investigation.

Effect of Nutrients

In some feeding experiments with ergot we injected into the pith of rye plants amino acids which were likely to take part in the synthesis of ergot alkaloids. We did not succeed in changing the mixture of alkaloids, nor were we able to influence the total quantity of alkaloids—perhaps with one exception: tryptophane seemed to increase the alkaloid content. These results agree with the fact that in the mature ergot all the amino acids involved in the alkaloid formation are already present, and therefore they cannot be a limiting factor in the alkaloid synthesis, except the tryptophane, which mostly seems to be missing[77]. The honey-dew, a product of the activity of the young mycelium, also contains all the essential amino acids[78]. It has not yet been proved how far these amino acids are assimilated from the rye plant or are synthesised in the mycelium itself[79,80].

At any rate the alkaloid composition cannot be changed by the character of the host[8], or by nutrition[72], or in saprophytic culture[81]. Therefore it is remarkable that injection of labelled tryptophane into the rye stem produces alkaloids labelled at the lysergic acid ring[82].

Concerning the root alkaloids, we studied the effect of some nutrients on the formation of nicotine in rooted leaf cuttings of *Nicotiana rustica*. In agreement with experiments with whole tobacco plants described in the literature we found that with increasing nitrogen supply the nicotine production increases both absolutely and relatively (dry weight basis).

A high nitrogen supply, however, can only be fully utilised if the plant is also sufficiently supplied with carbohydrates. If carbohydrates are deficient a high nitrogen supply inhibits both the root growth and the alkaloid formation[68,33]. A decrease in the concentration of general minerals increases the effectiveness of the N supply. This may essentially be conditioned by the promotion of the nitrogen uptake and the greater accumulation of soluble nitrogen containing compounds.

These results are interesting because, in contrast to intact plants, in leaf cuttings there is no formation of flowers to be inhibited, and no immature vegetative tissues to be developed by a high nitrogen supply. Alkaloid formation in ergot is also promoted by nitrogen-containing fertiliser, but we do not know why. Perhaps a good supply of nitrogen preserves the metabolic acitivity of the photosynthesising parts of the ear for a longer time.

There is a voluminous literature about the effect of potassium. We are especially interested in the statements that potassium deficiency brings about an increase in the acidity of the cell-sap, an increased nitrogen uptake and an accumulation of ammonia. We have made the same observations with leaf-cuttings. As potassium deficiency was found[83,84] to cause an accumulation of putrescine in barley and of arginine in flax, nicotine was investigated to see if it behaved similarly. This, however, is not the case. Potassium deficiency inhibits the nicotine synthesis, but an excess of K also effects a lower nicotine content. A "normal" potassium supply (0·215 g. K_2SO_4/1. nutrient solution) appears to be optimal[33].

An interesting problem is whether a nutrient-factor exists which in a specific way promotes alkaloid synthesis. One such factor seemed to be boron[85,86]. Boron deficiency was thought to bring about a high nicotine content in tobacco plants. The investigations of our laboratory with leaf-cuttings did not confirm this[87]. Boron deficiency inhibits the development of the root, and therefore the alkaloid synthesis. Possibly in the experiments of Steinberg the inhibition of flower formation by a slight boron deficiency gave rise to a promotion of root activity[88]. Excised roots of *Atropa* cultivated under conditions of boron deficiency also showed a very bad growth and a low alkaloid production[89].

All our investigations with leaf-cuttings resulted in our establishing that nicotine production is closely related to root growth. Growth itself, however, is a complex phenomenon. We must distinguish between growth of plasma, division of the nucleus and the cell, and enlargement of cells. We are not yet quite sure to which sort of growth the nicotine synthesis is associated. The branching of the root is not the decisive factor, as boron deficiency may effect a rich branching, but the nicotine synthesis is low under these conditions. To effect a full nicotine synthesis the growth must be vigorous and the roots must look white and healthy. Perhaps the experiments of Solt[32] and those of our own[33] with auxin indicate a closer relation of the nicotine synthesis to cell-elongation.

Relation between Alkaloid and Protein Synthesis

In most cases growth is linked to an increase of cell protein. Therefore it was supposed that nicotine synthesis was bound to protein synthesis[90], and an almost stoicheiometric ratio of the two substances was found in the plant: half a molecule of nicotine was supposed to arise for every one molecule of amino acid bound in the protein of the root. But we could not confirm this. The ratio total nicotine-N: root protein-N fluctuates considerably, from 0·1 to 1·5[33,39]. The occasional validity of this "rule" only seems to be an expression for the observation that growth is mostly combined with protein synthesis. But we have no explanation why alkaloid synthesis primarily takes place in the growing tissue; this is apparently a widespread phenomenon and not limited to root alkaloids. For example, it is true for the solanidines[13,14]. The relation between the alkaloid synthesis and the metabolism of growing organs leads to the question: what is the function of alkaloids in the life of plants? This problem has long been treated in a teleological way only.

Function of Alkaloids

Doubtless for many animals alkaloids are only poisons preventing plants from being eaten. By no means must we overlook the interesting special cases, where apparently alkaloids play a rôle in defence, for example, against the Colorado beetle (*Leptinotarsa decemlineata*) by *Solanum* species, the fungus *Phymatotrichum* by *Mahonia* and *Fusarium* by *Lupinus* or *Lycopersicum*. There are a number of such examples, but they should not be generalised. Certainly even alkaloidal plants have their animal and vegetable enemies. The general value for the selection of the character "alkaloidal" is therefore dubious. If the alkaloids had a great value in selection, the alkaloidal plants would constitute a higher percentage of the individuals living on the earth. But only rarely do they predominate in the vegetation, as for example on the alpine pastures of our mountains, where around the cottages of the shepherds the cattle crowd in large numbers, there are great amounts of *Veratrum*, *Delphinium* and *Aconitum*. Or in the more humid parts of the asiatic deserts, where the camel caravans make a halt and great herds of antelopes gather, *Anabasis* and other alkaloid-containing Chenopodiaceae grow. We can take it for granted that in these two examples the character "alkaloid" has a positive value for selection, since vegetation is threatened almost exclusively by mammals. But these are rare cases. They correspond to the extensive spread of thistles on our pastures. Perhaps it is the same problem that in free nature "sweet lupines" are rare and the alkaloid-containing strains predominate. On the other hand I stress the fact that a high content of alkaloid seems to decrease vitality. This is even probable for lupines and seems to be one more cause for the fact that these "poisonous plants" are not very frequent.

This prompts the examination of plant tissue to see whether alkaloids can accumulate to such an extent during the development of an individual that they gradually produce detrimental effects. But this examination is not easy. A substance applied externally to plant tissue may have a different mode of action from one that is enclosed in the vacuole and separated from the protoplasm by the tonoplast.

If we cultivate excised tobacco roots under aseptic conditions we find that nicotine in the concentration of 10^{-4} g./l. inhibits growth. In the plant, however, the concentrations can be much higher. An excised tobacco root continuously excretes nicotine into the medium. Possibly the inhibition of growth in the excised root is related to the accumulation of root-substances, which would normally pass into the shoot, producing a self-poisoning.

Another important question is whether alkaloids are essential and indispensable substances with important functions in metabolism and growth. Such functions have as yet not been proved, but we are not inclined to deny their existence. The fact that we did not succeed in producing artificial X-ray-mutants devoid of alkaloid might possibly indicate that such mutants come into existence but are not able to survive. Perhaps the low alkaloid races have a decreased vitality[91,92].

Fisher and Loomis describe a flower-promoting effect of nicotine sprays with soya-bean[93,94]. Our collaborator Dr. Ramshorn could not confirm this.

If *Cyphomandra* is grafted on *Nicotiana rustica* the scion flowers very soon, but the flower-promoting factor is not nicotine. If *Hyoscyamus muticus* is grafted on *Nicotiana rustica* the vegetative growth is remarkably promoted, but again the promoting factor is not nicotine.

Thus there remain but a few facts, which might indicate a possible physiological function of the alkaloids. Ramshorn[95,96], Solotnizkaja[97] and Izard[98] found that nicotine in a specific way affects the auxin-complex; in low concentrations it promotes growth of the *Avena* coleoptile, in concentrations more than 10^{-5} M it inhibits. Müller and Ramshorn found that nicotine influences the permeability for water during deplasmolysis in a specific way[99]. But those are effects on plants which do not contain nicotine, where nicotine is a drug and not a physiological substance. Bachmann ascribes an effect similar to auxin to all the pyridines[100]. It is very difficult to make similar experiments with tobacco, since the presupposition of such experiments is a comparable control plant devoid of nicotine, while all the tissues of tobacco contain this alkaloid.

There are many reports of pharmacological investigations on alkaloids, investigations concerning the effect upon the nervous system, and more detailed work on the effect upon enzyme systems and particularly the respiratory enzymes[101–108].

It is possible that we may erroneously conclude a physiological function from the pharmacological effect.

As nature excites us by a continuous play with colours and odours, as she stimulates the morphologist by an abundance of forms, which cannot be understood merely teleologically, thus as an artist she creates for the chemist an enormous number of the simplest and most complicated substances. Certainly each of these in the course of evolution may receive a function. But we should not look upon nature as so limited that she had to exhaust her creative genius purposefully.

Only a small part of the recent literature could be considered in this article. According to the subject given to him the author has put in the foreground the work of his own laboratory, for which he asks to be excused.

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