

# PLANT PHYSIOLOGY

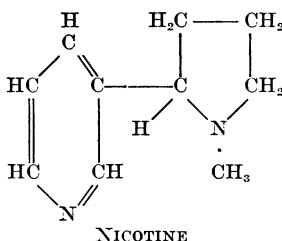
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## DEVELOPMENT OF SOME RECENT CONCEPTS IN THE PHYSIOLOGICAL CHEMISTRY OF THE TOBACCO ALKALOIDS<sup>1</sup>

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The plant alkaloids constitute a group of nitrogenous substances which possess great interest for the organic chemist because of their intricate molecular structure and for the pharmacologist because of the extraordinary physiological effects that many of them exert when introduced into the animal body. It is a regrettable fact, however, that so little is known of their physiological functions and of the mechanisms by which they are synthesized within the plant cell. Concerning these problems, the lack of an obvious experimental approach has led to extensive speculation. For instance, PICTET, who first synthesized nicotine, assumed that the alkaloids may represent degradation products of proteins, nucleic acids, and chlorophyll (35). The great JUSTUS VON LIEBIG implied their use as substitutes for the mineral bases within the plant body when he developed his "mineral theory" (3, 26). The most prevalent view at the present time has been well expressed by CROMWELL (5) and by VICKERY (42). This hypothesis holds that the alkaloids represent by-products of nitrogen metabolism in which irreversible reactions are involved and that they differ from the end-products of nitrogen metabolism in animals by their retention within the body of the organism.

Within the past decade a serious attempt has been made to ascertain the nature of nicotine production in the tobacco plant. Several factors have contributed to this choice, but the most important have undoubtedly been the



worldwide use of nicotine as a mild narcotic and the relative ease with which the alkaloid may be quantitatively assayed. From these investigations cer-

<sup>1</sup> The ninth STEPHEN HALES Address. 1945.

tain well-defined concepts have been developed concerning 1) the locus of nicotine synthesis, 2) the extent of translocation, and 3) the nature of nicotine accumulation in the growing plant body (9, 14, 19, 32). It has seemed worthwhile to retrace here the development of these concepts in order to call attention to the important new opportunities which they provide for basic research in the physiological chemistry of nicotine in particular and of the plant alkaloids in general.

#### Distribution of nicotine in the growing plant

The very excellent work of VICKERY and his collaborators (43, 45) has provided a picture of the accumulation of nicotine in the leaf and stem of the tobacco plant during its growth and developmental stages. The general pattern of accumulation begins with the seedling, nine to eleven days after germination, for the mature tobacco seed does not contain detectable quantities of the alkaloid. When the young plants have attained transplanting size, the differential nature of alkaloid distribution within the plant body has already become apparent. That is, nicotine is present in appreciable quantities in both root and stem, but by far the greatest proportion resides in the leaves. Furthermore, the proportion of the total nicotine of the plant to be found in the leaves throughout most of the period of growth (45) remains within the extremely limited range of 85 to 88 per cent. As VICKERY has pointed out, "The constancy of the proportion in the leaves is remarkable in view of the rapidly changing relative proportions of leaf and stem tissue as the plant grows, and raises an interesting question with regard to the kind of cells that are capable of synthesizing nicotine." It is also of interest to note that the most rapid rate of overall nicotine accumulation may occur at a time in the life of the plant when growth in terms of dry weight increase has all but ceased (45). Clearly, therefore, nicotine accumulation, in the aerial organs at least, is not directly associated with the growth processes of the tobacco plant. This conclusion has, indeed, already been expressed by MOTHEs (31). As VICKERY has suggested, the interpretation of these and of other aspects of nicotine accumulation in tobacco, particularly the quantitative relationship between nicotine nitrogen and total nitrogen in the leaf, is difficult "in view of our ignorance of the exact position of nicotine in the scheme of nitrogen metabolism in the tobacco plant."

Certainly, one of the important contributions of VICKERY and his associates lies in their emphasis upon the lack of information concerning the "kind of cells," that is, the exact locus or loci within the plant body where nicotine formation can occur. Hence it becomes apparent that the dilemma of interpretation can be dispelled only by going back to some more fundamental problems among which is the ultimate origin of nicotine.

#### The locus of nicotine formation

The first extensive physiological investigation of nicotine formation in tobacco was carried out by MOTHEs (31). Working within the framework

of older suppositions concerning the *in situ* nature of alkaloid synthesis and the doctrine of phloem-transport of organic compounds, MOTHEs attempted to alter or to block nicotine formation by manipulations of the environment of the plant. He found the process of nicotine accumulation to be an extremely stubborn object for experimentation. So long as the leaf was attached to the growing plant, it continued to acquire nicotine even under such drastic conditions as nitrogen deprivation and carbohydrate starvation. No appreciable transport of alkaloid from the leaf could be detected. It seems reasonable to conclude, therefore, that the nicotine metabolism of the tobacco leaf is characterized by a ponderous stability and that the usual methods of physiological experimentation can not be expected to yield information of much value.

The methodology of experimental morphology would seem to offer more promise. It has been noted above that as long as the tobacco leaf is attached to the growing plant, nicotine accumulates regardless of environmental circumstances. MOTHEs observed, however, that excised leaves ceased immediately to acquire the alkaloid in spite of the nature of their subsequent treatment. This phenomenon of accumulation interrupted by excision has, of course, long been known to occur in tobacco leaves which are stripped from the plant preparatory to processing for commercial use. The work of MOTHEs and the researches of VICKERY and PUCHER (44, 46) on the chemical changes that occur in excised leaves during culture under a wide variety of conditions have, nevertheless, brought into bold relief the simple fact that organic connection between leaf and plant is necessary to continued increase in the nicotine content of the leaf.

In logical continuation of this observation, the following points have been experimentally established. First, when tobacco shoots are excised and cultured in water, they, likewise, cease at once to accumulate nicotine (7). Secondly, if segments of tobacco stems are excised and placed in moist chambers, they send out lateral branches which are nicotine free (10). Thirdly, excised segments of tobacco stems placed in moist chambers also produce callous tissue at their basal surfaces. This callous tissue contains no alkaloid (10). Fourthly, the callous tissue that develops on the cut ends of the petioles of excised leaves held in moist chambers contains nicotine, but this nicotine is transported from the leaf blade and hence does not lead to an increase in the total amount of alkaloid in the leaf (unpublished data). Finally, nicotine does accumulate in extraordinarily large amounts in excised leaves that have been rooted in sand (9). As a result of these experiments, it is possible to conclude that not only is an organic connection between leaf and plant necessary for continued alkaloid accumulation, but also the plants (or the leaf) must bear roots. Consequently, it becomes necessary to look to the root for the source of nicotine or of its precursor(s).

NATH in India (33) and BERNARDINI in Italy (1) were among the first to report the remarkable results to be obtained by grafting tobacco upon tomato. These authors observed a great decrease in the concentration of

nicotine in the leaves of the tobacco scions. NATH reported the equally interesting observation that the reciprocal graft (i.e., tomato scion on tobacco stock) accumulated nicotine in both stock and scion. A year later HASEGAWA (18) confirmed these results. Subsequently, EVTUSHENKO (15), KUSMENKO and TIKHVINSKAYA (25), SHMUCK (37), SHMUCK, SMIRNOV and ILYIN (39), and SHMUCK, KOSTOFF, and BOROZDINA (38) brought forth extensive confirmation based upon grafts of tobacco with tomato and with a variety of other species. The experimental data collected by these investigators, fortunately, were characterized by more agreement than were the interpretations applied to them. The earlier report by NATH and the observations of BERNARDINI and HASEGAWA were based upon data too meager to permit more than speculation concerning their significance. It is difficult to understand, however, why the far more extensive and well planned work of SHMUCK and his colleagues was regarded by them to support the mystical notion of KRENKE (24) concerning the release of "hidden properties" within the scions under the influence of "new developmental conditions." SHMUCK definitely discounted the possibility of translocation of the alkaloid from root to shoot (38, 39).

More recent investigations (10) have considerably simplified the problem of interpretation. Tobacco stocks and scions were defoliated and cut as short as possible in order to reduce materially their initial content of nicotine. They were then reciprocally grafted with tomato. The tobacco leaves that subsequently developed upon tomato stocks were nicotine-free with the exception of the lowermost, which contained traces of the alkaloid. From the upper stem and the inflorescence of the tobacco scion there was isolated a base which could be determined as "nicotine" by the usual silicotungstic acid precipitation but which was not identical with this alkaloid. On the other hand, nicotine did accumulate extensively in tomato scions grown upon tobacco stocks. Such accumulation was greatest in the lower and older leaves, and extensive injury occurred to the tissues of these leaves. One of the most interesting observations, however, in connection with the problem of the origin of nicotine is found in the sectoral nature of nicotine accumulation in the tomato component of approach grafts of tobacco with tomato (10). Such a response could indicate only an upward transport of nicotine through the xylem. This view was confirmed by the isolation of nicotine from the sap that bleeds from the cut stumps of decapitated tobacco plants. The latter observation, likewise, demonstrated that it is nicotine itself that is transported to the leaf and not some precursor which can be converted to nicotine once it has reached the leaf cells. These findings have since been confirmed completely by the work of MOTHES and HIEKE (32) and in large part by the subsequent report of PAL and NATH (34).

To recapitulate, the evidence obtained from excised organs and from reciprocal grafts with non-alkaloid producing species has shown that nicotine is not produced *in situ* in the leaf and stem of the tobacco plant but rather is translocated to these organs there to accumulate.

The next question, of course, concerns the actual locus of the nicotine synthetic mechanism within the plant body. The absence of nicotine in tobacco stems and leaves when grafted close to the roots of tomato stocks obviously suggests that the alkaloid is formed only in the roots of the tobacco plant. Final proof for the correctness of this view was obtained when nicotine was isolated from both the tissues and the spent culture fluids of excised tobacco roots in sterile culture (11).

Many heretofore unexplained characteristics of nicotine content in tobacco now seem susceptible of interpretation. Among these may be mentioned the differential nature of alkaloid accumulation in leaves and stems during growth (45); the extensive enrichment of the leaves of topped tobacco plants with respect to nicotine; the low nicotine content of the rapidly grown sucker or ratoon crop (30); the abrupt termination of alkaloid accumulation in excised tobacco leaves and shoots; and the difficulty encountered by MOTHEs in his experiments with respect to altering the nicotine content of the leaves of intact plants by manipulating those environmental factors which influence principally the aerial shoot.

#### The extent and nature of nicotine transport

Evidence was presented in the preceding section to show that nicotine is translocated from root to shoot and that the pathway for at least the bulk of such transport is undoubtedly the xylem. It would seem reasonable to assume, therefore, that an examination of the distribution of the alkaloid in various parts of the stem and in leaves at different levels on the stem might reveal the major currents of such transport. MOTHEs (31) reported, and indeed general experience shows, that in the absence of senescent changes the total nicotine content per leaf decreases with increase in height of the leaf position on the stalk. That is, so long as they remain anabolically active, the older leaves contain the greatest quantities of alkaloid. The distribution of nicotine in the stems has also been examined (9). In this case, the greatest accumulation of the alkaloid is found in the cortex, although the pith and xylem contain appreciable amounts. Taking into consideration the additional fact that by far the greater proportion of the total nicotine of the aerial shoot is located in the leaves, it at once becomes apparent that the patterns of nicotine distribution within the shoot are identical, at least qualitatively, with what might be expected if the alkaloid were translocated in the transpiration stream. To state the matter in another way, the alkaloid can be considered to accumulate in the different regions of the shoot in proportion to the anticipated temporal duration and relative intensity of their transpirational losses. Based upon this viewpoint, a flow-diagram of nicotine transport and deposition within the plant body may be constructed with interesting results. It may be noted that such a diagram predicts the differential character of nicotine distribution in the tobacco leaf as determined experimentally by CICERONE and MAROCCHI (4).

At this point it seems desirable to raise the question of the state in which

nicotine occurs in the cells and cell fluids. In the above discussion, it has been assumed that the sole forces governing nicotine distribution within the plant body are diffusion and transpiration. If, however, nicotine were to associate electively in salt formation with any particular acidic substance or substances and this were to be followed by the appearance of a solid phase, then it might be expected that the foregoing interpretation of the nature of nicotine accumulation would be considerably in error. Almost without exception, texts and reference books contain the statement that nicotine is found in tobacco leaves as the salt of malic and citric acids. Now, the alkaloid is a relatively feeble di-acid base (20) and would be expected to undergo salt formation readily. In the very low concentration in which it occurs in the leaf tissue fluids (0.012 molar or approximately 0.20 per cent. in the experiments of VICKERY and PUCHER (45) at the 75-day collection), it is not likely that precipitation of nicotine as the salt of an organic acid would take place. On the contrary, since most of the salts of nicotine are readily soluble in water, it is far more likely that the alkaloid exists in the living cell in a complex series of equilibria with negatively charged particles any and all of which may undergo extensive changes in relative concentration with drift in time and in metabolic activity. Therefore, nicotine cannot be said to associate electively with any one or two acids but must be thought of as entering into the general buffering system of the plant cell. Indeed, to the limited extent that its low concentration in terms of normality in the cell sap permits, it may be said that its chemical properties demand recognition of such a function for this alkaloid in tobacco physiology.

#### The nature of the nicotine synthetic process

As an approach to the highly interesting but largely undocumented subject of the intermediary metabolism of nicotine, it is necessary to observe that the localization of the synthetic mechanism with respect to specific tissues or developmental zones within the tobacco root has not yet been investigated. Likewise, information concerning the nature of the environmental factors which can influence the rate of synthesis is meager and often conflicting. It seems fairly certain, however, that the extent of accumulation in the leaf and presumably, therefore, the overall rate of production by the root can be increased by growing the plants in a heavy soil as contrasted with a light soil (private communication), in a dry soil as compared with a moist soil (6), and by topping and suckering the plants during the growth period (30). Interpretation of these results is greatly complicated by the lack of suitable data on concomitant shifts in root-shoot ratio. The same may be said of the results obtained from the application of nitrogenous fertilizers (31).

Evidence has been obtained (45) which indicates that nicotine disappears as such during the profound redistribution of metabolites that occurs within the plant body following the onset of sexual reproduction. In the last stages of growth, therefore, the rate of nicotine accumulation in the

plant as a whole is determined by the progressive difference between the rate of synthesis and the rate of utilization or breakdown. This differential is probably augmented by a simultaneous diversion of the carbohydrate output of the leaves from root to inflorescence. Indeed, many observations (unpublished) point to the necessity of continued and adequate supplies of carbohydrates in maintaining maximum rates of nicotine output by the roots. In this connection, the remarkable changes that take place as a result of topping tobacco plants during the growth period are of interest. In such cases, there are no reproductive structures or fruits to monopolize the food supply. The result is that the leaves become greatly enlarged due to abnormal increases in the size of the parenchymatous cells (49). Associated with this change is usually a very considerable increase in alkaloid concentration (30). The magnitude of such increase seems to be greater than would be expected on the basis of an increase in the root-shoot ratio alone.

Aside from circumstantial evidence concerning the necessity of ample supplies of carbohydrate, nothing is known concerning the influence of temperature, oxygen tension, nitrogen supply, and similar variables on the absolute rate of nicotine synthesis in the roots. This field obviously provides much opportunity for future research.

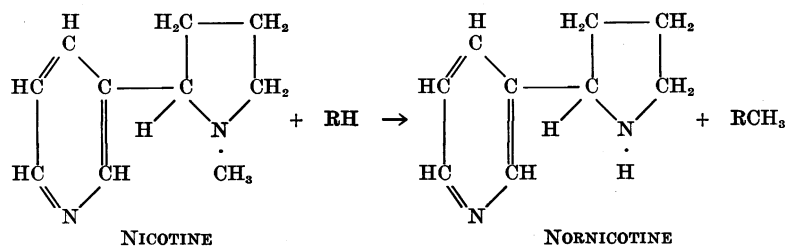
In connection with the identification of possible intermediates in alkaloid synthesis, TRIER (48) has suggested a purely hypothetical and rather improbable scheme which would yield the basic ring structure of nicotine by the simultaneous oxidative decarboxylation of proline and nicotinic acid. KLEIN and LINSER (23) have published data to show that excised tobacco leaves can make nicotine from proline alone. GORTER (17) repeated these experiments and, using a somewhat different method for the expression of data, failed to confirm the results. A third investigation of the problem has also been reported (7) in which even larger increases were obtained by feeding nicotinic acid to excised tobacco shoots than were obtained by feeding proline. The recent discovery that nicotine is not normally produced in important amounts in tobacco leaves and the failure of the investigators cited above to establish the validity of analytically determined increases in nicotine content by suitable statistical controls necessitates the adoption of a somewhat skeptical attitude toward such results. Although it seems very unlikely, the possibility cannot be denied that leaves may be found to manufacture at least small amounts of nicotine provided suitable intermediates in the total synthesis are supplied by the investigator and/or by the root of the intact plant. In this connection, a repetition of the experiments described above utilizing naturally nicotine-free tobacco leaves from appropriately grafted plants should clarify once and for all the position of nicotinic acid and proline in the alkaloid metabolism of the aerial organs.

VICKERY (41) has attempted to identify the possible precursors of nicotine by another approach. Rather than feed the plant with hypothetical intermediates, this investigator resorted to direct isolation from the seed meal of substances that might play a part in the formation of nicotine during

the germination of the seed. This interesting procedure led to no positive conclusions perhaps due to the lack of suitable methods for the isolation and separation of the rather large proportion of unknown nitrogenous substances that was encountered.

### The synthesis of secondary tobacco alkaloids

Nicotine is accompanied in many strains of tobacco by variable amounts of nornicotine. It is interesting to note that nornicotine accumulates most extensively in those varieties of cigarette tobaccos that have been selected for low nicotine content (28). That is, there is an intimation that as the nicotine producing capacity of the plant is reduced, its ability to manufacture nornicotine is correspondingly increased. It has recently been shown (13, 14) that nornicotine synthesis is, indeed, closely related to that of nicotine insofar as the former alkaloid is produced at the expense of the latter. The overall change involved in the transformation of nicotine into nornicotine is merely the substitution of a hydrogen atom for a methyl group. The simplest assumption to make with regard to the probable mechanism is that nicotine participates in a transmethylation reaction with an enzyme system and a methyl acceptor (Scheme I). In view of the fact that only nicotine



SCHEME I

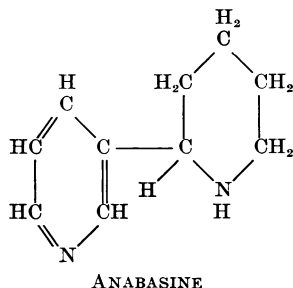
accumulates in tomato scions when these are grafted to the roots of species that normally contain nornicotine, it has been suggested that the roots of the strains and species in question produce nicotine in the usual manner. This is subsequently transported to the leaves where it is slowly demethylated (14). All that is known of the nature of the hypothetical transmethylation system is that it is inheritable and that it possesses a limited working capacity.

Regardless of the lack of detailed information concerning the real nature of the process by which nicotine is changed to nornicotine, it is perfectly clear that nornicotine arises by secondary processes within the leaf. There is as yet no evidence that any organ of the plant can carry out a total synthesis of nornicotine *in situ*, although the possibility that the formation of nicotine in the root may proceed through the reverse process (i.e., the methylation of nornicotine) to that found in the leaf must be investigated.

Many reports may be found in the literature to the effect that F<sub>1</sub> hybrids between *Nicotiana tabacum* and *N. glauca* and scions of *N. glauca* grafted upon *N. tabacum* stocks contain only anabasine in spite of the fact that nico-



tine synthesis in *N. tabacum* is usually far more intensive than is the production of anabasine in *N. glauca* (40). If it had been true, this relationship would have intimated, among other possibilities, a competition between the synthetic mechanisms of nicotine and anabasine for a common precursor. The stimulating prospect of discovering such a system has been eliminated by the demonstration (14) that anabasine formation does not predominate in the genetical sense over nicotine production in the various combinations



described above. Rather, the extensive secondary conversion in these combinations of nicotine to nornicotine and the difficulty encountered in identifying the latter substance in the presence of anabasine have contributed to erroneous interpretations. It should be noted that the demonstration of independent anabasine synthesis in both root and shoot of *Nicotiana glauca* (12) has shown that the total synthesis of alkaloids is not an inherently unique property of root tissues.

#### The physiological significance of nicotine

Insofar as it is justifiable to attempt to blueprint the physiology of the tobacco plant and to assign a function to each of the constituents thereof, it would seem well to list the more obvious alternatives with respect to nicotine. For instance it may be possible that nicotine originates as a by-product of a number of irreversible and physiologically useless reactions. Or it may be that it is formed for any one of several reasons as a by-product of chemical reactions that do play a rôle in cell metabolism. Again, the final steps in the synthesis of the alkaloid molecule may involve reactions of use to the cell; or the finished product may itself participate in important activities which are not at present recognized.

It will be noted that the views expressed prior to 1942 were based upon the assumption that nicotine is produced largely in the green leaf and that its physiological import must, therefore, be linked in some way with this organ. From this point of view, the great variation that occurs in nicotine content of different strains and crops of tobacco; the fact that tobacco scions can grow and develop quite normally on tomato stocks without more than minute traces of available nicotine; and the failure of nicotine fed through the cut stems of excised tobacco shoots to alter nitrogen metabolism appreciably (8), all support the conclusion that perhaps nicotine plays no important

rôle in the aerial organs. It is obvious, therefore, that an investigation of the effects of nicotine upon the roots of the tobacco plant is necessary. The results of some preliminary experiments, as yet unpublished, are available and are outlined here solely for the purpose of indicating the direction which future investigations of alkaloid physiology are expected to take in this laboratory.

In these experiments, Connecticut Broadleaf tobacco plants were grown in sand culture with a mineral nutrient solution that contained nitrogen only in the form of nitrate for approximately seven weeks. At that time, one-half the plants were given a supplement of 0.10 per cent. nicotine as the hydrochloride. This treatment was maintained for ten days: one-half of the nicotine-fed plants and one-half of the control plants were then harvested. For another nineteen days the remaining plants were watered only with tap water and received no more nutrient solution. These were likewise harvested. The results were rather astonishing.

In the first place, the roots of the plants to which nicotine was supplied very quickly acquired a violet-blue pigmentation which appeared to be localized in the vacuoles of the external cortical cells. No root injury of any sort was observed although the coloration was rather intense and in great contrast to the light cream color of the roots of the control plants. Some of the pigment was obtained in aqueous solution. It was insoluble in fat solvents, unchanged by zinc dust in dilute acid, but was destroyed by strong acid and strong base. The addition of silicotungstic acid to a portion of the original solution immediately led to destruction of the color and to the separation of a voluminous white precipitate. The supernatant solution was clear and colorless.

The second remarkable feature of these plants was the accumulation during the initial ten-day period of large amounts of nitrate nitrogen in the leaves and stems. Accompanying, or perhaps resulting from the accelerated nitrate uptake, was a proportionate increase in the reduced forms of nitrogen in root, stem and leaf (table I).

The plants that were supplied with tap water for a subsequent period of nineteen days gave further evidence of the changes that had been brought about. Under these conditions, the excess accumulation of nitrate largely disappeared, and a corresponding increase in other forms of water soluble and in hot-water-insoluble nitrogen fractions resulted. It was noted that the overall rate of nitrate assimilation during the period of nitrogen deprivation greatly exceeded that for ammonia assimilation (cf. 47).

It was observed that the roots of the nicotine-fed plants accumulated relatively much ammonia and amide nitrogen, whereas the amount of nitrate stored in these organs did not appreciably change. Clearly, the overall response to the presence of extra nicotine, so far as nitrogen metabolism is concerned, was the absorption by the roots of abnormally large amounts of nitrate nitrogen from the nutrient solution.

If the view is adopted that nicotine may function within the tobacco root as an accelerator of nitrate absorption, it then becomes necessary to inquire

into the possible mechanisms through which such an activity might be exercised. From the physiological point of view, alkaloid synthesis could conceivably increase the concentration of hydroxyl ion derived from water and thereby make possible increased acid exchange between root and soil. Furthermore, the alkaloid could serve as a buffer substance in the roots against the accumulation of dangerous amounts of nitric acid or of its immediate reduction product, nitrous acid. These suggestions break down under the fact that the ratio of nicotine to nitrate absorbed was so exceedingly small. For instance, in the experiment under consideration, only 1.2 m.e. of nicotine (calculated as mono-acid base) were absorbed from the substrate and accumulated without change, while 28.7 m.e. of nitrate were

TABLE I

CHANGES IN THE NITROGENOUS FRACTIONS OF TOBACCO PLANTS AS A RESULT OF (1) THE ADDITION OF NICOTINE HYDROCHLORIDE TO THE NUTRIENT SOLUTION FOR TEN DAYS AND (2) SUBSEQUENT WITHDRAWAL OF NICOTINE AND OF MINERAL NUTRIENTS FOR NINETEEN DAYS. FIGURES ARE IN MILLIGRAMS OF NITROGEN PER PLANT

	NITROGENOUS FRACTIONS					
	NH <sub>3</sub> -N	AMIDE-N	NO <sub>3</sub> -N	NICO-TINE-N‡	UN-KNOWN-N	INSOLU-BLE-N
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
(1) Nicotine supplied*						
Shoots .....	+ 20.2	+ 59.0	+ 119.4	+ 32.4	+ 35.8	+ 80.4
Roots .....	+ 13.1	+ 9.2	- 0.4	+ 1.0	+ 63.5	
(2) Nicotine withdrawn†						
Shoots .....	- 7.4	+ 4.0	- 246.2	+ 2.8	+ 128.6	+ 83.0
Roots .....	- 11.3	- 6.1	- 1.0	+ 0.6	- 38.9	

\* Figures express the differences between plants receiving nicotine for ten days and those receiving none.

† Figures express the differences between plants from which nicotine and all mineral nutrients were withdrawn for nineteen days and plants which had been supplied with both nicotine and mineral nutrients for an earlier ten-day period.

‡ During the period of nicotine feeding, each plant received 86.5 mgm. of nicotine nitrogen in 500 ml. of nutrient solution per day.

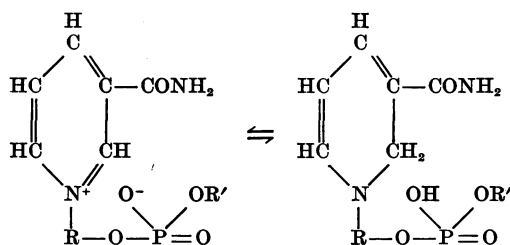
obtained from the same source (the solution contained no ammonium nitrogen).

A second possibility would seem to be that nicotine may alter the permeability of the root tip cells to the calcium, magnesium or potassium salt of nitric acid. This effect should be readily detected by ash analyses of the root, stem, and leaf tissues of the plants concerned.

The most suggestive possibility, however, would seem to follow the observation (table I) that, while much nitrate presumably traversed the root on its way to storage in leaf and stem, none was accumulated by the root cells as such. Instead, these cells contained relatively large amounts of ammonia, amide, and the undetermined forms of nitrogen including protein. While no estimate of the actual intensity of nitrate reduction in the roots is available from the existing data, it seems not unlikely that the presence of extra nicotine may have resulted in a greatly increased rate of reduction of nitrate to ammonia in the roots. This, in turn, may have conditioned an increase in

the rate of nitrate absorption by providing not only an extra supply of hydroxyl ion for acid exchange with the nutrient solution but also an equivalent amount of ammonium ion for preserving a favorable pH within the root cell fluids.

It seems worthwhile here to point out some of the chemical properties of nicotine by means of which effects such as those described above could be brought about. For instance, the nicotine molecule contains two feebly basic trivalent nitrogen atoms through which it is entirely conceivable that salt linkages might serve as points of attachment to specific proteins. The pronounced narcotic action of nicotine in the animal body lends weight to such a suggestion and, indeed, indicates the need for an investigation of the behavior of the alkaloid in both the animal and the plant cell from the point of view of the systems described by JOHNSON, EYRING, and WILLIAMS (22); JOHNSON, EYRING, and KEARNS (21); and MCELROY (29). If the narcotic action of nicotine in the animal body is based upon its ability to displace reversibly the prosthetic groups of one or more enzymes or upon its ability to denature reversibly the protein component of such enzymes, then the prospect must also be envisaged that nicotine or a metabolite of nicotine in

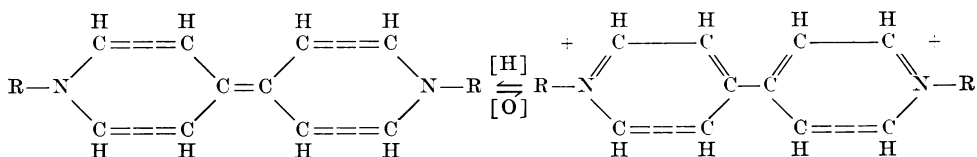


SCHEME II

combination with a specific protein may either catalyze or inhibit physiologically useful reactions in the tobacco root cells. In the event that evidence were forthcoming for such a mechanism, however, it would also be necessary to explain the fact that so much of the nicotine is lost from this combination and is permitted to be carried in the transpiration stream to those parts of the plant in which its physiological activity seems to be at a minimum [cf. the dissociation of nicotinamide-protein systems (36)].

The possible combination of nicotine (or of nicotine metabolites) with specific proteins raises many interesting possibilities for experimentation. For instance, although WARBURG has studied the properties of heme in combination with nicotine, no one seems to have attempted to obtain a biologically active substance by substituting nicotine for nicotinic acid amide in model substances related to the codehydrogenases. The codehydrogenases themselves are derivatives of pyridine, and their enormous biological importance needs no emphasis here (36). It is of interest to note, however, that the pyridine ring nitrogen of these compounds seems peculiarly fitted for the facile change from trivalency to pentavalency and back again as electrons are shifted from one catalyst to another in the course of cellular respiration (Scheme II). The general properties of pyridinium compounds and the

possibility of the existence of semiquinoid intermediates (27) in the reversible hydrogenation of nicotinic acid amide lead to the suggestion that, even in the absence of established combination with specific proteins, the potential biological importance of pyridine derivatives including nicotine deserves more widespread attention. From the point of view of the present discussion, the formation of quinoid structures such as those of the blue-colored N,N-dialkyldihydrodipyridyls is of great interest (16). The latter are easily oxidized with silver nitrate and less readily with atmospheric oxygen, followed by treatment with hydrochloric acid to form the dialkyl halogenide of 4,4'-dipyridyl. These, in turn, may be reduced back to the original blue mother compound with zinc dust and glacial acetic acid (Scheme III).



SCHEME III

While it seems likely that the pyridine ring in the nicotine molecule could contribute to such biochemical processes as the reduction of nitrate, it must not be overlooked that the pyrrolidine ring also possesses interesting properties. For instance, nicotine can be oxidized by mere exposure to air and to sunlight to the corresponding pyrrolidine-N-oxide containing a pentavalent nitrogen atom. With silver oxide in warm water, nicotine is dehydrogenated to nicotyrine (2). To carry speculations such as these to their logical conclusion, it becomes necessary to assume that electron shifts, in which nicotine or a metabolite of nicotine participates, could result either directly or indirectly in the absorption of relatively large amounts of nitrate ion from the soil solution without disturbing the electrostatic balance of the root tissue fluids. Whether or not this alkaloid, or any other alkaloid, can perform such a direct function in the plant that produces it remains to be determined by actual experimentation. These relationships are suggested here merely to call attention to the fact that the field is by no means devoid of problems for investigation.

Finally, it seems possible that careful study will reveal equally interesting but relatively minor biological rôles for nicotine in tobacco stem and leaf tissues. For instance, it is now definitely established that a certain small fraction of the nicotine content of excised leaves disappears as such during curing and during culture (44, 46). The extensive mobilization and disappearance of nicotine during fruiting and senescence has been noted above (45). Experiments have also been performed in which nicotine was supplied to excised tobacco shoots during culture in light (8). In all cases, approximately 25 per cent. of the nicotine absorbed by these shoots was converted into some other form of nitrogen which was water-soluble but not volatile with steam from alkaline solution. Certainly, the chemical nature

of the alkaloid would seem to fit it for many possible rôles in cellular metabolism including oxidations and reductions, both reversible and irreversible, and combinations with other compounds such as the proteins. It is, therefore, a great mistake to relegate it without further study to the scrap-heap of "waste-products" of tobacco metabolism and thence to consider the matter of little further importance.

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