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THE METABOLISM OF NICOTINIC ACID IN THE GREEN PEA AND ITS CONNECTION WITH TRIGONELLINE

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§ 1. THE PROBLEM OF THE BIOSYNTHESIS OF NICOTINIC ACID

In plants two groups of pyridine compounds are found. To the first group belongs the vitamin B_6 complex i.e. pyridoxine, pyridoxal and pyridoxamine. The function of these substances has as yet not been established. To the second group belong the nicotinic acid derivatives and the phosphopyridine nucleotides. These phosphopyridine nucleotides, which consist of two bases, the amide of nicotinic acid and adenine, two molecules ribose and phosphoric acid, are indispensable ingredients in the metabolism of every cell, because they act as hydrogen carrying coenzyms in respiratory processes. Therefore, the nicotinic acid (abbreviated as *niacin*), is found in all the plants, hitherto examined.

The pathway of the biosynthesis of the pyridine nucleus is not yet established. As we obtained no indications that the plant is able to transform pyridoxine into niacin, we restricted our investigations to the problem of the biosynthesis of niacin in plants.

The niacin cannot, however, be produced by all the organisms themselves. Those which have lost the capacity of synthesizing niacin, may be called *niacin-heterotrophic*. Such organisms, therefore, have to obtain niacin as an indispensable nutrient, a vitamin or a growth-factor. It is evident that by investigating the metabolism of niacin-

heterotrophic organisms, no further information can be obtained about the pathway in which the pyridine nucleus is formed naturally.

A large number of organisms, mammals as well as bacteria and the green plants are able to synthesize niacin from simpler compounds. These *niacin-autotrophic organisms* can only be used to investigate the biosynthesis of niacin. Broadly outlined two different methods of esearch have been followed.

The first method is based on a *comparative physiological examination* of organisms, in which the pathway of the biosynthesis of niacin is somewhere blocked. Then a number of feeding experiments are performed to investigate which compounds can replace niacin as a factor of growth.

Such investigations were performed with the aid of Neurospora mutants. In this case it was shown that *Neurospora crassa* mutant 65001, which needed niacin for growth, could also be grown when the medium contained indol, tryptophan, kynurenine or 3-hydroxy-anthranilic acid (BEADLE e.a. 1947; MITCHELL and NYC 1948). From this it was concluded that these compounds could be precursors in the biosynthesis of niacin (fig. 1).

In the second method of research an attempt was made to *influence directly the pathway* of the niacin metabolism in complete niacin-autotrophic organisms by adding supposed precursors to the organism. To perform this, the investigator has to determine quantitatively the amounts of substances concerned with the reactions.

Several mammals are able to produce large amounts of niacin when tryptophan is added to the food. These organisms, thereby, stabilize the niacin content in their tissues to a certain level by an increased excretion of niacin derivatives, especially N-methyl niacinamide (ROSEN e.a. 1946; SINGAL e.a. 1946; SARETT e.a. 1947; SCHWEIGERT 1947). The conclusion is often published that mammals transform tryptophan partly into niacin. However, tryptophan as well as niacin are intensively concerned in the metabolism of amino acids (KREHL 1949). This makes the interpretation of such experiments rather difficult. Furthermore, it was shown that the addition of ornithine to a suspension of Escherischia coli had a favourable effect on the niacin production, whereas this was not the case when tryptophan was added (ELLINGER and ABDEL KADER 1948–49).

Furthermore researches were made by means of *isotopic compounds*. The essence of the isotope technique in biochemical research consists in the preparation of a compound in which one or more of the atomic components have an abnormal isotope concentration. Thereupon "labeled" compounds are administered to the organism, and it is tested in which metabolic products the labeled atoms will be found back.

The question under discussion was traced by HEIDELBERGER e.a. (1948–49) with the use of labeled tryptophan. They gave several animals labeled tryptophan and tested the urine for labelled compounds. A young mature rabbit was injected subcutaneously with dl-tryptophan-β-C^{14} in isotonic saline and kynurenine was isolated from the urine with the radioactive C-atom in the β position. Further-

more young dogs were given dl-tryptophan-β-C^{14}, and kynurenic acid was isolated from the urine. The labeled C-atom now was present in the 3-position of kynurenic acid. Through these investigations the breakdown of tryptophan to kynurenine and kynurenic acid, already known,

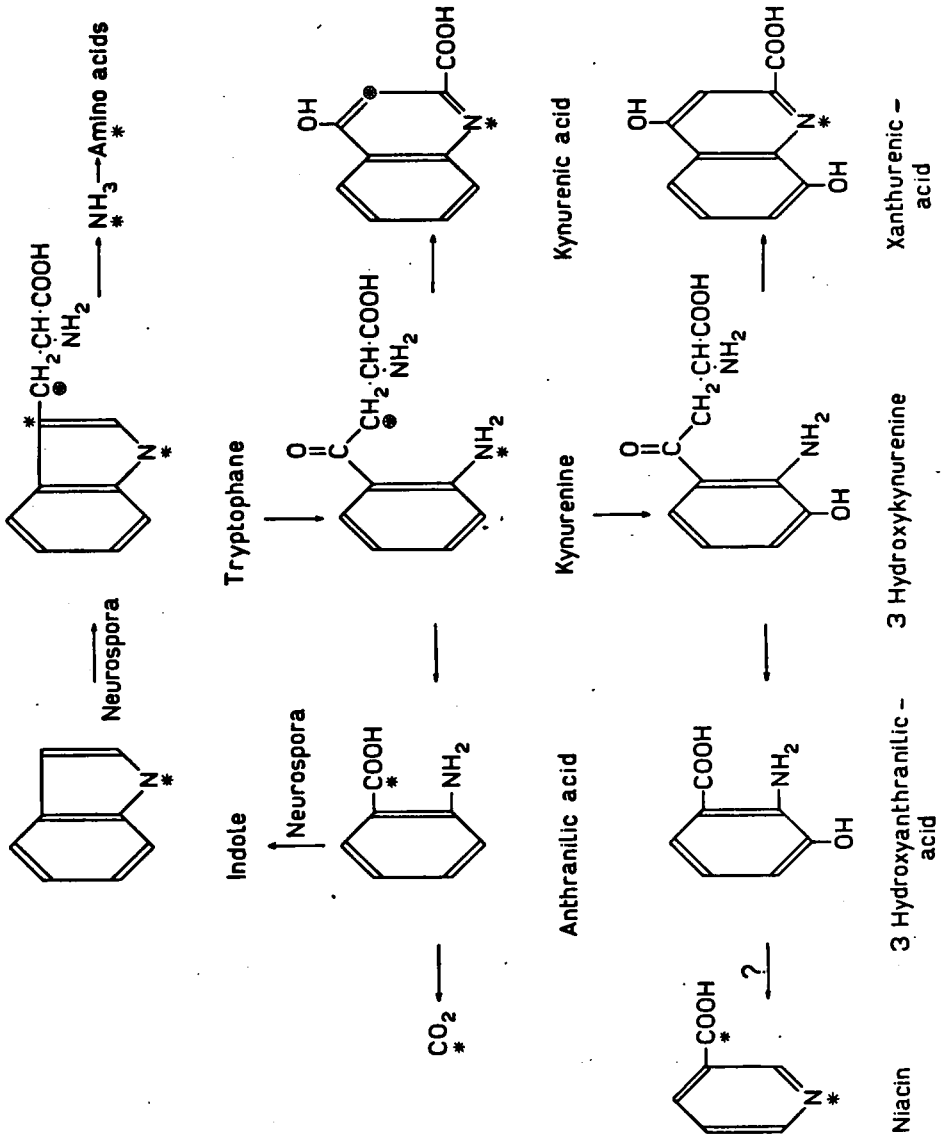

Fig. 1. Tryptophan and biochemically related compounds.

was ascertained. When finally dl-tryptophan-β-C^{14} was brought into the stomach of rats and the urine tested for N-methyl niacinamide, no radioactivity of this compound could be demonstrated. From the

above it results that in an eventual conversion of tryptophan into niacin the lateral chain of tryptophan must get lost. Later on 225 mg dl-tryptophan-3-C^{14} was given by stomach tube to rats after which 13,4 mg niacin with radioactive carboxylgroup was isolated from urine. In this way it is proved that this C-atom can be assimilated somehow in the carboxylgroup of niacin.

Then HEIDELBERGER e.a. point out that their results agree with those of the Neurospora investigations about the niacin synthesis from tryptophan. Therefore, the following conception was made:

tryptophan → kynurenine → kynurenic acid

↓

3-hydroxyanthranilic acid → niacin → N-methyl niacinamide

This kind of investigations, however, has some drawbacks. In the first place the labeled compounds prepared must, of course, be of such a nature that the isotopic atom will not be transferred by mere exchange. This for example may be the case with the carboxyl group and the aminogroup of amino acids.

Furthermore, there is an important breakdown of products added to the organism. This is also shown for indol, tryptophan and anthranilic acid (LEIFER e.a. 1950; SCHAYER 1950; NYC and MITCHELL e.a. 1949). The products of this breakdown may be assimilated again in the synthesis of niacin.

The conception of HEIDELBERGER e.a. is not the only explanation for the synthesis of niacin (BONNER and WASSERMAN 1950).

A few investigations are known in which the experiments were performed on *green plants*. Several investigators showed a strong niacin synthesis in germinating seeds and seedlings of the pea, the bean and cereals (BURKHOLDER e.a. 1942; TERROINE 1947–48; KLATZKIN 1948 and BANERJEE 1950).

The question may be asked whether the niacin synthesis in the green plant follows the same pathway as does the synthesis in mammals. The green plant does not excrete waste products like the animal does. An investigation into the precursors of niacin, therefore, is only possible in the following way. The plant is forced to absorb the compounds to be investigated, e.g. tryptophan or ornithine, and then the investigator has to analyse the plant whether an increase of niacin derivatives can be shown.

Such investigations were performed on leaves of cabbage, on those of broccoli and of tomato plants (GUSTAFSON 1949) on corn embryos in sterile culture (NASON 1949–1950) and on germinating seeds of *Phaseolus mungo* (BANERJEE 1950). Only a small rather insignificant increase of the niacin content after feeding tryptophan could be shown.

Contrary to the above mentioned investigations TERROINE e.a. (1948) could not show any increase of the niacin production in embryos of *Phaseolus vulgaris* in sterile culture when the culture solution contained tryptophan.

In this connection the earlier investigations may be mentioned of KLEIN and LINSER (1932–33) in which a favourable effect of ornithine on the content of *trigonelline*, a methyl betaine of 3-pyridine carboxylic acid, was communicated. (fig. 2). They supposed, like ACKERMANN

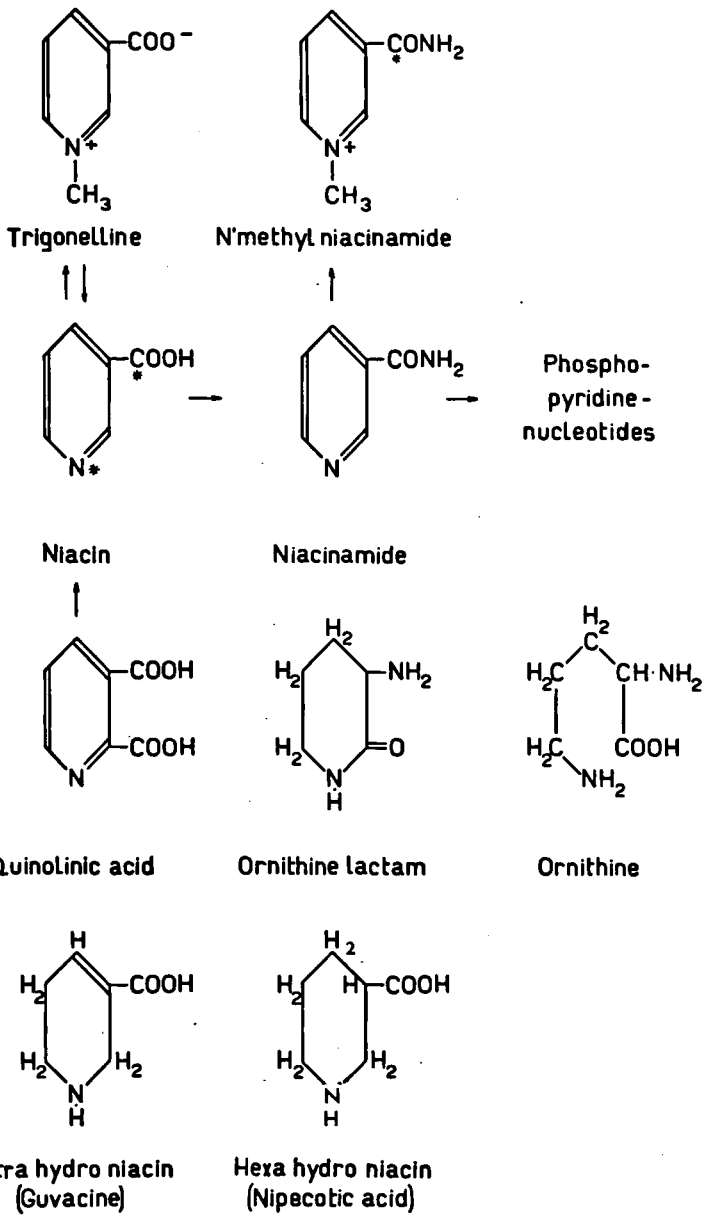

Fig. 2. Nicotinic acid (niacin) and biochemically related compounds.

(1913) had done, that trigonelline, which commonly occurs in plants, is formed niacin. Though even HENRY (1949) refers to these sources, the definite proof of this supposition has not yet been given.

The results of the investigations were in consequence contradictory as there are doubtful indications showing that besides tryptophan also ornithine may be involved in niacin synthesis by plants. Therefore, it will be important to examine which of the compounds above mentioned can effect the niacin synthesis in green plants.

Moreover, it is interesting to examine the conversion of niacin into trigonelline in green plants.

§ 2. THE DETERMINATION OF NICOTINIC ACID AND TRIGONELLINE

Nicotinic acid or *niacin* crystallizes in fine needles with a melting point of 229–232° C. It is soluble in water and ethanol, not, however, in ether. It is not destroyed by boiling with acids. All niacin derivatives which occur in plants are transformed into niacin by boiling with acids, except trigonelline and vitamin B_6.

Shortly after 1937 when the importance of niacin had become known, several investigators tried to find a quantitative determination. As early as 1943, a number of biological, spectrographic, colorimetric and microbiological determinations are mentioned in a survey made by ELVEHJEM and TEPLY. Only the microbiological and colorimetric determinations have been elaborated in such a way that they come in useful for our purpose.

A good *microbiological method* was found by SNELL and WRIGHT (1941) through the use of *Lactobacillus arabinosus* 17–5. For this microorganism niacin is a specific growth-factor; therefore, the growth of Lactobacillus in a medium, in which all other nutricious matter is present to excess, is a measure for the quantity of niacin. Niacin and niacinamide have equal activities for this organism. Cozymase and nicotinuric acid can also be used as a source of niacin, but trigonelline is inactive, like picolinic acid, isonicotinic acid and quinolinic acid (BARTON–WRIGHT 1944).

The *colorimetric determinations*, which are still in use, are based upon the reaction of König for pyridine, in which this compound reacts with CNBr and an aromatic amine, to give a yellow colour. This reaction has been used most extensively for the chemical estimation of niacin. The specificity of this reaction has been discussed by KRINGSTAD and NAESS (1939) and by WAISMAN and ELVEHJEM (1941). Amino acids, trigonelline, cozymase and vitamin B_6 do not cause this reaction. Pyridine and the 3-pyridine derivatives with a free 2-position, such as niacin, niacinamide, nicotinuric acid, nicotine, 3-picoline and nipecotic acid (hexahydro nicotinic acid), give a coloured reaction product. The course of the reaction is not identical for all compounds mentioned. The extinction-coefficient first increases and afterwards decreases. For different pyridine derivatives the maximum extinction, however, is obtained at other moments (KRINGSTAD and NAESS 1939, LAMB 1943). Therefore in plantextracts niacin derivatives must be transformed into niacin by hydrolysis. In the other way it is even

possible to determine niacin and niacinamide side by side in one solution (LAMB 1943; MELNICK and OSER 1943). The extinction of the reaction product in precisely fixed circumstances is directly proportional to the quantity of niacin. SWAMINATHAN (1938) established this for 10–50 micrograms in 25 ml. KRINGSTAD and NAESS (1939) indicate the proportion for 2–15 micrograms per ml. We did the same for 0.5–15 micrograms per ml.

Though the microbiological determination seems to be more specific and also allows a determination of smaller quantities of niacin, the complicated composition of the culture medium and the long incubation period of 72 hours is indeed a great drawback. The chemical determination proceeds along simpler and quicker lines. Disturbing compounds, however, have to be taken into consideration here. There is a great similarity between the niacin values obtained with the microbiological method and those obtained with the colorimetric method. So we used the latter method.

In combination with CNBr as aromatic amine we chose aniline, because a solution of this compound has no colour of its own and because it may be preserved for a long time if kept in the refrigerator.

The details concerning these niacin determinations have been published in our former publication (ZEIJLEMAKER 1951).

Trigonelline is a white crystalline compound, the melting point of which is 215–218° C. The hydrochloric addition product which is often used, has a melting point of 258° C. Trigonelline is soluble in water and ethanol. It is hydrolized with NaOH in high concentrations, but not with acids.

Trigonelline is found in 18 different families, belonging to divergent orders. So WEEVERS (1933–36) thought it was therefore very probable that trigonelline could be derived in a straightforward manner from a common product of metabolism. This might be niacin.

As is recorded in the literature upon the subject trigonelline should be found too in animals. ACKERMANN (1913) fed a dog with niacin and by means of mercuric chloride he obtained from the urine a precipitate which was considered to have been caused by trigonelline. As no blank test had been performed in which a dog was fed without niacin, it is not certain that the trigonelline found had been derived from the niacin. The greater part of the trigonelline found in urine appears to be derived from plant materials in the food (MELNICK et al. 1940). The animal organism probably does not produce any trigonelline, for ELLINGER and ABDEL KADER (1949) could not find trigonelline in the urine of several mammals when the food did not contain this compound. Meanwhile it was proved that practically all the methylated niacin previously determined and designated as trigonelline was indeed N-methyl niacinamide (HUFF and PERLZWEIG 1943). The processes of methylation which take place in the liver, apparently cannot include niacin, whereas they can include niacinamide (BACH 1945).

Different attempts were made to determine trigonelline quantitatively. A method as yet little used but very specific is the polaro-

graphic method. In this connection a number of pyridine derivatives have been examined by TOMPKINS and SCHMIDT (1942–43). It appeared that trigonelline could be determined polarographically with good results. Of the compounds which have been proved to occur in plants, only niacinamide can disturb the determination. However, this compound is converted into niacin by hydrolysis, after which process it no longer hinders. In conclusion we for our trigonelline determinations chose the polarographic method (ZEIJLEMAKER 1951).

The determinations of niacin and trigonelline *in plant material*, were always performed with series of 20 plants. The roots, the stems, the leaves and the cotyledons of each series of plants were cut off to work up similar parts together. Cutting the leaf-blades and the stipules of the leaves took so much time that in later investigations we worked up both stems and leaves of one series together. The plant material was ground in a mortar after the addition of 96 % ethanol, and transferred quantitatively into a beaker. The homogeneous mixture was weighed. A sample of it was extracted, hydrolized and prepared for the determinations (ZEIJLEMAKER 1951).

§ 3. THE PLANT MATERIAL

We used for our investigations *Pisum sativum* L. We chose a variety of the green pea with round yellow seeds, placed upon the market as "Ronde gele, Stam of Kruip" (height 40 cm). Among several varieties which could be investigated we were unable to establish any important differences of the niacin content (table I).

TABLE I

The niacin content in micrograms of a few varieties of Pisum sativum L.

Varieties examined	Per g material	Per pea	Weight of one pea
Stam of Kruip	12.4	2.9	0.232 g
Zelka	13.4	4.9	0.362 g
Unica	13.4	3.8	0.284 g
Mansholt	14.0	4.1	0.295 g
Stijfstroo CB	13.0	4.0	0.306 g
CB 3954	13.8	4.4	0.322 g

Before our experiments the seeds were selected according to the similarity of size and shape. We caused the peas to germinate between moist filterpaper in a room of constant temperature at 22° C. In summer the germinating percentage was about 100, only a few percents could not be nursed to full growth.

The seedlings of 4–5 days old were further bred on water cultures in the greenhouse. The culture solution was composed according to HOAGLAND and SHIVE (table II). About 10 plants were grown on each culture cylinder containing 2.5 liters of liquid. The solutions were aerated daily and renewed weekly (HOAGLAND 1937). In winter the cultures were given an extra illumination in the day time of 2

Neon high tension tubes and 2 white fluorescence tubes (Philips TL-tubes) at 75 cm distance.

The roots were not infected by *Bacterium radicicola.*

Before starting the experiments plants of the same size were selected.

TABLE II

Composition of the culture solution for green plants according to HOAGLAND and SHIVE. To 1 liter of the solution A 1 ml of the solution B is added.

Solution A contains per l		Solution B contains per l	
$Ca(NO_3)_2.4aq$	— 1.180 g	H_3BO_3	— 2.86 g
$MgSO_4.7aq$	— 0.493 g	$MnCl_2.4aq$	— 1.81 g
KNO_3	— 0.506 g	$ZnSO_4.7aq$	— 0.22 g
KH_2PO_4	— 0.136 g	Na_2MoO_4	— 0.07 g
Fe-tartrate	— 0.005 g	$CuSO_4.5aq$	— 0.08 g

§ 4. THE METABOLISM OF NICOTINIC ACID DURING THE DEVELOPMENT OF THE PEA

Certain quantities of a substance present in different plants or parts of plants, may be calculated in relation to the fresh weight or the dry weight. The dry weight of the plant is changed by assimilation, by dissimilation and by transport. The fresh weight, moreover, depends on the water content. When calculating the quantity of a substance present on *a basis per plant*, this is independent of water content and assimilation.

In this case, however, *the variability* of the plants or the parts of the plants must be taken into account. Therefore, we determined the quantity of niacin as an average out of a series of 20 plants. We had shown that with the rigorous selection of the seeds as well as of the plant material, this number of plants was sufficient to get constant results in parallel series.

We grew our plants on water cultures, because then the plants could be made more homogeneous, the roots more suitable for analysis, and it was easier to manipulate investigations with controlled feeding.

We determined the amount of niacin in the plant in several stages of the development, to get an impression of the niacin metabolism in the pea (fig. 3). The total amount of niacin increases rapidly during the development of the pea. It is interesting to note a slow increase of the niacin content in the first 3 weeks followed by a sharp increase thereafter. The plants with an age of 3 weeks are, therefore, most suitable for our investigations. These plants were about 15 cm long and the seventh or eighth leaf had just developed. The maximum amount of niacin reached in our plants was about 20 times the value of the beginning. The increase of the niacin content was relatively small. The increase with plants grown in soil can certainly be greater. The plants which we had grown in waterculture developed in a normal way indeed, but had filled out less than would have been the case in soil. The amount of niacin again decreases towards the end of the period of vegetation. From this it is concluded that niacin may be converted into other compounds than the phosphopyridine

nucleotides, which we determined with our method as niacin too. As will be established in further investigations, niacin proved to have been converted into trigonelline. Thus the curve is the resultant of the synthesis and the conversion of niacin.

Moreover, it is of importance to know the distribution of the niacin content present in the different parts of the plant. Therefore, we

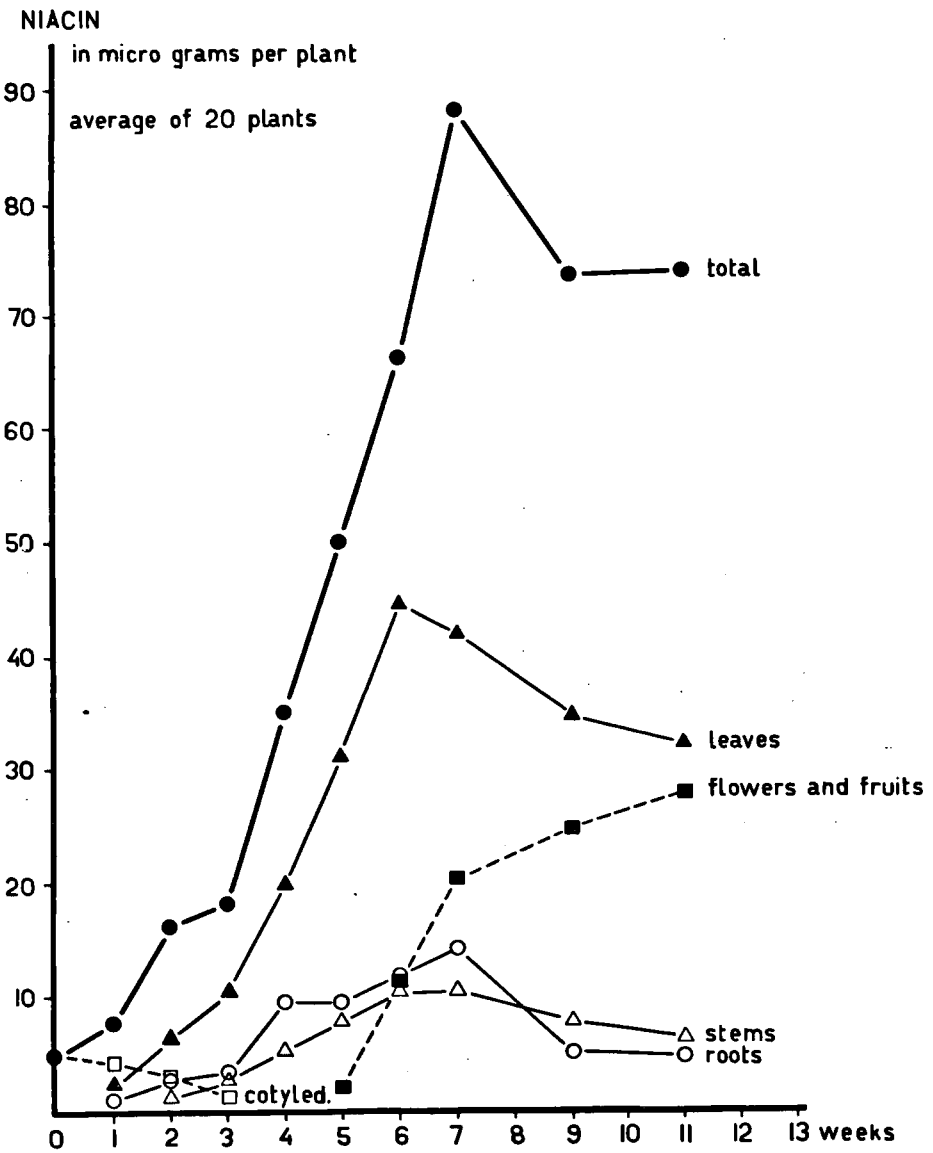

Fig. 3. The course of the nicotinic acid content in the green pea.

determined separately the quantity of niacin, both free and bound, present in the cotyledons, the roots, the stems, the leaves, the flowers and the fruits. The distribution of the amount of niacin over the various parts of the plant shows the following details (fig. 3). The quantity of niacin in the cotyledons decreases sharply, immediately after germination, whereas the quantity of niacin in the roots and leaves increases. Later on in the development of the plant after about 7 weeks, that is also the case with the roots, the stems and the leaves. In the niacin determination of the leaves those fallen off and dried were included. Although a conversion may appear at the same time it is noteworthy that this decrease takes place just at that time, when there is an increase of niacin in the fruits. So we presumed on good grounds that there is a *transport* in the seedling from the cotyledons into the other parts of the plant, and afterwards from the leaves into the fruits.

That there should appear a transport from the cotyledons into the seedling has also been shown in the experiments of BONNER (1938) in which isolated pea embryos could be grown in vitro, on condition that niacin was added as a growth-factor. Evidently the embryos of the normal plant obtain niacin from the cotyledons.

In other investigations it was shown that isolated pea roots in sterile culture cannot synthesize niacin (BONNER and DEVIRIAN 1939; ADDICOTT and DEVIRIAN 1939). So the increase of the niacin content we have shown in the roots is likely to be caused by a transport from higher parts of the plants. Whether such a transport really takes place, has not yet been established.

The sharp increase of the niacin content *in the leaves indicates an important synthesis.* This conclusion will be proved afterwards with the aid of cut plants.

§ 5. THE INFLUENCE OF EXTERNAL CIRCUMSTANCES ON THE METABOLISM OF NICOTINIC ACID

With a view to the experiments still to be described, in which different compounds are absorbed with the transpiration stream in cut plants, we examined *the influence of cutting* on the niacin metabolism of the plants. In cut plants the niacin metabolism may be disturbed, for the transport of compounds from the upper parts of the plant is eliminated.

In our experiments the stems were cut between the cotyledons and the first squamose leaves and placed in a 10 times diluted culture solution as indicated by HOAGLAND. These cut plants can be kept in good condition for several days. Further investigations, however, forced us to apply a shorter time for the experiment. An experiment lasting 3 days clearly showed that cut plants have the same niacin production as normal plants (table III, column 3). In both cases the increase of niacin amounts to 20 to 30 %. From this experiment we concluded that the niacin synthesis takes place in the leaves. For our further investigations *we can therefore use cut plants.*

Now we examined *the effect of light* on the production of niacin

(table III and IV). It appeared that in cut plants as well as in normal plants niacin is formed in the light, whereas in the dark no niacin is formed at all. Growth, however, was not inhibited in the dark. This does not include, of course, that the production of niacin itself would be a photochemical reaction. It is also possible that in the photo-

TABLE III

The influence of light and dark on the production of niacin in cut pea plants on a 10 × diluted culture solution. Niacin content calculated per plant as the average of the leaves of 20 plants.

Date of sowing Beginning of the experiment	21/2 21/3	7/3 8/4
	Niacin in micrograms per plant	
Cut pea plants		
Beginning of the experiment	7	10
After 3 days in dark	7	—
After 3 days in light	—	13
Normal pea plants analyzed		
After 3 days	10	12

TABLE IV

The influence of light and dark on the production of niacin in normal pea plants. Niacin content calculated per plant as the average of 20 plants without roots.

Date of sowing Beginning of the experiment	20/2 20/3	27/2 27/3	20/3 13/4
	Niacin in micrograms per plant		
Normal pea plants			
Beginning of the experiment	11	10	12
After 2 days in dark	10	11	11
After 2 days in light	—	13	14

synthesis compounds are formed which are indispensable for the synthesis of niacin.

The investigations of TERROINE and DESVEAUX–CHABROL (1947) also proved that niacin synthesis in Phaseolus vulgaris only takes place in the light.

The quantity of *nitrogenous* compounds which the plant has at its disposal, is obviously of great importance in the synthesis of niacin. When replacing, however, the culture solution of the water cultures by a nitrogen free solution, the production of niacin still proceeds for another couple of days. After about 5 days the plants show clear marks of lack of nitrogen. Then the lower leaves turn yellow, and the niacin content does not increase any further. It is not yet known, whether the synthesis of niacin is inhibited or whether the transformation is accelerated.

§ 6. The transformation of nicotinic acid into trigonelline

It is well known that niacin is for the greater part used to synthesize the pyridine nucleotides. We have already pointed out that we estimated these compounds which are decomposed into niacin by hydrolysis, as the total amount of niacin present in the plant. For our further experiments it is necessary to investigate whether niacin can be converted into other compounds than those already mentioned. For this purpose a recorded quantity of niacin has to be absorbed by a plant. After some time we can analyse whether this quantity in the plant has decreased. If this is so, it has to be investigated by further analysis which compounds have been formed and which quantities are concerned with this process. In other words an attempt has to be made to draw up *a niacin balance.*

In order to make a series of 20 plants absorb a recorded quantity of a compound under controlled circumstances we constructed a new apparatus. The details of the construction are clearly shown in fig. 4. The level of the liquid is kept constant by an automatic siphon which is connected with the flask containing the stock-solution.

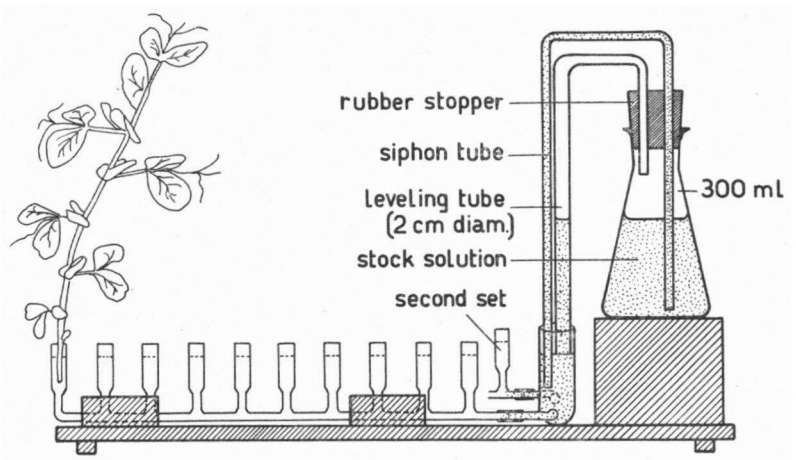

Fig. 4. The apparatus to feed cut plants with recordable quantities of the compounds to be investigated.

This apparatus proved to be very satisfactory in practice. All plants receive the same culture solution. During the experiment no culture solution has to be added. In this solution the compounds which are to be investigated may be dissolved. The quantity of the substance absorbed can be determined by weighing the apparatus before and after the experiment and from the concentration of the solution. The evaporation may be neglected, for during the experiment no change of the concentration in the liquid occurs. E.g. in one experiment a solution containing 18.4 microgram of niacin per ml was brought into the apparatus. After two days we determined 18.1 microgram of niacin per ml.

Now we made some series of plants absorb à recorded quantity of niacin, whereas parallel series were placed on the diluted culture solution only. After 2 days the plants were analysed to determine the niacin content. Table V shows that about 70 to 95 % of the niacin absorbed cannot be traced in the plant as niacin anymore and is consequently transformed by the plant. In this connection it may be remarked that important quantities of the niacin can be found back.

TABLE V

The conversion of niacin under different circumstances in cut pea plants. Niacin content calculated per plant in micrograms as an average of 20 plants. The experiment lasted 2 days.

Date of sowing Beginning of the experiment	15/8 5/9	22/8 12/9	10/10 5/11	8/8 23/8	26/9 8/10	3/10 26/10
External circumstances	light	light	light	N-free	dark	dark
Pea plants without niacin added						
Leaves	9.5	9.4	—	6.7	—	—
Stem	2.1	2.7	—	2.3	—	—
Total	11.6	12.1	12.7	9.0	11.8	9.2
Niacin absorbed in 2 days	100	55	107	45	171	81
Pea plant with niacin added						
Leaves	10.6	9.9	—	7.8	—	—
Stem	7.8	5.4	—	4.8	—	—
Total	18.4	15.3	37.9	12.6	65.2	29.2
Recovered niacin	6.8	3.2	25.2	3.6	53.4	20.0
Lost in %	93	94	76	92	69	75

This may be explained by the assumption that the transformation of the niacin occurs for the greater part in the leaves, whereas the stems of the cut plants could absorb niacin till the moment of analysis. Therefore, a part of the niacin absorbed will be found again unchanged in the stems.

In order to establish this fact accurately the leaves and the stems of some series were analysed separately. It appeared that although a large quantity of niacin was absorbed and brought to the leaves by the transpiration stream, the niacin content in the leaves remained practically constant. This points to a conversion of niacin in the leaves. The quantity of niacin recovered is for the greater part found in the stems. The increase of niacin in the stems is about 2 to 4 times the original quantity. This increase is probably due to the absorption of niacin for transport to the leaves.

The same experiments were made with normal plants in the dark and with plants lacking nitrogen in the light. The results were completely similar to those of the experiments already described (table V).

In a stage of development in which a distinct niacin synthesis takes place the plant is capable of converting large quantities of the niacin added.

For a long time now the hypothesis has been assumed that trigonelline could be formed from niacin. This, however, has not been proved satisfactorily. As was shown in the above mentioned experiments that an intensive niacin metabolism occurs in the plant the question arose whether trigonelline could be formed.

TABLE VI

The conversion of niacin into trigonelline in light with cut pea plants of an age of 3 weeks. Calculated per plant as an average of 20 plants.

	Niacin		Trigonelline		Total
	micro-grams	10^{-3} mmol	micro-grams	10^{-3} mmol	10^{-3} mmol
Beginning of the experiment . . .	12.7	0.10	179	1.31	1.41
Blank plant 2 days on a 10 × diluted culture solution					
A Found per plant.	16.6	0.13	237	1.73	1.86
Test plant 2 days on a 10 × diluted culture sol. with niacin					
B Absorbed per plant	301	2.45	—	—	2.45
C Found per plant.	103	0.84	484	3.53	4.37
Conversion C — (A + B)	— 215	— 1.74	+ 247	+ 1.80	+ 0.06

We investigated this in the way already described. Niacin was added to cut plants and the quantity absorbed was determined. Thereafter we analized the quantity of niacin and trigonelline present in these plants. These experiments also confirmed a strong conversion of niacin in the plant. Furthermore we could show a clear increase of the trigonelline content. As is shown in table VI the quantities of niacin and of trigonelline increase, too, in plants which were not fed with niacin. The total amount of niacin per plant does not change very much in this synthesis (from 12.7 to 16.6 micrograms), but the amount of trigonelline increases by 50 micrograms. In drawing up the balance we charged this synthesis by comparing the plant which had absorbed niacin for 2 days with the blank plants. The result was that 1.74×10^{-3} mmol of the niacin absorbed must have been converted and 1.80×10^{-3} mmol trigonelline has been formed. The difference is about 4 %, this is within the limits of error.

So we may conclude that in experiments over a short period *the conversion of niacin in the pea is limited practically to a production of trigonelline.*

This calculation is based on two suppositions. Firstly, the normal niacin synthesis in the pea is not influenced by the addition of a large amount of niacin. Secondly, all pyridine derivatives, belonging to the niacin group and taking part in the metabolism in plants, are determined in our chemical analyses either as niacin or as trigonelline.

These suppositions were justified when it was shown that the niacin balance squared. The blank plants contained 1.86×10^{-3} mmol pyridine derivatives (estimated as niacin and trigonelline); the test plants, which had absorbed 2.45×10^{-3} mmol niacin contained 4.37×10^{-3} mmol pyridine derivatives, whereas 4.31×10^{-3} mmol was calculated.

The purpose of our investigations was further to make researches into the niacin metabolism. As transformation of niacin into trigonelline appears to be so rapid, the trigonelline metabolism must be included. Therefore, besides the niacin determinations, trigonelline determinations have to be performed also. This necessity is particularly urgent because the niacin level of the plant under the normal physiological circumstances, retains a very constant value. This has been established in all our experiments.

§ 7. The effect of several compounds on the production of nicotinic acid

It has become clear from the discussion of the literature on the subject that two groups of compounds are to be considered precursors in the synthesis of niacin. In the first place tryptophan and the compounds biochemically related with it e.g. kynurenine, kynurenic acid, 3-hydroxyanthranilic acid and quinolinic acid. The second group includes ornithine, citrulline and arginine.

In many experiments we investigated whether after the addition of dl-tryptophan or l-ornithine an increased synthesis of niacin or trigonelline could be obtained, with normal plants in the light. For this purpose we had solutions of these compounds absorbed by the transpiration stream into cut pea plants. After 2 days the niacin content and the trigonelline content of the test plants and the blanks were determined. We could not establish any increase of the niacin and trigonelline production either in plants of 2 weeks or in plants of 3 weeks old (table VII and VIII).

TABLE VII

The addition of ornithine and tryptophan to cut pea plants, aged about 2 weeks. Ornithine: 36.9 mg l (+) ornithine.2HCl dissolved in 500 ml diluted culture solution. Tryptophan: 33.1 mg dl-tryptophan dissolved in 500 ml diluted culture solution. Cut pea plants. Date of sowing 26/6. Beginning of the experiment 12/7. Calculated per plant as an average of 20 cut plants.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Beginning of the experiment	—	7.7	149
Cut pea plants			
After 2 days on blank culture solution	8.7 ml	8.7	243
After 2 days and absorbed 675 μg ornithine	9.2 ml	9.1	241
After 2 days and absorbed 622 μg tryptophan	9.4 ml	9.0	239

These results agree with those of the investigations of TERROINE et al. (1948) and VOLCANI and SNELL (1948). Our results do not correspond with those of GUSTAFSON (1949), NASON (1949–50) and BANERJEE e.a. (1950).

In accordance with the method described we further examined the effect of pyridoxine and pyrivic acid on the production of niacin

TABLE VIII

The addition of ornithine and tryptophan to cut pea plants, aged about 3 weeks. Ornithine: 103.3 mg l (+) ornithine.2HCl dissolved in 500 ml diluted culture solution. Tryptophan: 100.6 mg dl-tryptophan dissolved in 500 ml diluted culture solution. Cut pea plants. Calculated per plant as an average of 20 plants. Date of sowing 31/7. Beginning of the experiment 21/8.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Cut pea plants after 2 days in blank culture solution	8.5 ml	13.7	262
absorbed 2.53 mg ornithine . . .	12.3 ml	12.5	209
absorbed 1.58 mg tryptophan . .	7.9 ml	12.0	256

and trigonelline in the pea. These compounds were tested separately and in combination with tryptophan, ornithine and citrulline (table IX and X). In some experiments the addition of vitamin B_6, pyruvic acid and ornithine caused indeed a small increase of about 15 % of the trigonelline content, whereas the amount of niacin remained constant. But these results were not found in all our experiments. This is in agreement with the investigations of NASON (1949).

TABLE IX

The addition of citrulline and pyridoxine to cut pea plants. Citrulline: 22.2 mg l (+) citrulline dissolved in 500 ml diluted culture solution. Pyridoxine: 25.3 mg dissolved in 500 ml diluted culture solution. The combination contained: 21.6 mg l (+) citrulline and 26.0 mg pyridoxine dissolved in 500 ml diluted culture solution. Cut pea plants of 3 weeks age, in the light. Calculated per plant as an average of 20 plants.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Cut pea plants after 2 days in blank culture solution	10.5 ml	13.3	271
absorbed 404 μg citrulline. . . .	10.4 ml	13.9	241
absorbed 524 μg pyridoxine . . .	9.8 ml	13.6	268
absorbed 421 μg citrulline and 507 μg pyridoxine . . .	9.1 ml	14.2	268

The niacin synthesis was totally inhibited almost directly by putting the plants in the dark, as was shown in par. 5. It may be assumed for experiments lasting a short time that the enzyme system is still present and entirely intact. There must then be a lack of an essential product. We fed plants with ornithine in the dark, but could not

show an increased niacin or trigonelline production (table XI). A drawback of the experiments in the dark, however, is the etiolation of the plants.

Furthermore niacin synthesis is inhibited by a lack of nitrogenous compounds. To obtain nitrogen deficient plants they must be grown under these circumstances for a longer period. Then the objection

TABLE X

The addition of ornithine or tryptophan together with pyridoxine and pyruvic acid to cut pea plants. Solution B: 45.1 mg l (+) ornithine.2HCl, 48.0 mg pyridoxine and 135 mg pyruvic acid dissolved in 1 liter diluted culture solution. Solution: C: 23.10 mg dl-tryptophan; 21.8 mg pyridoxine and 69.2 mg pyruvic acid dissolved in 500 ml diluted culture solution. Cut pea plants of 3 weeks age, in the light. Calculated per plant as an average of 20 cut pea plants.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Cut plants after 2 days			
A in blank culture solution	11.8 ml	15.7	270
B absorbed 571 μg ornithine 607 μg pyridoxine and 1708 μg pyruvic acid .	12.8 ml	15.1	274
C absorbed 647 μg tryptophan 610 μg pyridoxine and 1938 μg pyruvic acid .	14.0 ml	14.1	274

TABLE XI

The addition of ornithine to cut pea plants in the dark. Ornithine: 34.8 mg l (+) ornithine.2HCl dissolved in 500 ml diluted culture solution. Cut pea plants aged 3 weeks. Experiment in the dark. Calculated per plant as an average of 20 plants.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Beginning of the experiment	—	22	319
Cut plants in 2 days in the dark			
in blank culture solution	12.8 ml	21	292
absorbed 863 μg ornithine . . .	12.4 ml	20	246

can be made that the enzyme systems are influenced. Moreover, the compounds added are, at least for the greater part, very probably converted, first into other substances, which are necessary for the plant. Although these experiments are of less importance in relation to our problem, yet we performed a few experiments with plants lacking nitrogenous compounds. Such plants we fed ornithine, citrulline or tryptophan in combination with pyridoxine and pyruvic acid. We could not show any increase of the amount of niacin and trigonelline (table XII).

Therefore we must conclude that none of the compounds examined determine the rate of the niacin production under the circumstances investigated.

TABLE XII

The addition of ornithine or tryptophan to cut pea plants with a lack of nitrogenous compounds. Ornithine: 107.3 mg l (+) ornithine.2HCl dissolved in 500 ml diluted culture solution. Tryptophan: 103.5 mg dl-tryptophan dissolved in 500 ml diluted culture solution. Pea plants with a clear lack of nitrogen. Experiment in the light. Calculated per plant as an average of 20 plants.

	Culture solution absorbed	Micrograms niacin	Micrograms trigonelline
Beginning of the experiment	—	9.8	235
Cut pea plants after 2 days			
in blank culture solution	11.2 ml	—	290
absorbed 2.55 mg ornithine . . .	12.4 ml	—	275
absorbed 2.32 mg tryptophan . .	13.5 ml	10.8	253

§ 8. Summary

1. An active nicotinic acid metabolism has been established during the development of the green pea (fig. 3).

2. The nicotinic acid production was inhibited by putting the plants in the dark or when the plants lack nitrogenous compounds.

3. We constructed an apparatus with which it was possible to determine in a simple way the amount of a substance absorbed by cut plants (fig. 4).

4. Cut pea plants were proved to have a normal nicotinic acid synthesis, therefore the leaves were supposed to synthesize nicotinic acid.

5. Above a definite level the nicotinic acid absorbed by the leaves is quantitatively transformed into trigonelline.

6. The balance-sheet of niacin was drawn up (table VI). From this we concluded that all pyridine compounds which take part in metabolism, are determined in our analyses, either as trigonelline or as nicotinic acid.

7. The nicotinic acid level of the pea retains a very constant value under normal physiological circumstances.

8. The addition of tryptophan, ornithine, citrulline, pyridoxine and pyruvic acid separately or in combination did not cause any increase of the nicotinic acid or trigonelline content.

9. The addition of tryptophan or ornithine with nitrogen free plants and of ornithine with plants in the dark gave the same results.

10. Therefore we concluded that the compounds mentioned above did not determine the rate of the nicotinic acid synthesis.

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