SPECIFICITY OF NICOTINIC ACID AS A GROWTH FACTOR FOR ISOLATED PEA ROOTS[1]

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(WITH ONE FIGURE)

It is known that nicotinic acid is essential to the growth of numerous species of isolated roots (ADDICOTT and BONNER, **1**, BONNER and DEVIRIAN, **4**). The present paper is concerned with the chemical specificity of nicotinic acid as a root growth factor.

Methods

The pea roots (variety Perfection) which were used exclusively in the present experiments were cultured according to the methods outlined in earlier papers (BONNER and ADDICOTT, **3**; BONNER and DEVIRIAN, **4**). Four-mm. tips were cut from the roots of aseptic pea seedlings and transferred to nutrient medium containing per liter of redistilled water 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 36 mg. $MgSO_4 \cdot 7H_2O$; 81 mg. KNO_3; 65 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. ferric tartrate; and 40 gm. of sucrose. After the tips had remained in the medium for one week at 25° C., and had grown into roots several cm. long, they were subcultured by tranferring 1-cm. tips to fresh nutrient solution. This process of subculture was repeated weekly. Vitamin B_1, which is essential to the continued growth of isolated pea roots, was added at the rate of 0.1 mg. per liter to all of the nutrient solution for transfers later than the first. Nicotinic acid, or related substance to be tested for ability to replace nicotinic acid, was added at the rate of 0.5 mg. per liter, since this concentration has been found to be approximately optimal for pea roots (ADDICOTT and DEVIRIAN, **2**).

A rigid selection for uniformity in root growth rate was carried out at the end of the first week of culture in order to assure the greatest possible uniformity in the roots actually used in each experiment. In a single experiment 400–500 roots were cultured and 20–50 roots were used for testing the activity of each substance reported below. In each experiment the roots were maintained through 5 or more weekly transfers.

Experimentation

The ability of decreasing concentrations of nicotinic acid to support the continued growth of isolated pea roots is shown in figure 1. Although 0.5 γ nicotinic acid per liter of nutrient solution supports modest growth, a ten times higher concentration is needed to support growth at a significant level. Still ten times higher concentration (50 γ per liter) supports growth

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at a maximum level, beyond which further increases result in no further increases in growth rate. The standard concentration, 0.5 mg., of nicotinic acid used in these experiments is therefore at least 100 times as high as is needed for a detectable promotive influence on the growth of isolated pea roots. In the experiments reported later analogs of nicotinic acid were also used in a concentration of 0.5 mg. per liter. An analog inactive at this concentration may then be concluded to possess less than 1 per cent. of the activity of nicotinic acid in supporting the growth of isolated pea roots.

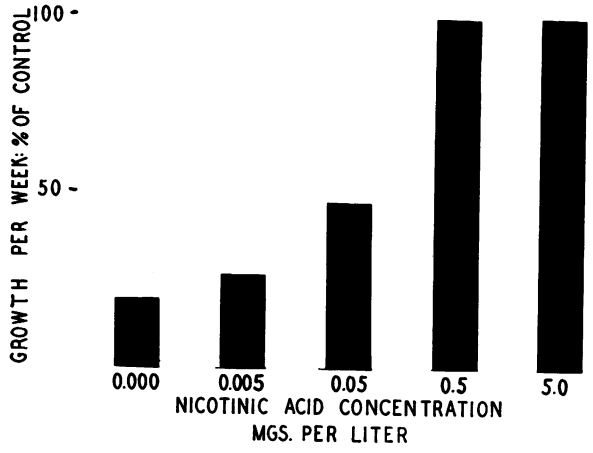

FIG. 1. Growth of isolated pea roots in medium containing adequate amounts of vitamin B_1 and varying amounts of nicotinic acid. The growth rates given are those for the fifth week of roots which had already been maintained through five weekly transfers in a medium of the composition shown.

Table I gives as an example one experiment in which methyl nicotinate and β-picoline respectively were substituted for nicotinic acid in the basic medium. In the absence of nicotinic acid growth in length decreases in each successive transfer. In medium containing nicotinic acid on the other hand, the growth rate is maintained at essentially a constant level. After 5 weekly transfers the roots which received nicotinic acid grew approximately 5 times as much per week as roots which did not receive nicotinic acid. Table I shows that the methyl ester of nicotinic acid also supports the growth of isolated pea roots and it may be concluded that methyl nicotinate is capable of replacing nicotinic acid as a growth factor for the isolated pea root. β-picoline, on the other hand, is clearly incapable of replacing nicotinic acid since roots supplied with β-picoline grew no better than the control roots which received vitamin B_1 alone.

All of the substances discussed below were tested, in experiments similar to that exemplified in table I, for ability to support the growth of pea roots through at least 5 weekly transfers. Each substance was either clearly inac-

TABLE I

ACTIVITIES OF 2 SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE CONTINUED GROWTH OF ISOLATED PEA ROOTS. 30–40 ROOTS IN EACH SERIES. ROOTS CULTIVATED FOR A PRELIMINARY WEEK IN MEDIUM CONTAINING VITAMIN B_1 AND NICOTINIC ACID

SUPPLEMENTS TO MEDIUM	GROWTH PER WEEK DURING:				
	WEEK III	WEEK IV	WEEK V	WEEK VI	WEEK VII
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
Vitamin B_1, 0.1 mg./l.	63	42	35	22	16
" + nicotinic acid, 0.5 mg./l.	79	78	74	86	80
" + methyl nicotinate, 0.5 mg./l.	85	78	81	96	78
" + β-picoline, 0.5 mg./l.	58	41	31	22	16

tive and supported growth at no higher level than nicotinic acid-free medium or it was completely active and supported growth essentially as well as nicotinic acid itself. Table II gives the activities in supporting isolated pea root growth of 23 substances related to nicotinic acid. Nicotinamide, esters of nicotinic acid, and nicotinuric acid were the only compounds among the substances tested which were active in supporting the growth of isolated pea roots. These active substances are all ones which yield nicotinic acid on simple hydrolysis. It must be concluded therefore that of the substances tested, only those which readily yield nicotinic acid by hydrolysis *in vivo* are capable of replacing the latter substance in the nutrition of isolated pea roots.

TABLE II

ACTIVITIES OF SUBSTANCES RELATED TO NICOTINIC ACID IN SUPPORTING THE GROWTH OF ISOLATED PEA ROOTS. + = ACTIVE. 0 = INACTIVE. DATA OBTAINED BY EXPERIMENTS OF TYPE SHOWN IN TABLE I

1. Nicotinic acid	+	10. Picolinic acid[1]	0
2. Nicotinamide	+	11. Quinolinic acid[1]	0
3. Coramine (N-diethyl amide of 1)[1]	0	12. Dinicotinic acid[1]	0
4. Methiodide of 2[1]	0	13. Nicotinuric acid[1]	+
5. Methyl nicotinate[1]	+	14. Ethyl nicotinoylacetate[1]	0
6. Ethyl nicotinate[1]	+	15. Arecoline[1]	0
7. Propyl nicotinate[1]	+	16. β amino pyridine[1]	0
8. Butyl nicotinate[1]	+	17. Nicotino-3-nitrile[1]	0
9. Isonicotinic acid[1]	0	18. β-picoline[1]	0
		19. Thiazole-5-carboxylic acid[2]	0
		20. Amide of 19[2]	0
		21. Benzoic acid	0
		22. Pyrazine, 3, carboxylic acid[2]	0
		23. Pyrazine, 2–3, dicarboxylic acid[2]	0

[1] Obtained through the courtesy of Prof. FELIX SAUNDERS and Dr. ALBERT DORFMAN, University of Chicago.

[2] Obtained through the courtesy of Dr. FRANZ C. SCHMOLKES, Research Division, Wallace & Tiernan Products, Inc., Belleville, New Jersey.

Inspection of table II shows that the steric requirements for nicotinic acid activity are strict. Thus isonicotinic acid and picolinic acid are completely inactive, although they differ from nicotinic acid only in the position of the carboxyl group relative to the heterocyclic nitrogen atom. Substitution of a second carboxyl group as in dinicotinic acid and quinolinic acid also renders the molecule inactive. It is noteworthy that the N-diethyl substituted amide of nicotinic acid, coramine, is inactive, indicating that this substance is not hydrolyzed *in vivo* by the pea root.

The carboxylic acids of certain cyclic substances other than pyridine are unable to replace nicotinic acid in the nutrition of pea roots. Thus benzoic acid is inactive. Thiazole-5-carboxylic acid and its amide are both inactive even though in this substance, as in nicotinic acid, the carboxyl group is β to the cyclic N atom. Pyrazine-3-carboxylic acid is likewise inactive.

Vitamin B_6, which like nicotinic acid is a pyridine derivative, is completely incapable of replacing the latter in the nutrition of isolated pea roots. Similar results have been obtained with isolated tomato roots (**10**).

Discussion

In relation to the general question of which reactions are capable and which incapable of execution *in vivo,* it is of interest that the pea root can neither hydrolyze nicotino-3-nitrile nor oxidize the methyl group of β-picoline to form nicotinic acid.

The specificity of nicotinic acid in the nutrition of pea roots is in substantial agreement with the specificity of this substance in the nutrition of *Staphylococcus aureus* (**7, 8, 9**), dysentery bacillus (**5, 6**) and the dog (**11**), with the particular exception that β-picoline is claimed to possess curative activity for black tongue.

The present data do not permit of a decision as to whether it is nicotinic acid *per se* which is required as a growth factor by the isolated pea root, or whether it may not be nicotinamide which, as a constituent of the codehydrogenases (**12**), is the true active substance. On the assumption that nicotinamide is the substance actually utilized by the pea root, it must be concluded that the root is capable of ready amide formation *in vivo.*

Summary

The ability of 23 substances chemically related to nicotinic acid to replace the latter substance as a growth factor for isolated pea roots has been determined. Among those tested, only substances which yield nicotinic acid by simple hydrolysis were found to be active.

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