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Nicotinate, Quinolinate and Nicotinamide as Precursors in the Biosynthesis of Nicotinamide–Adenine Dinucleotide in Barley

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1. The relative efficiencies of nicotinate, quinolinate and nicotinamide as precursors of NAD^+ were measured in the first leaf of barley seedlings. 2. In small amounts, both [^{14}C]nicotinate and [^{14}C]quinolinate were quickly and efficiently incorporated into NAD^+ and some evidence is presented suggesting that NAD^+ is formed from each via nicotinic acid mononucleotide and deamido-NAD. 3. [^{14}C]Nicotinamide served equally well as a precursor of NAD^+ and although significant amounts of [^{14}C]NMN were detected, most of the [^{14}C]NAD^+ was derived from nicotinate intermediates formed by deamination of [^{14}C]nicotinamide. 4. Radioactive NMN was also a product of the metabolism of [^{14}C]nicotinate and [^{14}C]quinolinate but most probably it arose from the breakdown of [^{14}C]NAD^+. 5. In barley leaves where the concentration of NAD^+ is markedly increased by infection with *Erysiphe graminis*, the pathways of NAD^+ biosynthesis did not appear to be altered after infection. A comparison of the rates of [^{14}C]NAD^+ formation in infected and non-infected leaves indicated that the increase in NAD^+ content was not due to an increased rate of synthesis.

The pathway of NAD^+ biosynthesis from nicotinate, which was originally demonstrated in erythrocytes and hepatic tissue by Preiss & Handler (1957, 1958*a*,*b*), has now been found in brain (Deguchi, Ichiyama, Nishizuka & Hayaishi, 1968) and in several micro-organisms (Ohtsu, Ichiyama, Nishizuka & Hayaishi, 1967) and it is likely that this pathway has a wide distribution in Nature. Nonetheless, the amount of nicotinate present in mammalian cells is normally very small compared with that of nicotinamide (Chaykin, Dagani, Johnson & Samli, 1965; Chaykin, Dagani, Johnson, Samli & Battaile, 1965). In recent studies on the formation of NAD^+ in hepatic tissue, Pallini & Ricci (1965) and Ijichi, Ichiyama & Hayaishi (1966) have shown that the incorporation of nicotinamide into NAD^+ occurs by way of nicotinate intermediates after its deamination. The enzyme nicotinamide deaminase is widely distributed (Joshi & Handler, 1960; Petrack, Greengard, Craston & Kalinsky, 1963; Marki & Greengard, 1966; Ohtsu *et al.* 1967) and the utilization of nicotinamide via nicotinate intermediates could well be a major pathway of NAD^+ biosynthesis in many tissues.

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In mammary gland, the nicotinate pathway of NAD^+ biosynthesis is completely replaced by a pathway in which nicotinamide is converted without deamination into NAD^+, by way of NMN (Greenbaum & Pinder, 1968). Grunicke *et al.* (1966) and McDonald & Stewart (1967) have also found evidence of this pathway in Ehrlich ascites-tumour cells, although in this case the nicotinate pathway also appeared to operate. Nishizuka & Hayaishi (1963) found an enzyme in liver that could catalyse the conversion of quinolinate into nicotinic acid mononucleotide, an intermediate in the nicotinate pathway of NAD^+ biosynthesis. Although the efficiency of quinolinate as a precursor of NAD^+ is not as clearly established as in the case of nicotinate and nicotinamide, the conversion of tryptophan into quinolinate and its subsequent incorporation into NAD^+ may represent an important pathway for NAD^+ biosynthesis *de novo* (see Chaykin, 1967).

Thus, in mammalian cells at least, several pathways exist for the synthesis of NAD^+ and in some instances, alternative pathways are present within the same tissue. In plant cells, however, the pathway(s) of NAD^+ biosynthesis have not yet been established. In *Ricinus communis* L., Waller, Yang, Gholson, Hadwiger & Chaykin (1966) have recently observed some conversion of nicotinamide into nicotinate and an incorporation of nicotinate into NAD^+ *in vitro*. Hadwiger, Badiei, Waller & Gholson (1963) have also demonstrated a conversion

of quinolinate into nicotinate compounds in this tissue. A notable feature of *Ricinus communis* L., however, is the high concentration of ricinine that accumulates. Waller *et al.* (1966) have presented evidence that ricinine, a pyridine alkaloid, is formed from either the nicotinamide moiety of NAD^+ or a closely related pyridine compound and the possibility must be considered that, as a result of this, the metabolism of NAD^+ in *Ricinus* may differ significantly from that in other plant species.

The present study was undertaken to establish more clearly the pathways of NAD^+ biosynthesis in plants. By using the excellent anion-exchange chromatographic technique described by Ijichi *et al.* (1966), the radioactive metabolites formed from nicotinate, quinolinate and nicotinamide have been investigated in barley leaves, and a scheme for NAD^+ biosynthesis is presented (Scheme 1). Further, a comparison was made between the formation of NAD^+ in non-infected barley leaves and in leaves infected with the powdery mildew fungus where the concentration of NAD^+ is markedly increased (Ryrie & Scott, 1968).

METHODS

Plant material. *Hordeum vulgare* L. var. *Manchuria* (barley), highly susceptible to infection by *Erysiphe graminis* var. *hordei* Marchal, race 3 (powdery mildew) was grown in river sand under greenhouse conditions as previously described (Ryrie & Scott, 1968). Infected material was produced by inoculating 5-day-old seedlings with fresh spores of powdery mildew and leaves were harvested for experimental use 5 days later. The top 5cm. of the primary leaf was used.

Reagents. [*carboxyl*-^{14}C]Nicotinic acid (27·9mc/m-mole), [*carbonyl*-^{14}C]nicotinamide (13·2mc/m-mole) and [6-^{14}C]-quinolinic acid (29·3mc/m-mole) were purchased from The Radiochemical Centre (Amersham, Bucks.).

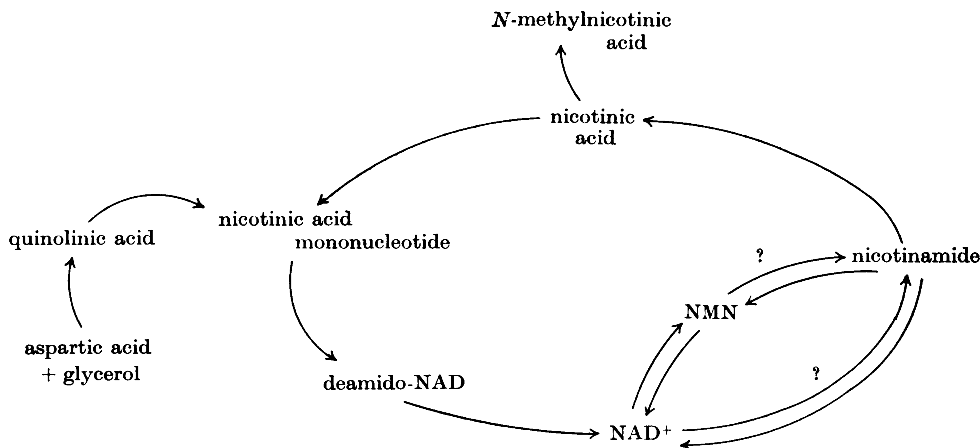
NAD^+, $NADP^+$, quinolinic acid and NMN were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.) and Dowex 1 (X2; formate form; 200–400 mesh) was purchased from Bio-Rad Laboratories (Richmond, Calif., U.S.A.). *N*-Methylnicotinic acid was prepared by making methyl iodide and nicotinic acid react under pressure as described by Sarett, Perlzweig & Levy (1940). The product was twice recrystallized from ethanol and shown to be free of nicotinic acid by t.l.c. on silica gel, by using chloroform–methanol (2:1, v/v). Polyethyleneimine anion-exchange paper was prepared from Whatman no. 1 paper as described by Verachtert, Bass, Wilder & Hansen (1965).

Nicotinic acid and nicotinamide were recrystallized in water from A.R.-grade chemicals before use.

Uptake of precursors. Each precursor was administered to the leaves by transpiration from small angled grooves in a Perspex block by the method of Graham & Walker (1962). Experiments were conducted at $28 \pm 1°$ and at a constant light-intensity of 5000 lux. Almost quantitative uptake of the precursor was attained within 2hr. by this method.

Unless otherwise stated 5·0nmoles of each radioactive precursor were offered/g. fresh wt. of leaves. Either 0·2 or 2·0μmoles/g. fresh wt. were offered in experiments with non-radioactive precursors.

Separation and counting of radioactive compounds. At preselected times, 0·75g. of leaf material was plunged into liquid N_2 and extracted in 0·1M-HCl as previously described (Ryrie & Scott, 1968). The supernatant from the neutralized extract was passed through a column (diam. 0·8cm., length 40cm.) of Dowex 1 (X2; formate form; 200–400 mesh) together with authentic compounds (2–8μmoles) as markers, and elution was carried out with a formic acid gradient as described by Ijichi *et al.* (1966), with the following modifications. Radioactive compounds were eluted at 3° with 250ml. of water in the mixing reservoir and successive reservoirs of 150ml. of 0·01M-formic acid, 250ml. of 0·25M-formic acid, 550ml. of 2·0M-formic acid and 150ml. of 4·0M-ammonium formate. Fractions (7·5ml.) were collected at a rate of 32ml./hr.; those corresponding to E_{260} peaks were combined and freeze-dried to remove formic acid and then redissolved in small volumes (usually



Scheme 1. Pathways of NAD^+ biosynthesis in barley leaves.

2·0 ml.) of 0·02 M-HCl. Portions of each sample were applied to Whatman no. 3 paper and chromatographed with the authentic compounds in three different solvent systems: (1) 1 M-ammonium acetate–ethanol (3:7, v/v, pH 5·0); (2) aq. 60% (v/v) propan-1-ol or aq. 85% (v/v) propan-2-ol; (3) 0·1 M-Na_3PO_4, pH 6·8, containing 60% (w/v) ammonium sulphate and 2% (v/v) propan-1-ol. [^{14}C]NAD$^+$ was also chromatographed with 0·3 M-LiCl on polyethyleneimine anion-exchange paper. The strips containing radioactivity were cut into 0·5 in. segments and suspended in vials containing 0·4% (w/v) 2,5-diphenyloxazole and 0·01% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene. The radioactivity was measured in a Packard Tri-Carb model 3375 liquid-scintillation spectrometer.

Nicotinamide and *N*-methylnicotinic acid were eluted as a single peak from Dowex 1 (formate form) and were separated as a routine by chromatography in solvents 1, 2 and 3 before radioactivity counting.

Measurement of the specific radioactivity of [^{14}C]*NAD*$^+$. After freeze-drying, [^{14}C]NAD$^+$ was redissolved in 0·30 ml. of 0·02 M-HCl; duplicate samples (0·05 ml.) were applied to Whatman no. 3 paper and developed in solvent 1 before their radioactivity was counted. A further sample (0·10 ml.) was diluted to 20·0 ml. in 5 mM-tris–HCl buffer, pH 7·4, and NAD$^+$ was measured by the spectrophotometric method previously described (Ryrie & Scott, 1968).

Formation of nicotinic acid mononucleotide and deamido-NAD. [^{14}C]Nicotinic acid (5·14 nmoles, 173 400 c.p.m.) was continuously administered to barley leaves (0·75 g.) for 1 hr. before extraction of ^{14}C-labelled compounds in 0·1 M-HCl and separation on Dowex 1 (formate form). Two radioactive peaks appeared immediately before the elution of standard quinolinic acid. The fractions from each peak were combined and concentrated by freeze-drying and then hydrolysed in 0·1 M-KOH as described by Pallini & Ricci (1965). As shown by chromatography in solvents 1, 2 and 3, all radioactivity was present as [^{14}C]nicotinate in both cases.

Nicotinamide deaminase. The deamination of [^{14}C]-nicotinamide to [^{14}C]nicotinate was measured *in vitro* by the method of Joshi & Handler (1960). Barley leaves were ground at 3° in 2 vol. of 0·01 M-tris–HCl buffer, pH 7·4, by using glass TenBroeck hand homogenizers and homogenates were centrifuged at 30000*g* for 20 min. in the cold. Deaminase activity was then measured in the clear supernatant, as described in the text.

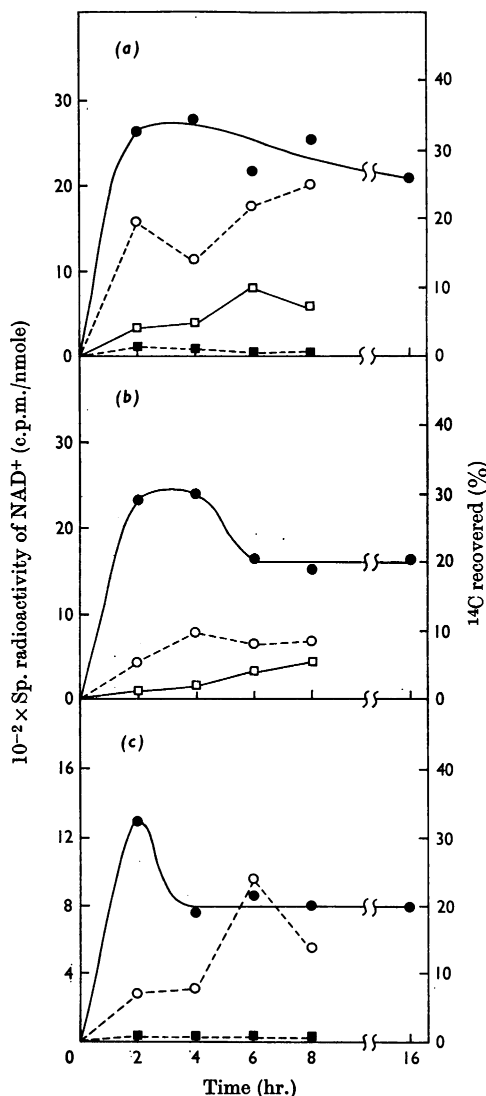


Fig. 1. The biosynthesis of NAD$^+$ and related compounds from [^{14}C]nicotinate, [^{14}C]quinolinate and [^{14}C]nicotinamide in non-infected barley leaves. Each precursor was administered to detached barley leaves by transpiration and the radioactive products formed were analysed as described in the text. (*a*) [^{14}C]Nicotinate; (*b*) [^{14}C]quinolinate; (*c*) [^{14}C]nicotinamide. ●, NAD$^+$; ○, NMN; □, *N*-methylnicotinate; ■, nicotinate.

RESULTS AND DISCUSSION

Metabolic fate of [^{14}C]*nicotinate in barley leaves.* As shown in Fig. 1(*a*) small amounts of [^{14}C]-nicotinate (5 nmoles/g. fresh wt.) were rapidly metabolized in barley leaves with the formation of [^{14}C]NAD$^+$. In agreement with the results of Ijichi *et al.* (1966) for liver and Deguchi *et al.* (1968) for brain the specific radioactivity of [^{14}C]NAD$^+$ quickly reached a maximum and then gradually declined. The analysis of radioactive compounds formed by metabolism of [^{14}C]nicotinate showed two radioactive peaks on elution from Dowex 1 (formate form) (Fig. 2), corresponding to the elution positions of nicotinic acid mononucleotide and deamido-NAD as described by Ijichi *et al.* (1966). Both compounds produced [^{14}C]nicotinate when hydrolysed under conditions that cause splitting of the pyridine–ribose bond. Thus, like erythrocytes and liver (Preiss & Handler, 1958*a*,*b*), it appears that higher plants can synthesize NAD$^+$ from nicotinate and that incorporation occurs by

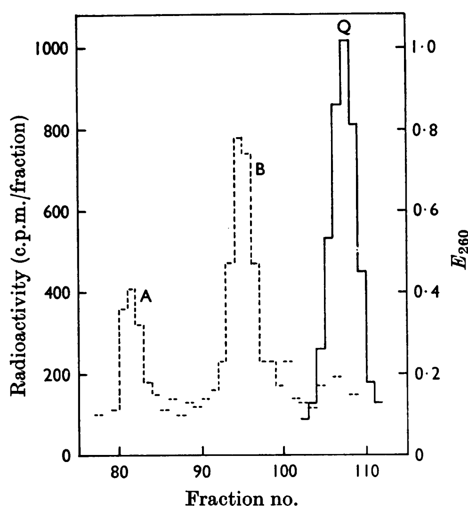


Fig. 2. The occurrence of two radioactive peaks corresponding to nicotinic acid mononucleotide and deamido-NAD after the separation of products of [14C]nicotinate metabolism on Dowex 1 (formate form). [14C]Nicotinate was administered to non-infected barley leaves for 1hr. as described in the text; the radioactive compounds were extracted in 0·1M-HCl and chromatographed together with 8μmoles of quinolinic acid. Fraction size was 7·5ml. Peaks A and B were tentatively identified as nicotinic acid mononucleotide and deamido-NAD respectively by their elution positions and the formation of [14C]nicotinate on alkaline hydrolysis. ----, Radioactivity; ——, E_{260}. Q, Quinolinic acid.

way of nicotinic acid mononucleotide and deamido-NAD.

The appearance of about 20% of the radioactivity from nicotinate in [14C]NMN (Fig. 1*a*) was unexpected. The only pathways known for NMN formation *in vivo* are the breakdown of NAD^+ and a synthesis from nicotinamide, catalysed by NMN pyrophosphorylase (see Scheme 1). The following considerations favour the former pathway in barley leaves: (1) a pyrophosphatase, which catalyses the formation of NMN from NAD^+, has been demonstrated in plant tissues (Kornberg & Pricer, 1950); (2) the direct formation of NMN from nicotinamide would require the prior conversion of administered nicotinate into nicotinamide, but enzymic reactions for the direct amination of nicotinate are unknown; the amide group of NAD^+ is incorporated into deamido-NAD by NAD synthetase; (3) results presented in the section on nicotinamide metabolism also suggest that the synthesis of NAD^+ from nicotinamide does not involve the formation of NMN.

Significant amounts of radioactivity from [14C]-nicotinate were also found in the alkaloid *N*-methylnicotinate (trigonelline). This result confirms previous observations made on plant extracts (Joshi & Handler, 1960) that higher plants can convert nicotinate directly into *N*-methylnicotinate.

Metabolic fate of [14C]*quinolinate*. The demonstration by Nishizuka & Hayaishi (1963) of an enzyme in liver catalysing the direct conversion of quinolinate into nicotinic acid mononucleotide provided the first evidence that quinolinate is utilized in NAD^+ biosynthesis without prior conversion into nicotinate. This enzyme, quinolinate transphosphoribosylase, has subsequently been found in other mammalian tissues (Ikeda *et al.* 1965), in several micro-organisms (Andreoli, Ikeda, Nishizuka & Hayaishi, 1963; Saxton *et al.* 1968) and in higher plants (Hadwiger *et al.* 1963; Waller *et al.* 1966). However, it is not known whether this reaction is important in cells that can efficiently utilize nicotinate and nicotinamide as precursors of NAD^+. Recent attempts to compare the efficiencies of quinolinate, nicotinate and nicotinamide as precursors of NAD^+ (Ijichi *et al.* 1966; Hagino, Lan, Ng & Henderson, 1968) have failed because of the limited uptake of quinolinate by liver cells. In barley leaves, [14C]quinolinate was rapidly metabolized and most of the radioactivity appeared in NAD^+ and metabolically related compounds. As shown in Fig. 1(*b*), the formation of [14C]NAD^+ from quinolinate closely paralleled that from nicotinate, both in the rate of formation and in the percentage incorporated. By 6hr., 77% of the quinolinate taken up by the leaves had been converted into other compounds.

The appearance of *N*-[14C]methylnicotinate (Fig. 1*b*) and traces of [14C]nicotinate (approx. 0·3% of that offered) from radioactive quinolinate is evidence that a pathway exists in barley leaves for the conversion of quinolinate into nicotinate. Waller *et al.* (1966) have found some evidence of a cyclic interrelationship of pyridine compounds in higher plants, which might account for these results. However, nicotinic acid mononucleotidase activity could provide an alternative route by which quinolinate might first be converted into nicotinic acid mononucleotide and then into nicotinate without the formation of NAD^+. Although a cyclic interconversion is tentatively included in Scheme 1, our results allow no definite conclusion about whether it is functional in barley leaves.

Metabolic fate of [14C]*nicotinamide*. From the results in Fig. 1(*c*) it is clear that barley leaves can also utilize nicotinamide in the biosynthesis of NAD^+ and that its efficiency as a precursor equals that of nicotinate and quinolinate under these conditions. The specific radioactivity of NAD^+ quickly increased to a maximum but unlike the [14C]NAD^+ derived from nicotinate and quinolinate the specific radioactivity declined in this case by

almost 50% between 2 and 4hr. This result was unexpected for it seemed that the turnover of NAD+ should normally be independent of the pathway utilized in its synthesis. Deguchi *et al.* (1968) have reported a corresponding dis-similarity between the [14C]NAD+ derived from nicotinamide and that from nicotinate in brain tissue. The reasons underlying these results, however, are not as yet clear.

The appearance of small amounts of [14C]-nicotinate from [14C]nicotinamide metabolism suggested that the deaminase previously identified in other plant tissues (Joshi & Handler, 1960; Waller *et al.* 1966) might also be active in barley. This was confirmed by direct measurement of [14C]nicotinamide deamination *in vitro*. From the results in Table 1, it appears that barley leaves deaminate nicotinamide at rates that exceed the requirements of NAD+ biosynthesis.

In view of this, the possibility was considered that nicotinamide might be deaminated in barley leaves and incorporated into NAD+ via nicotinic acid mononucleotide and deamido-NAD. If such a pathway operates, the simultaneous administration of unlabelled nicotinate together with [14C]-nicotinamide would be expected to decrease the concentration of [14C]NAD+ and increase the amount of radioactivity in nicotinate compounds. If incorporation occurs without deamination, unlabelled nicotinate should have no effect. As shown in Table 2, the administration of 1·0nmole of [14C]nicotinamide with either 5·0 or 25·0nmoles of unlabelled nicotinate/g. fresh wt. resulted in a fall in [14C]NAD+ content and a corresponding increase in amounts of radioactive nicotinate and *N*-methylnicotinate. Notably, [14C]NMN content also decreased. These results indicate that at least a large proportion of the NAD+ derived from nicotinamide is synthesized through the Priess–Handler pathway after deamination.

Biosynthesis of NAD+ after infection. The infection of barley leaves by powdery mildew has previously been shown to result in a marked increase in NAD+ content (Ryrie & Scott, 1968) and evidence was presented suggesting that this coenzyme mediates the respiratory increase that accompanies infection. From results in Fig. 3 it appears unlikely that this increase in NAD+ is due to any loss of control over NAD+ biosynthesis. The uptake of a large amount of nicotinate, quinolinate or nicotinamide (2·0μmoles/g. fresh wt.) produced an increase of only about 20nmoles of NAD+/g. fresh wt. after 5hr. (Fig. 3*a*). Similar experiments with one-tenth this amount of precursor produced an increase of only about 5nmoles/g. fresh wt. (I. J. Ryrie & K. J. Scott, unpublished work). When similar quantities of precursor were administered to infected leaves the increase in NAD+ content was no greater than that observed in non-infected material (Fig. 3*b*). These results point to an effective regulation of the concentration of NAD+ in both non-infected and infected barley leaves.

An examination of the biosynthesis of NAD+

Table 1. *Nicotinamide deamination in barley extracts*

The reaction mixtures contained 200nmoles of [14C]-nicotinamide (67600 c.p.m.), 20μmoles of potassium phosphate, pH7·4, and 0·20ml. of extract in a total volume of 0·6ml. Each reaction was carried out at 37° and was terminated by the addition of 1·0ml. of 1M-HClO4. [14C]-Nicotinate was separated from [14C]nicotinamide on Dowex 1 (formate form) as described in the text. For the 'infected' results, *E. graminis* is an ectoparasite and much of the fungal growth was removed before homogenization by brushing with damp cotton swabs.

Time (min.)	Nicotinate formed (nmoles)	
	Non-infected	Infected
5	2·90	3·97
15	6·58	9·30
45	18·25	27·70

Table 2. *Effect of unlabelled nicotinate on the biosynthesis of NAD+ from* [14C]*nicotinamide*

Either 5·0 or 25·0nmoles of nicotinate were administered to non-infected barley leaves together with 1·0nmole of [14C]nicotinamide/g. fresh wt. At 2hr. after the commencement of feeding the leaves were homogenized in 0·1M-HCl and the radioactive compounds separated on Dowex 1 (formate form).

Nicotinate administered (nmoles/g. fresh wt.)	Recovery of radioactivity (%)				
Product ...	[14C]NAD+	[14C]NMN	[14C]-Nicotinamide	[14C]-Nicotinate	*N*-[14C]Methyl-nicotinate
0	44	14	1·2	0·31	4·9
5	39	12	1·2	0·49	9·3
25	24	6	1·2	1·12	31·0

with physiological amounts of radioactive precursors did not show an increase in the rate of [^{14}C]NAD$^+$ formation after infection. As shown in Fig. 4, radioactive nicotinate, quinolinate and nicotinamide were each metabolized by infected leaves with the formation of [^{14}C]NAD$^+$. The major difference between these results and those in non-infected leaves centred on the rate of [^{14}C]-NAD$^+$ formation; in non-infected leaves [^{14}C]NAD$^+$ concentration reached a maximum within 2hr. (Fig. 1) whereas infected leaves required at least 6hr. (Fig. 4). Because of the high percentage incorporations that were attained (see Table 3), it seems unlikely that the decreased rate of [^{14}C]NAD$^+$ formation after infection is due to larger endogenous pools of nicotinate, quinolinate and nicotinamide.

In view of this, it appeared that the increase in NAD$^+$ content after infection might result from a decrease in the rate of NAD$^+$ breakdown. However, the decline in specific radioactivity of NAD$^+$

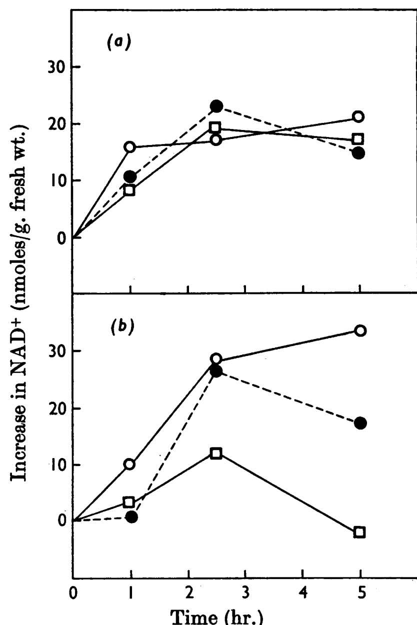


Fig. 3. The effect of nicotinate, quinolinate and nicotinamide on the concentration of NAD$^+$ in barley leaves. Each precursor (2·0μmoles/g. fresh wt.) was administered by transpiration and the amounts of NAD$^+$ were determined in acid extracts by the spectrophotometric method previously described (Ryrie & Scott, 1968). The values obtained were compared with those in leaves that were allowed to transpire only water. The amounts of NAD$^+$ initially present were about 25 and 45nmoles/g. fresh wt. for non-infected leaves and infected leaves respectively and these values were not changed by detachment over the 5hr. period examined. (*a*) Non-infected; (*b*) infected. ●, Nicotinate; □, quinolinate; ○, nicotinamide.

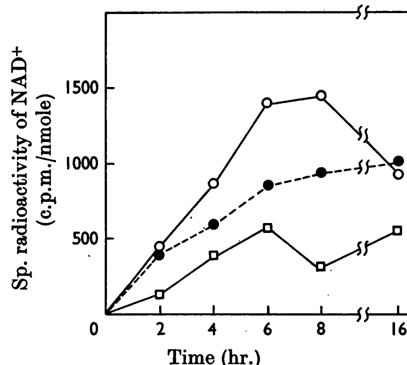


Fig. 4. The incorporation of [^{14}C]nicotinate, [^{14}C]quinolinate and [^{14}C]nicotinamide into NAD$^+$ in barley leaves infected with *E. graminis*. Precursors were administered to the detached leaves and the [^{14}C]NAD$^+$ was separated from other radioactive compounds on Dowex 1 (formate form). The percentage incorporation of each precursor after 6hr. is shown in Table 3. ●, Nicotinate; ○, quinolinate; □, nicotinamide.

Table 3. *Metabolic fates of radioactive nicotinate, quinolinate and nicotinamide in infected barley leaves*

Each precursor was administered to detached leaves by transpiration. After 6hr. the leaves were homogenized in 0·1M-HCl and the radioactive compounds separated on Dowex 1 (formate form). The unknown compound(s) were eluted as a neutral fraction from Dowex 1 (formate form). [^{14}C]Nicotinamide accounted for no more than 2% of the administered radioactivity in each case. —, Values not determined.

	Recovery of radioactivity (%)		
Precursor	[^{14}C]Nicotinate	[^{14}C]Quinolinate	[^{14}C]Nicotinamide
[^{14}C]NAD$^+$	20	23	23
[^{14}C]NMN	14	6	11
[^{14}C]Nicotinate	1·56	0·32	0·59
N-[^{14}C]Methylnicotinate	14	5	—
[^{14}C]Nicotinamide + ^{14}C-labelled unknown(s)	13	4	32
[^{14}C]Quinolinate	—	23	—

shown in Figs. 1 and 4 is not a good measure of this, as the [^{14}C]nicotinamide derived from NAD^+ breakdown may be utilized again in the formation of NAD^+. As pointed out by Gholson (1966) a cycling of the pyridine moiety in this way would maintain the specific radioactivity of NAD^+ even if rapid turnover was occurring.

Further analysis of the products formed by the metabolism of these precursors in infected leaves indicated the same pattern of incorporation as in non-infected leaves. As shown in Table 1, extracts of infected leaves readily deaminated nicotinamide to nicotinate; further, the metabolism of [^{14}C]-nicotinate was accompanied by the formation of two similar peaks to those shown in Fig. 2, indicating that nicotinic acid mononucleotide and deamido-NAD are probable intermediates in the conversion of nicotinate into NAD^+ *in vivo*. As in non-infected leaves, the biosynthesis of [^{14}C]NAD^+ from radioactive nicotinate, quinolinate or nicotinamide was accompanied by the formation of [^{14}C]NMN (Table 3). In some experiments, *N*-[^{14}C]methylnicotinate was also detected. It appears therefore that although the leaf content of NAD^+ is markedly increased by infection, there is a close similarity between the pattern of NAD^+ synthesis in non-infected and infected leaves.

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