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The Deamidation of Nicotinamide by Bacteria

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In a previous paper from this laboratory it was shown that nicotinamide was rapidly broken down to nicotinic acid by washed suspensions of *Lactobacillus arabinosus* 17-5 (Hughes & Williamson, 1952a). In the present paper it is shown that the breakdown of nicotinamide in this micro-organism is hydrolytic, nicotinic acid and ammonia being formed stoichiometrically. Cell-free extracts which deamidate nicotinamide have been prepared and some properties of the enzyme investigated. A number of other species of lactobacilli were tested and all found to deamidate nicotinamide. In contrast to this, none of eleven other species of bacteria, with the single exception of a strain of *Staphylococcus albus*, were active.

EXPERIMENTAL

Organisms and growth media. *Lactobacillus arabinosus* 17-5, *Lb. bulgaricus*, National Collection of Type Cultures, strain 76, *Lb. delbrueckii*, NCTC, strain 5125, *Lb. casei*, NCTC, strain 8001, and *Lb. helveticus*, NCTC, strain 8103, two locally isolated strains of *Staphylococcus aureus* and NCTC, strain 6775, several locally isolated strains of *Staph. albus* and haemolytic streptococci were grown at 37° on an enzymic casein hydrolysate medium (Nossal, 1951) adjusted to pH 7.6. In some experiments *Lb. arabinosus* was grown on the semi-synthetic medium used for the microbiological assay of nicotinic acid (McIlwain & Hughes,

1948). Twelve strains of lactobacilli isolated from beer by Dr T. K. Walker, Manchester College of Technology, were grown at 30° on the casein hydrolysate medium adjusted to pH 5.0. *Acetobacter aceti*, *Aceto. acetosum*, *Aceto. capsulatum*, *Aceto. suboxydans* and *Aceto. turbidens* (supplied by Dr T. K. Walker) were grown at 30° on the yeast extract-glucose medium described by Walker & Tosic (1945). *Proteus morgani*, NCTC, strain 2812, and a local strain of *P. vulgaris* and *Escherichia coli* were grown on a medium consisting of acid-hydrolysed casein (McIlwain & Hughes, 1944) and 2% yeast autolysate. A mutant strain of *Aerobacter aerogenes*, exacting for nicotinic acid, was grown on the inorganic salt medium described by Devi & Higginbottom (1949). Three locally isolated strains of *Pseudomonas fluorescens* were grown on the medium of Koser & Baird (1944).

The organisms were harvested by centrifugation, washed twice and suspended in 0.9% (w/v) NaCl to give a suspension containing from 5 to 40 mg. dry wt./ml.

Estimation of enzyme activity. The rate of deamidation of nicotinamide was estimated by measuring ammonia formation: 0.01 M nicotinamide (0.5 ml.), and 0.2 M phosphate buffer, pH 7.0 (1.0 ml.) were placed in one side of a double side-arm test tube (Hughes & Williamson, 1952b) and the enzyme solution (0.5 ml. previously adjusted to pH 7) in the other. After 5 min. incubation in a water bath at 40° the contents of the side arms were mixed. The reaction was stopped after varying periods of incubation by the addition of 1.0 ml. of 2N-H₂SO₄ and ice-cooling. The ammonia was distilled in a modified Parnas apparatus, the distillate nesslerized, and the colours read in a Beckman spectrophotometer at 625 mμ. Ammonia was also estimated in the

reagents and enzyme material. Formation of ammonia was found to proceed linearly when the enzyme concentration was such as to decompose from 10 to 80% of the added 5 μ moles of nicotinamide in 60 min. (Fig. 1). The rate of reaction was proportional to enzyme concentration under these conditions (Fig. 2). In experiments with organisms other than the lactobacilli and where the rate of reaction did not exceed 0.5 μ moles NH_3 /mg. dry wt. cells/hr.; 5 times the amount of the above reagents and up to 20 mg. dry wt. cells were incubated up to 2 hr. The results throughout the paper are expressed as μ moles NH_3 /mg. dry wt. cells/hr.; where extracts were used results are expressed in terms of mg. dry wt. of the cells from which the extract was made.

Fig. 1. Formation of ammonia from nicotinamide by various enzyme preparations. Each tube contained 0.5 ml. 0.01M nicotinamide, 0.5 ml. 0.8M phosphate buffer, pH 7.0, and 1.0 ml. enzyme preparation as indicated. ○—○, intact cells; ●—●, acetone powder; ×—×, cell-free extract.

Fig. 2. The effect of enzyme concentration on the deamination of nicotinamide. Tubes containing: 0.5 ml. 0.01M nicotinamide; 0.5 ml. 0.1M phosphate buffer, pH 7.0; 1.0 ml. enzyme preparation as indicated were incubated 10 min. at 40°. ○—○, intact cells; ×—×, extract of cells prepared by grinding with powdered glass.

Chemicals. Nicotinic acid and nicotinamide were the purified specimens used in previous work (Hughes & Williamson, 1952a). Diphosphopyridine nucleotide (DPN) (50% pure) was found to be contaminated with traces of acetone which interfered with the ammonia estimations. To remove acetone the DPN was ground in ice-cold ethanol and washed with ice-cold ether in a centrifuge tube, then dried for 3 days over P_2O_5 in a vacuum desiccator. All other chemicals were commercial specimens and were not purified further.

Preparation of cell-free extracts. In preliminary experiments, extracts were prepared from cells of *Lb. arabinosus* broken by grinding with powdered glass or by crushing at -20° in the bacterial press (Hughes, 1951). In later experiments it was found more convenient to prepare the extracts from cells dried from the partially frozen state over P_2O_5 (Hughes & Williamson, 1952b). The final method for extraction was as follows: dried cells (500 mg.) were ground in a glass pestle and mortar with 6–8 ml. of 0.2M phosphate buffer, pH 6.0. The volume was made up to 40 ml. with phosphate buffer; 6 drops of toluene were added and the suspension was incubated at 37° in a stoppered flask for 22 hr. It was then centrifuged and the supernatant was decanted. The residue was washed with 10 ml. water on the centrifuge. The combined supernatants contained 80% of the original enzyme activity. They were cooled to 0° to -2° and stirred with 5 vol. ice-cold redistilled acetone. After standing for 10 min. the precipitate was collected by centrifugation, washed with ice-cold acetone and dried in a desiccator over P_2O_5 . The dried powder weighed 40–50 mg. and contained 50–60% of the original enzymic activity. About 10% of the enzymic activity was lost from the dried acetone powder and from the dried cells after storage for 10 months at 2° . For subsequent use the dried powder (50 mg.) was triturated in cold distilled water (2.0 ml.), left at 2° for 1 hr. and then centrifuged to remove a little insoluble material and the clear supernatant diluted to 5 ml.

Estimation of nicotinic acid in the presence of nicotinamide. As shown by Larson & Haag (1944) many pyridine compounds when treated with CNBr form yellow or orange derivatives with typical absorption spectra in the region of 400–450 $\text{m}\mu$. It was found that this yellow derivative from nicotinic acid treated with NaOH formed material absorbing strongly in the near ultraviolet. It is probably material which acts as an internal filter and quenches the fluorescence of nicotinamide treated under similar conditions (Chaudhuri & Kodicek, 1949). The absorption spectrum of the reaction produced from nicotinic acid has a peak at 372 $\text{m}\mu$. (ϵ_{372} , where ϵ is molar extinction coefficient = 3.02×10^4), that of nicotinamide is weakly absorbing in this region and decreases and becomes negligible at 400 $\text{m}\mu$. (Hughes & Williamson, 1952a). By choosing suitable wavelengths, nicotinic acid and nicotinamide may be separately estimated and nicotinic acid may be estimated in a solution containing nicotinamide.

Preparation of the CNBr reagent. The reagent as generally prepared (Chaudhuri & Kodicek, 1949) gave erratic results mainly owing to differences in pH between batches and to changes in pH during the reaction. Consistent and maximum values were obtained when the pH of the reagent was buffered strongly at pH 5.2–5.8. The most suitable buffer was a mixture of 0.05M acetate and phosphate; succinate and citrate buffers inhibited the reaction. To prepare the CNBr solution, 25 ml. of bromine water were cooled to 2° and 2M-NaCN was added dropwise until the colour just dis-

appeared. A solution containing 12.5 ml. of 0.5M phosphate buffer, pH 6 and 12.5 ml. of 0.2M acetate buffer, pH 5 was then added and the pH finally adjusted to 5.5 (glass electrode) with either NaOH or 10% acetic acid. The reagent when stored in a dark bottle at 2° remained stable for 4-5 days.

Reaction with CNBr. The optimum concentration of CNBr reagent was found to be higher than that generally used in the Koenig reaction. Consistent values were found when 1.0 ml. of the test solution containing from 0.3 to 400 μ moles of nicotinic acid were allowed to react with 1.0 ml. of the buffered reagent. The optimum temperature for the reaction was 70°. Under these conditions the ϵ_{372} increased rapidly up to 15 min., reached a maximum value in 18 to 20 min. and decreased again if heating was continued longer than 25 min.

Reaction with NaOH. Upon addition of the optimum concentration of 6N-NaOH (0.5 ml.) after reaction with CNBr solutions became yellow, the yellow colour then faded and ϵ_{372} increased and reached 90% of the maximum value in 45 min. Thereafter the rate of increase was slow and the maximum value was reached in 2.5-3 hr.; the ϵ_{372} then decreased and after 16-20 hr. at room temperature had fallen to 80% of the maximum value. With more NaOH the reaction was faster but did not reach the maximum value; with less NaOH the reaction was slow and again did not reach maximum value.

Method. To 0.2-1.0 ml. of the test solution containing from 0.3 to 400 μ moles of nicotinic acid in a stoppered test tube were added 1.0 ml. of the CNBr reagent and water to make the volume to 2.0 ml. The mixture was heated 20 min. at 70°, cooled in running water; 0.5 ml. of 6N-NaOH was added and the final vol. was made to 3.0 ml. with water. The tubes were then placed in the dark at room temperature for 45 min. and the optical density was measured in a Beckman spectrophotometer.

The nicotinic acid content of solutions was estimated by comparing the ϵ_{372} with nicotinic acid standards set up at the same time. Solutions containing from 0.3 to 400 μ moles nicotinic acid obeyed Beer's Law (Fig. 3) and duplicate determinations agreed to $\pm 0.5\%$. Results for solutions containing both nicotinic acid and nicotinamide did not agree with the expected values when calculated by the use of Morton & Stubbs (1946) equations. This is probably owing to the fluorescence of nicotinamide and the quenching effect of nicotinic acid (Chaudhuri & Kodicek, 1949). In the present work, however, advantage was taken of the fact that during the enzymic reaction, it was the proportion of acid to amide that changed whilst the substituted pyridine content of the solutions remained constant. Standard solutions containing varying proportions of nicotinamide and nicotinic acid were treated with CNBr and NaOH and their extinctions measured at wavelengths between 350 and 400 μ . An approximately straight line was given at 400 μ . (Fig. 4). The nicotinic acid content of solutions from the enzymic deamidation of nicotinamide was therefore estimated from such a standard curve set up at the same time. Duplicate estimations agreed within 2% and the method thus compared favourably with the more tedious microbiological method previously used (McIlwain & Hughes, 1948; Hughes & Williamson, 1952a).

Interfering substances. When treated with CNBr and NaOH as described, nicotinamide riboside, nicotinamide ribotide and DPN had absorption spectra like that of nicotinamide. The spectra of nicotinyl hydrazide, nicotinic acid and 5-fluoronicotinic acid were identical with that

Fig. 3. Standard curve for estimating nicotinic acid prepared by reacting 0.5 ml. of solution with CNBr + NaOH. Final vol. 3.5 ml. $E_{1\text{ cm.}}$ was measured at 372 μ . in a Beckman or Unicam spectrophotometer against a reagent blank.

Fig. 4. Standard curves of a mixture of nicotinic acid and nicotinamide reacted with CNBr + NaOH and read at various wavelengths.

of nicotinic acid. Nicotinamide N^1 -methochloride, trigonelline, pyridoxine, pyridoxal, 2-fluoronicotinic acid and 6-fluoronicotinic acid did not react with CNBr and NaOH to give material adsorbing in the regions studied. Thiamine reacted to give an absorption spectrum with an ill-defined peak in the region of 370 μ . Solutions of various salts and casein hydrolysate did not interfere, but solutions containing glucose which had been autoclaved at 120° adsorbed too strongly for nicotinic acid to be estimated.

RESULTS

The deamidation of nicotinamide by various micro-organisms

Deamidation by lactobacilli. Under the standard conditions already described, *Lb. arabinosus* and *Lb. bulgaricus* formed ammonia at rates of 1-2 μ moles/mg. dry wt./hr. (Table 3). All the other lactobacilli tested formed ammonia, but at slower

rates (0.1–0.8 μ mole/mg. dry wt./hr.). Because *Lb. arabinosus* was consistently the most active the following experiments were done with this organism.

To test the stoichiometry of the reaction, ammonia and nicotinic acid were measured at time intervals during reaction between nicotinamide and acetone powder of an extract of *Lb. arabinosus*; both products were formed stoichiometrically (Table 1). Similar results were obtained with washed cells and other cell-free extracts. In addition, nicotinic acid was measured in some of the tests with the other lactobacilli and was always found to be formed to the same extent as the ammonia.

The identity of nicotinic acid was confirmed by isolation from the reaction fluid, 50 ml. of 0.01M nicotinamide in 0.05M phosphate buffer, pH 7.0, being incubated at 37° with 50 mg. of acetone powder preparation until ammonia estimation showed the reaction to be complete (16 hr.). The mixture was made acid to pH 4.0 with N hydrochloric acid, evaporated to dryness *in vacuo* at 40° and dried by evaporating it to dryness twice with acetone. The dried material was then extracted with three successive portions of boiling acetone. The combined extracts were filtered and taken to dryness in a test tube. The bottom of the test tube was immersed in an oil bath at 210° and the white solid which sublimed was collected, re-crystallized from water twice and dried *in vacuo* over phosphorus pentoxide: m.p. 198°; mixed m.p. 198°. The absorption spectra of solutions of the crystals in N hydrochloric acid and of solutions in N sodium hydroxide were identical with similarly prepared nicotinic acid. Tests by the paper-chromatographic methods of Kodicek & Reddi (1951) also supported the evidence that the crystals were those of nicotinic acid. The deamidation of nicotinamide is thus a hydrolytic reaction in which ammonia and nicotinic acid are formed. Under the conditions of the test nicotinamide is decomposed completely. There was no inhibition of the rate of reaction in the presence of high concentrations of either nicotinic acid or ammonia (0.1M). Likewise no formation of nicotinamide could be detected when the various enzyme preparations were incubated with ammonium nicotinate either alone or with adenosine triphosphate, glutamine and salts of magnesium and manganese.

The effect of growth conditions on the deamidation of nicotinamide. No significant difference was found in the rate of ammonia formation from nicotinamide by *Lb. arabinosus* when the cells were grown on the semi-synthetic medium or the casein-hydrolysate medium to which had been added nicotinic acid, nicotinamide or DPN in concentration from 1×10^{-3} to 5×10^{-7} M. The ability of the cells to deaminate nicotinamide does not therefore depend on the concentration of nicotinic acid or its derivatives in the

Table 1. *Nicotinic acid and NH₃ formation from nicotinamide*

(Each double-arm tube contained 0.5 mg. acetone powder from *Lb. arabinosus*, 8.0 ml. 0.2M phosphate buffer pH 7.0 in one side arm and 2 ml. 0.01M nicotinamide in the other arm. The contents were mixed after 5 min. at 40° and the reaction was stopped by the addition of 2 ml. 5N-H₂SO₄ at the times indicated. Nicotinic acid was determined in a 1 ml. sample by reaction with CNBr; NH₃ was determined in 8.0 ml.)

Time after mixing (min.)	NH ₃ formed (μ moles/10 ml.)	Nicotinic acid formed (μ moles/10 ml.)
0	1.1	1.2
15	2.1	2.3
45	3.8	4.3
90	7.8	8.2
150	11.8	11.8
180	13.2	13.8

Table 2. *The effect of age of culture on the rate of deamidation by Lactobacillus arabinosus 17-5*

(Hydrolysed casein medium (1 l.) was inoculated with 1 ml. of a 24-hr. culture and shaken at 37°. Samples were taken for the turbidimetric estimation of growth at 0.5 hr. periods and larger samples for the estimation of enzyme activity were taken as indicated. The cells were collected and washed and their enzyme activity was estimated as described.)

Time (hr.)	Growth (mg. dry wt. bacteria/ml. medium)	Rate of deamidation (μ moles NH ₃ /mg. dry wt./hr.)
5	0.25	1.3
8	0.40	1.1
10	0.45	1.4
12.5	0.8	1.8
14.5	0.9	1.3
24	1.0	1.3

medium as was found to be the case with DPN synthesis (Hughes & Williamson, 1952a). Nor did the age of the cells (5–24 hr., Table 2), the absence of glucose or pH of the media, have any marked effect. Similar results were obtained with *Lb. bulgaricus*. Nicotinamide deamidase thus may be considered to be a 'constitutive' enzyme of lactobacilli.

Deamidation of nicotinamide by micro-organisms other than lactobacilli. As shown in Table 3 none of the other organisms tested deamidated nicotinamide at a significant rate (0.05 μ mole/mg. dry wt./hr.) with the single exception of one strain of *Staph. albus*. It is noteworthy that some of the organisms tested and found not to deaminate nicotinamide (e.g. staphylococci and streptococci), decompose DPN with the formation of nicotinamide as one of the end products (McIlwain & Hughes, 1948). In contrast to this, *Lb. arabinosus* does not decompose DPN but is able to deaminate nicotinamide at a rapid rate. It seems possible that the nicotinamide deamidase is concerned in the regulation of the

DPN content of the cells (see Hughes & Williamson, 1952*a*). The inability of the three strains of *Ps. fluorescens* to deaminate nicotinamide explains why these organisms do not oxidize nicotinamide when they are adapted to oxidize nicotinic acid. Attempts to isolate from the soil organisms which would grow upon nicotinamide as a sole source of carbon and nitrogen were not successful (cf. Koser & Baird, 1944; Jakoby, Schatz, Hutner & Weber, 1952.)

Table 3. *Deamidation of nicotinamide by various bacteria*

(Organisms were grown for 16 to 24 hr. on the media described in the text, collected, washed and suspended in saline to give 5–20 mg. dry wt./ml. Enzyme activity was measured as described in the text. Maximum and minimum rate of at least three different batches are given.)

Organism	Rate of deamidation (μ moles NH_3 /mg. dry wt./hr.)
<i>Lb. arabinosus</i>	2.0–3.0
<i>Lb. casei</i>	0.5–1.0
<i>Lb. delbrueckii</i>	0.5–1.5
<i>Lb. helveticus</i>	0.5–1.0
<i>Lb. bulgaricus</i>	0.5–1.0
<i>Lactobacillus</i> sp. (from yogurt)	1.0–2.0
<i>Lactobacillus</i> strains W1, W2, K	0.3
<i>Lactobacillus</i> strains D4, W6, E4	0.5–1.0
<i>Lactobacillus</i> A4	0.4
<i>Lactobacillus</i> 15	0.1
<i>Staph. albus</i> sp.	0.8–1.5
<i>Staph. albus</i> (7 strains)	<0.05
<i>Staph. aureus</i> (5 strains)	<0.05
<i>Strep. haemolyticus</i> (2 strains)	<0.05
<i>Cl. welchii</i>	<0.05
<i>Esch. coli</i>	<0.05
<i>Aceto. suboxydans</i> , <i>Aceto. capsulatum</i> , <i>Aceto. aceti</i>	<0.05
<i>Aerobacter aerogenes</i>	<0.05
<i>Ps. fluorescens</i>	<0.05

Fig. 5. pH-activity curve of various enzyme preparations. Each tube contained: 0.5 ml. 0.01*M* nicotinamide; 0.5 ml. 0.8*M* acetate buffer (from pH 3.5 to 5.0) or 0.5 ml. 0.8*M* phosphate buffer (from pH 5.0 to 8.0); 1.0 ml. enzyme preparation as indicated. Incubated 10 min. at 40°. ○—○, intact cells; ●—●, acetone powder; ×—×, cell-free extract.

Properties of the enzyme

The pH-activity curve. The deamidation reaction in intact cells, in extracts of cells prepared by grinding them with powdered glass, and in extracts of the acetone-dried cells were identical and had a broad optimum between pH 8.0 and 4.5. Below pH 4.5 the activity of the enzyme fell abruptly (Fig. 5).

Substrate concentration curves. The initial rates of reaction at substrate concentrations between 0.025*M* and 0.0005*M* in intact cells and cell-free extracts obeyed the Michaelis-Menten equation, and the apparent Michaelis constant was 0.0015*M*.

Specificity. No ammonia was produced when the acetone-dried cells or the extracts prepared from them were incubated with the following substances: glutamine, asparagine, arginine, adenine, urea, nicotinuric acid, nicotinamide methochloride, trigonelline, nicotinamide riboside, DPN, pyridine-3-sulphonamide, 5-fluoronicotinamide. Because the enzyme did not deaminate the nicotinamide moiety of DPN and nicotinamide riboside, some experiments were made to test the usefulness of the enzyme for determining free nicotinamide in extracts of tissues. Recovery of added nicotinamide (0.1–10 μ moles) from aqueous extracts of various rat tissues was satisfactory but the ammonia blanks were high. The experiments were discontinued since the method appeared to be less convenient than chemical estimations on fractions separated chromatographically (Kodicek & Reddi, 1951; Wollish, Schmall & Shafer, 1951).

Stability. Aqueous suspensions of the freshly washed cells lost about 15–25% of their original enzyme activity in 7 days when stored at 2°. The cell-free preparation was stable under these conditions. All enzyme preparations lost their activity completely in 3 min. at 100°; about 50% of activity was lost after 15 min. at 60°.

Solutions of the enzyme did not lose activity when dialysed for 2 days at 2° against tap or distilled water, 0.05*M* phosphate buffer, pH 7.0, or 0.05*M* acetate buffer, pH 5.0. Treating the enzyme solutions with ethanol, ammonium sulphide or acid and alkaline solutions of ammonium sulphate resulted in irreversible loss of activity. There was no evidence of any cofactor in the preparations.

The effect of inhibitors. The most effective inhibitors of deamidation in intact cells and cell-free extracts of *Lb. arabinosus* were ions of heavy metals, copper sulphate, mercuric chloride and silver nitrate, which inhibited deamidation completely in concentrations between 1×10^{-3} and 5×10^{-4} *M*. Substances related to nicotinamide such as pyridine-3-sulphonic acid, pyridine-3-sulphonamide, nicotinyl hydrazide, 5-fluoronicotinic acid, 2-fluoronicotinic acid, 6-fluoronicotinic acid did not inhibit in concen-

trations up to 5×10^{-2} M. The phenolphthaleins and sulphonophthaleins which inhibit the glutaminase of *Clostridium welchii* (Hughes & Williamson, 1952b) and the acridines, euflavine and atebine which inhibit renal glutaminase (Archibald, 1944) did not affect deamidation of nicotinamide in concentrations up to 4×10^{-2} M. Sodium azide, hydrazine sulphate, sodium nitrate, sodium iodoacetate, cetyltrimethylammonium bromide, and sodium dodecyl sulphate were also without effect.

DISCUSSION

Although nicotinamide can give rise to nicotinic acid in the intact animal (Ellinger & Abdel-Kader, 1949; Johnson & Lin, 1951; Leifer, Roth, Hogness & Corson, 1951) no enzyme hydrolysing the amide has as yet been described. The present experiments are the first demonstration of a nicotinamide deamidase. It is remarkable that the distribution of the enzyme in bacteria appears to be restricted mainly to the genus *Lactobacillus* where the rate of deamidation can be such that the bacteria decompose their own weight in nicotinamide in 1 to 2 hr. It seems likely that part at least of the nicotinic acid formed from nicotinamide in the intact animal may be formed by the action of lactobacilli present in the digestive tract. It has recently been claimed that nicotinamide is deamidated *in vitro* by rat liver (Quagliarello, Gueritore & Porcellati, 1952). The activity of liver in this respect cannot however be high because nicotinamide is stable enough in the liver to be isolated as an end product of the breakdown of cozymase (Mann & Quastel, 1941) and to serve as a methyl acceptor in experiments on methylation (Ellinger & Armitage, 1953).

The method for the determination of nicotinic acid described in the present paper was elaborated before the chromatographic methods (Kodicek &

Reddi, 1951; Wollish *et al.* 1951) were known. The latter methods are more convenient in many ways and of wider application. But there is still scope in the present method as an independent one for identifying and estimating metabolic products of pyridine derivatives (Chaudhuri & Kodicek, 1949; Hughes, 1951).

SUMMARY

1. Washed suspensions and cell-free extracts of *Lactobacillus arabinosus* 17-5 contain an enzyme which hydrolyses nicotinamide to ammonia and nicotinic acid.

2. The enzyme does not attack other naturally occurring nicotinamide derivatives such as diphosphopyridine nucleotide, nicotinamide riboside and nicotinamide ribotide.

3. The pH optimum of the enzyme is broad and between pH 5 and 8. The apparent Michaelis constant is 0.0015 M.

4. The formation of nicotinamide deamidase in *Lb. arabinosus* and *Lb. bulgaricus* is not affected by the composition of the growth medium; the enzyme is constitutive.

5. Seven species of lactobacilli tested formed the enzyme. With the exception of a strain of *Staphylococcus albus*, strains of eleven species of other bacteria did not possess the enzyme.

6. A modified Koenig reaction was used to estimate the quantity of nicotinic acid in the presence of nicotinamide. The use of the method for identifying and estimating pyridine derivatives is briefly discussed.

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