6·5 g. of crystalline material were obtained which was found to be unchanged hydrate of benzoyl-histidine. It had m.p. 245° (decomp.) and $[\alpha]_D = -47·1°$ ($c = 2·1$). The authentic specimen had m.p. 247° (decomp.) and $[\alpha]_D = -47·3°$ ($c = 2·2$).

Decarboxylation of compound B *to* 1:2:5:6-*tetrahydropyrido*-3:4-*iminazole.* Compound *B* (1·5 g.) was heated to 130° until all the water of crystallization had been removed and was mixed with 4·5 g. of melted fluorene. The mixture was then slowly heated to 265° and kept between 265° and 270° for 30 min. The mixture was cooled, extracted twice with 15 ml. 2N-HCl, the acid solution extracted with ether and then made strongly alkaline by addition of 10N-NaOH. The alkaline solution was extracted with amyl alcohol (150 ml.) in three portions, the combined alcoholic extracts dried over Na_2SO_4, filtered and extracted five times with N-HCl. The combined acid extracts were evaporated *in vacuo* and the residue recrystallized twice from 95% aqueous ethanol. It amounted to 35 mg. and had m.p. 276–277°. A specimen mixed with material prepared from histamine (Dale & Dudley, 1921) showed no depression. (Found: N, 21·2. Calc. for $C_6H_{11}N_3Cl_2$: N, 21·47%.) 25 mg. were converted into the picrate, which gave m.p. 212–214° alone or mixed with the dipicrate of the product from histamine.

Preparation of 3:4-*dibenzamido*-N-*benzoyl*-1:2:5:6-*tetrahydropyridine.* 1:2:5:6-Tetrahydropyrido-3:4-iminazole (0·9 g.) prepared from histamine (Dale & Dudley, 1921) was dissolved in N-NaOH (10 ml.) and benzoylated with benzoyl-chloride (4 g.) and 2N-NaOH (16 ml.) in the usual way. The oil which formed during the reaction was extracted with chloroform, the extract washed successively with N-NaOH, water and 2N-HCl, dried and evaporated *in vacuo*. Twice crystallized from 60% aqueous ethanol, the product had m.p. 215°. (Yield, 25%.) (Found: C, 73·2; H, 5·2; N, 10·1. Calc. for $C_{26}H_{23}O_3N_3$: C, 73·4; H, 5·4; N, 9·9%.)

The mother liquor from the benzoylation was acidified and most of the benzoic acid removed by filtration. The solution was then steam-distilled. The distillate was made just alkaline with NaOH and concentrated to small volume. The residue was then acidified with H_2SO_4 and again distilled. The distillate was strongly acid, although apparently free from H_2SO_4, and reduced $AgNO_3$ under acid conditions.

SUMMARY

1. It is shown that histidine reacts with one mol. of formaldehyde to give a tetrahydropyrido-iminazole carboxylic acid. The structure of this acid has been demonstrated by decarboxylation followed by a Bamberger-Berlé fission. In presence of excess formaldehyde a very insoluble methylol derivative of this product is obtained.

2. The dissociation constants of the two compounds have been measured and compared with those of histidine.

3. The kinetics of the reaction have been examined and the bearing of the results on the formaldehyde titration of histidine been discussed.

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The Microbiological Assay of Nicotinic Acid in Cereals and Other Products

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We have had difficulty in the assay of nicotinic acid in cereals and cereal products by the chemical method described by Kodicek (1940), owing to the presence of interfering substances. On the other hand, the microbiological method originally introduced by Snell & Wright (1941), later modified by Krehl, Strong & Elvehjem (1943), and now further modified, has been found to be expeditious and accurate.

The chief drawback to the Snell & Wright method as originally published was the non-linearity of the standard curve with concentrations of nicotinic acid

above 0·15–0·2 μg./10 ml. This difficulty was overcome by Krehl *et al.* who raised the concentration of glucose and buffer (sodium acetate) to 2%. This increase in concentration of glucose and buffer led to an increase in acid production and enhanced the linearity of the standard curve. Further modifications introduced by Krehl *et al.* were to double the concentration of cystine and halve the concentration of biotin (i.e. 0·2 μg. instead of 0·4 μg. biotin/1000 ml. of medium). In the present investigation the modifications that have been found to give the best results in addition to those described by Krehl *et al.* are: (1) to double the concentration of casein hydrolysate (1% instead of 0·5%), (2) to maintain the concentration of biotin at 0·4 μg./1000 ml. medium, (3) to add xanthine and (4) to add 0·1% xylose.

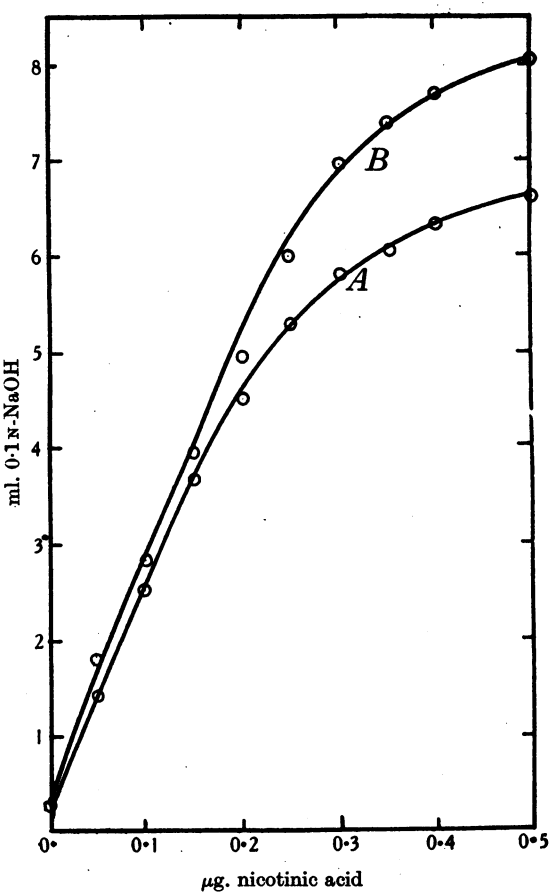

Fig. 1. *A*. Standard curve obtained on original Snell and Wright medium. *B*. Standard curve obtained on modified medium with 2% glucose and 2% sodium acetate.

Fig. 1 shows a standard curve (*A*) obtained in the original Snell & Wright medium and a standard curve (*B*) obtained on the modified medium described here.

A difficulty encountered with the method in the past has been the incidence of high blanks, due to three main causes: (i) it was difficult to rid the casein hydrolysate of the last traces of nicotinic acid; (ii) natural *l*-tryptophan, unless carefully purified, is liable to be heavily contaminated with nicotinic acid. One sample of natural *l*-tryptophan was found to contain as high a concentration as 20 μg./g. of nicotinic acid, which will vitiate any microbiological assay; (iii) the crude concentrates of biotin which were at one time the only available sources of this vitamin were frequently contaminated with nicotinic acid. This last difficulty no longer exists, as pure crystalline biotin is now available.

EXPERIMENTAL

Organism. The organism employed was *Lactobacillus arabinosus* 17/5 from Snell & Wright's original strain obtained from the American Type Culture Collection, Georgetown University, Washington, D.C. Stock cultures of the organism were carried on a yeast-water-glucose agar to which had been added 0·6% of sodium acetate. The cultures were preserved in a refrigerator at a temperature of 4° approx. and renewed at fortnightly intervals.

Composition of basal medium. This is given in Table 1.

Table 1. *Composition of basal medium*

Component	Weight/ml. of basal medium
Acid-hydrolyzed casein	10 mg.
dl-Tryptophan	0·1 mg.
l-Cystine	0·2 mg.
Glucose	20 mg.
Sodium acetate	20 mg.
Xylose	1 mg.
Calcium pantothenate	0·1 μg.
Pyridoxin	0·1 μg.
Riboflavin	0·2 μg.
p-Aminobenzoic acid	0·1 μg.
Biotin	0·0004 μg.
Adenine	0·01 mg.
Guanine	0·01 mg.
Uracil	0·01 mg.
Xanthine	0·01 mg.
Sodium chloride	5 mg.
Inorganic salts—solution A	See text
Inorganic salts—solution B	

Preparation of stock solutions

The stock solutions described below are preserved in the presence of a thin layer of toluene and stored in a refrigerator. All stock solutions and extracts are prepared with glass-distilled water. Possible contamination of any of the constituents of the medium with nicotinic acid was always tested for by using *Proteus vulgaris* in Stephenson's (1938) medium of known composition with sodium lactate as carbon source.

Acid-hydrolyzed casein. The various 'vitamin-free' caseins on the market all contain sufficient nicotinic acid to give high blanks with *L. arabinosus*. The following method of preparation of the hydrolysate consistently gave low blanks. 100 g. of a 'vitamin-free' casein are shaken twice for 20 min. with 300 ml. of 85% ethanol and filtered. The casein is

then added gradually to distilled water (1 l.) at 50° with constant stirring. The mixture is stirred for 30 min. and a 30% NaOH solution is slowly added, still with constant stirring, until the casein passes into solution. The viscous mass is stirred for 20 min., the mixture then adjusted to pH 4·6 with 10% HCl, the precipitate allowed to settle, and the whole then filtered. This operation is repeated three or four times. The alkali-extracted casein is then well washed with water at 50° and hydrolyzed with 500 ml. of 25% w/v H_2SO_4 for 10 hr. at 15 lb. pressure in an autoclave, or refluxed for 16–20 hr. on a sand-bath. 200 g. of litharge are added to the hydrolysate and the precipitate of lead sulphate filtered off. Barium hydroxide is now added to neutralize the remaining sulphuric acid and the barium sulphate removed by filtration. The hydrolysate is adjusted to pH 3, the volume made up to 700 ml., shaken for 30 min. with an active charcoal, e.g. Norite A, and filtered. The pH is adjusted to 6·8 with barium hydroxide. The resulting hydrolysate should be almost colourless. The solution is adjusted by evaporation or dilution to contain 100 mg./ml. of dry matter and is preserved under toluene in a refrigerator. It is usual after a few days for tyrosine to precipitate; this can be disregarded.

dl-*Tryptophan.* Natural *l*-tryptophan was so variable in its behaviour, owing to contamination with nicotinic acid, that its use was abandoned and the synthetic product prepared by Glaxo Laboratories, Ltd. was always used. *dl*-Tryptophan (2·0 g.) is suspended in approximately 500 ml. of hot water and concentrated HCl added drop by drop until solution is effected. The solution is then made up to a final volume of 1000 ml.

l-*Cystine.* *l*-Cystine (4·0 g.) is suspended in 500 ml. of hot water, 5 ml. of concentrated HCl added, and the solution made up to a final volume of 1000 ml.

Glucose, sodium acetate, xylose and *sodium chloride* are weighed out as required. Hydrated sodium acetate was used in preference to the anhydrous salt (1·66 g. of $CH_3.COONa.3H_2O \equiv 1$ g. CH_3COONa).

Biotin. An ampoule containing 25 μg. of the free acid is made up to 250 ml. with water. 1 ml. of inorganic salt solution A (see below) is also added before the solution is made up to volume. The solution thus contains 0·1 μg./ml. of biotin. *L. arabinosus* is insensitive to methyl biotin. If the methyl ester only be available it must be hydrolyzed to the free acid. The contents of an ampoule containing 25 μg. of the ester is added to 0·50 ml. of 0·1 N-HCl and the whole hydrolyzed for 1 hr. at 15 lb. pressure. 1 ml. of salt solution A is added and the pH adjusted to 6·8 with NaOH solution and the volume made up to 236 ml.

p-*Aminobenzoic acid.* *p*-Aminobenzoic acid (0·1 g.) is dissolved in 100 ml. of water containing two or three drops of glacial acetic acid. Before use this solution is diluted to one in ten so that each ml. contains 100 μg. The addition of *p*-aminobenzoic acid to the medium was first recommended by Isbell (1942), as this substance is removed from the casein hydrolysate by active charcoal. It has since been found (cf. Shankman, 1943) that there are two different types of strain of *L. arabinosus*, one of which requires *p*-aminobenzoic acid, while the other does not. In this investigation *p*-aminobenzoic acid was found to have a slight stimulating effect and was always added. It is recommended that the concentration of the acid in the final medium be not increased beyond that given here (0·1 μg./ml.); higher amounts, e.g. 1 μg./ml., have a definite depressing effect at the higher levels of nicotinic acid used for the standard curve.

Aneurin, calcium pantothenate and pyridoxin. A stock solution of these substances is prepared by dissolving 0·1 g. of each in 100 ml. of water. The stock solution is diluted one in ten before use.

Standard nicotinic acid. Pure nicotinic acid (0·1 g.) is dissolved in 100 ml. of water. This solution is diluted to give a standard solution containing 0·1 μg./ml.

Specificity. Nicotinic acid is a specific growth factor for *L. arabinosus*. Runs were set up with picolinic acid (pyridine-2-carboxylic acid), isonicotinic acid (pyridine-4-carboxylic acid) and quinolinic acid (pyridine-2:3-dicarboxylic acid) and no growth was obtained even when the concentration of these substances was raised to 1 μg./10 ml. of medium.

Riboflavin. A solution of riboflavin is prepared to contain 100 μg./ml. in 0·02 N-acetic acid.

Adenine, guanine and uracil. The stock solution of these three substances contains 1 mg./ml. Solution is effected by prolonged heating in the presence of a few drops of concentrated hydrochloric acid.

Xanthine. This solution also contains 1 mg./ml. Solution is effected by the addition of a few drops of strong ammonia.

The various stock solutions of vitamins, with the exception of biotin, are renewed at weekly intervals and the adenine, guanine, uracil and xanthine solutions are renewed at fortnightly intervals.

Inorganic salts, solution A. This is composed of 25 g. of K_2HPO_4 and 25 g. of KH_2PO_4 in 250 ml. of water.

Inorganic salt, solution B. This solution was modified to contain 10 g. $MgSO_4.7H_2O$, 0·5 g. $MnSO_4.4H_2O$ and 0·03 g. $FeCl_3$ in 250 ml. of water. Five drops of concentrated hydrochloric acid are added to the mixture.

Preparation of inoculum

A heavy growth of inoculum is required for a successful assay by this method. The best results were obtained when a transfer was made from the agar stab culture to a tube of the Snell & Strong riboflavin medium (see Barton-Wright & Booth, 1943). This is incubated for 18–20 hr. at 37°, centrifuged aseptically and resuspended in twice the volume of 0·9% saline solution. Particularly heavy growth is obtained in this medium.

The fermentations are carried out in ordinary chemical or bacteriological tubes (18 × 150 mm.) and the acid produced is titrated against 0·1 N-NaOH, with bromothymol blue as indicator. The titrations are carried out as described by Barton-Wright & Booth (1943).

Preparation of extracts

Dry materials are finely ground and 5 g. amounts are suspended in 50 ml. of N-HCl and autoclaved for 15 min. at 15 lb. pressure. Although *L. arabinosus*, unlike *L. helveticus*, is not stimulated by the presence of fats, materials which contain a high proportion of fat, e.g. wheat germ, maize, oats, soybean, meat, etc., should nevertheless be defatted by a preliminary Soxhlet extraction with light petroleum for 10 hr. This procedure prevents the formation of oily emulsions which may hinder complete extraction. After cooling, the extract is adjusted to pH 6·8 with 30% NaOH and the volume is made up to 100 ml. The concentration of the final solution of the extract should contain as nearly as possible 0·05 μg./ml. of nicotinic acid. This is effected by pipetting the necessary volume of sample from the initial 100 ml. of solution into distilled water and making up the volume to 500 ml. This solution is preserved under toluene.

Assay procedure

The following amounts of the stock solutions will give sufficient medium for 100 tubes:

Acid-hydrolyzed casein	100 ml.
l-Cystine solution	50 ml.
dl-Tryptophan solution	50 ml.
Anhydrous glucose	20 g.
Anhydrous sodium acetate	20 g.
Xylose	1·0 g.
Sodium chloride	5·0 g.
Biotin solution	4·0 ml.
p-Aminobenzoic acid solution	1·0 ml.
Aneurin, calcium pantothenate and pyridoxine solution	1·0 ml.
Riboflavin solution	2·0 ml.
Adenine + guanine + uracil solution	10·0 ml.
Xanthine solution	10·0 ml.
Inorganic salt solution A	5·0 ml.
Inorganic salt solution B	5·0 ml.

After mixing, the medium is adjusted to pH 6·8 with sodium hydroxide and the volume made up to 500 ml. with water. This gives a mixture having twice the concentration of the final assay medium. 5 ml. of the medium are transferred to each tube. Sixteen tubes are kept for the blanks and the determination of the standard nicotinic acid curve. To the series of tubes are added serially 5 ml. of water (=blank), 0·5, 1·0, 1·5, 2·0, 2·5, 3·0, 3·5 ml. of standard nicotinic acid solution (0·1 μg./ml.). In each case sufficient distilled water is added to bring the final volume of each tube to 10 ml. The remaining tubes are used for the assay of the extracts.

It is necessary to carry out each assay at four different levels. It will be found convenient to take 1·0, 2·0, 3·0, 4·0 ml. of each extract for an assay. All concentration levels of standard nicotinic acid solution and extracts are set up in duplicate. After the volume of the tubes has been adjusted to 10 ml. they are plugged with cotton-wool and autoclaved for 10 min. at 10 lb. pressure, cooled and inoculated with one drop of inoculum per tube from a sterile pipette. The tubes are incubated for 72 hr. at 37° and then titrated. Good results can be obtained after 48 hr. incubation, but it was found to be more convenient in routine work to maintain a standard incubation time of 72 hr.

The standard curve (Fig. 1) is plotted in the usual way and the nicotinic acid content of each concentration level of an extract determined from it. The mean of at least three readings, not differing among themselves by more than ±10%, should be taken.

ASSAY RESULTS

A wide range of materials was assayed for nicotinic acid, including different varieties of wheat, various fractions of the wheat grain, wheaten flours, other cereals, e.g. maize, rye, oats and barley, as well as yeast, dried meat, ordinary and dried milk, beer, vinegar, coffee, cocoa and tea. The results of a number of different determinations on various cereals and preparations therefrom are given in Tables 2–7; the results are calculated on the normal water contents of the products.

A survey of the nicotinic acid content of the milling fractions of five different Manitoba wheats specially milled by my colleague Mr A. G. Simpson for this work gave the results shown in Table 4.

Table 2. *Nicotinic acid content of wheat and wheat grain fractions*

	Sample	Nicotinic acid content (μg./g.)
1	Wheat, English	48·0
2	Wheat, Manitoba	55·0–66·0
3	Wheat, germ, commercial sample 1	55·0
4	Wheat, germ, commercial sample 2	75·0
5	Wheat, germ, commercial sample 3	77·0
6	Wheat, bran	267–325
7	Flour, National (85% extraction)	12·0–18·7

Table 3. *Nicotinic acid content of wheat flour fractions*

	Sample English wheat (%)	Nicotinic acid content (μg./g.)
Extraction	45	5·0
	70	7·5
	75	8·0
	80	9·0
	85	10·5
	100	48·0
	Manitoba wheat (%)	
Extraction	40	7·0
	70	8·4
	75	9·6
	80	11·0
	85	13·3
	100	55·0

A number of beers and vinegars was also assayed for nicotinic acid content. The results are shown in Table 8. It is of interest to compare the nicotinic acid content of modern beers, samples 1–7, with those brewed 45, 70 and in one case 150 years ago. The specimen called 'Chancellor' is abnormally high in nicotinic acid. The same is true of its riboflavin content (3·9 μg./g.).

The opportunity was taken to assay the nicotinic acid of various hopped and unhopped worts and to compare the values with the gravities of the liquors (Table 9).

It will be seen from Table 9 that there is a direct correlation between the nicotinic acid content and the gravity of the liquors. The values for miscellaneous products assayed for nicotinic acid are given in Table 10.

DISCUSSION

Lactobacillus arabinosus is less exacting than *L. helveticus* (*L. casei* ϵ) in its vitamin requirements. Only three growth-factors need be given for normal growth, namely nicotinic acid, pantothenic acid and biotin, whereas *L. helveticus* requires in addition riboflavin, pyridoxin (or 'pseudopyridoxin') and the at present ill-defined factor or factors contained in 'folic acid' concentrates, which are to be found

Table 4. *Nicotinic acid content of different milling fractions of Manitoba wheat*

Sample	%	Nicotinic acid content (μg./g.)	Product
A. No. 1 Northern Manitoba export standard, 1943 crop			
Grist	100	62·5	6250
Flour	74·5	13·5	1006
Middlings*	2·9	100·0	290
Fine bran†	10·0	120·0	1200
Coarse bran‡	12·6	298·0	3755
	Total		6251
	Difference		+1
B. No. 2 Northern Manitoba export standard, 1943 crop			
Grist	100	62·5	6250
Flour	75·1	12·8	961
Middlings*	2·4	105·0	252
Fine bran†	10·0	142·0	1420
Coarse bran‡	12·5	281·0	3510
	Total		6143
	Difference		−107
C. No. 3 Northern Manitoba export standard, 1943 crop			
Grist	100	62·8	6280
Flour	73·2	13·6	995
Middlings*	3·5	97·5	340
Fine bran†	11·4	150·0	1710
Coarse bran‡	12·0	267·0	3210
	Total		6255
	Difference		−25
D. No. 4 Northern Manitoba export standard, 1943 crop			
Grist	100	62·5	6650
Flour	72·2	11·5	834
Middlings*	3·8	81·0	308
Fine bran†	11·8	145·0	1710
Coarse bran‡	12·2	325·0	3910
	Total		6762
	Difference		+112
E. No. 5 Wheat (Manitoba) export standard, 1943 crop			
Grist	100	66·0	6600
Flour	68·7	14·0	962
Middlings*	5·7	75·0	427
Fine bran†	13·3	131·0	1740
Coarse bran‡	12·35	285·0	3520
	Total		6649
	Difference		+49

* 'Middlings' contains all the remaining residual endosperm and also some germ.

† 'Fine bran' contains a little more endosperm and a fair proportion of the germ of the wheat.

‡ 'Coarse bran' is defined as the outer coat of the grain practically free from endosperm.

in peptone, liver, kidney and yeast. In the present investigation several runs were made in which aneurin, riboflavin and pyridoxin were omitted from the medium. In agreement with previous work (cf. Bohonos, Hutchings & Peterson, 1941; Snell & Wright, 1941; and Krehl *et al.* 1943), perfectly satisfactory results were obtained. It is therefore possible that these factors are unnecessary. Nevertheless, it was considered undesirable, in all the circumstances, to omit these substances at this stage of the investigation.

Table 5. *Nicotinic acid content of barleys and malts*

Sample	Nicotinic acid content (μg./g.)
1. Barley. Unnamed commercial variety	91·5
2. Barley. Block	85·0
3. Barley. Irish Archer	147·0
4. Barley. Plumage	124·0
5. Barley. Arch Plume	120·0
6. Barley. Spratt	131·0
7. Barley. Archer Goldthorpe	120·0
8. Barley. Spratt Archer	120·0
9. Barley. Irish Goldthorpe	120·0
10. Commercial malt. Sample 1	100·0
11. Commercial malt. Sample 2	108·0
12. Commercial malt. Sample 3	108·0
13. Commercial malt. Sample 4	91·0

(Samples 3–9 were unhusked grains.)

Table 6. *Nicotinic acid content of starchy and sweet maizes*

Sample	Nicotinic acid content (μg./g.)
A. Starchy maize	
1. Manalta	11·5
2. Beacon	18·0
3. Howes Alberta	11·5
4. Jehu	20·0
5. B 45	18·8
6. Quebec 28	15·0
7. Mixed seed variety trial	14·2
Mean	15·6
B. Sweet maize	
8. John Innes hybrid no. 1	28·0
9. John Innes hybrid no. 2	33·0
10. Canada Gold	37·0
11. C 13	26·0
Mean	31·3

Table 7. *Nicotinic acid content of rye and oat varieties*

Sample	Nicotinic acid content (μg./g.)
Oats, Victory	6·4
Oats, Monarch	6·5
Oats, Star Seed	6·3
Oats, Royal Scot	6·5
Oats, Golden Rain	5·5
Oats, Ayr Commando	4·9
Oats, Ayr Bounty	6·8
Oats, Bell	7·0
Rye, Carter's Giant Winter	10·0
Rye, unnamed variety	9·3
Rye, unnamed variety	7·7

According to Brown, Thomas & Bina (1943), oxidation with hydrogen peroxide destroys approximately 50 % of the nicotinic acid content of wheat.

Table 8. *Nicotinic acid content of beers and vinegars*

Sample	Nicotinic acid content (μg./g.)
1. Modern commercial samples	7·8
2. Modern commercial samples	8·2
3. Modern commercial samples	9·5
4. Modern commercial samples	12·0
5. Modern commercial samples	12·5
6. Modern commercial samples	14·4
7. Modern commercial samples	17·0
8. 'Chancellor'. (All Souls College, Oxford, brewed 1938)	45·6
9. Worthington Strong Ale (brewed 1798)	16·5
10. Anglo-Bavarian Beer (brewed 1872)	10·3
11. Reid's Stout (brewed 1899)	16·0
12. Combes Export Brown Stout (brewed 1900)	15·3
13. Stock Vinegar	9·5
14. Tarragon Vinegar	9·0

Extraction of cereal samples with water alone will not remove the whole of the nicotinic acid content of the grain. There is also present in cereals a nicotinic acid precursor (cf. Melnick, 1942), which is converted to nicotinic acid in the presence of alkali in the cold, but with acid only on heating. It is probable that Brown *et al.* failed to extract the whole of the nicotinic acid from their samples, since they used water alone for this purpose.

The low nicotinic acid content of rye and oats is surprising. It might have been expected that the values would have been of the same order as that of wheat and barley. In the case of maize the nicotinic acid content of starchy maize is approximately 50 % of that of sweet varieties. It has been known for some time that the nicotinic acid content of maize is low and maize is said to be a component of a pellagra-producing diet (maize, salt meat and molasses). Nevertheless, compared with rye and oats the nicotinic acid content of maize is relatively high and it would be of interest to know whether the incidence of pellagra in rye-consuming countries, e.g. Italy and the Balkans, is connected with the low nicotinic acid content of this cereal.

Table 9. *Nicotinic acid content and gravity of hopped and unhopped worts*

Sample	Nicotinic acid content (μg./ml.)	Gravity
1. *Unhopped wort.* Samples drawn at intervals and mixed to represent liquors drained off from the mash tun at various stages	19·5	1096·58
2.	15·1	1071·46
3.	6·5	1014·50
4. Mixture of above representing the whole extract used in the brew	10·5	1045·71
5. *Hopped wort.* Mixture of different strengths of wort samples above but *after* boiling with hops and before adding yeast	11·0	1045·85
6. Beer	8·2	1034·0

Table 10. *Miscellaneous products*

Sample	Nicotinic acid content (μg./g.)
1. Dehydrated meat	100–110
2. Yeast extract 1 (*Torula utilis*)	2077
3. Yeast extract 2	655
4. Dried brewer's yeast	600
5. Yeast extract 4	275–288
6. Coffee	132
7. Cocoa	16
8. Tea	61
9. Milk powder	9·2
10. Milk	0·8–1·0 (μg./ml.)

Krehl *et al.* (1943) were unable to confirm this statement. A repetition of this work in the present investigation also failed to confirm this suggestion.

SUMMARY

1. A number of modifications and improvements have been made in the original Snell & Wright medium for the microbiological assay of nicotinic acid, using *Lactobacillus arabinosus* 17/5.

2. The nicotinic acid content of cereals, cereal products and other foods has been assayed.

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