88. ESTIMATION OF NICOTINIC ACID IN ANIMAL TISSUES, BLOOD AND CERTAIN FOODSTUFFS

I. METHOD[1]

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SINCE the importance of nicotinic acid as the blacktongue- and pellagra-preventing vitamin and as an essential part of coenzyme systems—the phosphopyridine nucleotides—has been established, a reliable method for its estimation in various substances is needed; this should help to deepen the understanding of its physiological and biochemical role in the living organism.

Methods for the estimation of nicotinic acid in tissues and foodstuffs

The following reagents have been proposed for the estimation of nicotinic acid in animal tissues, blood or foodstuffs:

(1) 2:4-Dinitrochlorobenzene was used by Karrer & Keller [1938, 1, 2; 1939] and by Vilter *et al.* [1938]; it gives a colour reaction with pyridine derivatives [Vongerichten, 1899; Zincke, 1904]. This method is much less sensitive than the following and much more laborious.

(2) Aniline was used by Swaminathan [1938, 1, 2], Kringstad & Naess [1938; 1939], Pearson [1939], Ritsert [1939]. It produces a colour reaction of the type described by König [1904] and by Strafford & Parry Jones [1933], i.e. pyridine derivatives react with cyanogen bromide plus an aromatic amine to yield a coloured substance.

(3) A similar procedure was followed by Bandier & Hald [1939] and by Bandier [1939], who however used metol instead of aniline.

(4) v. Euler *et al.* [1938] described a modification of Barta's [1935] method in which naphthylamine is the reagent employed.

(5) In blood, nicotinic acid has also been estimated quantitatively by its growth-promoting action on various micro-organisms [e.g. Querido *et al.* 1939].

Use of p-*aminoacetophenone and comparison with metol and aniline*

In the work described in this paper *p*-aminoacetophenone, introduced by Harris & Raymond [1939, 1, 2], was used as the aromatic amine for the König reaction. This method seems to be more satisfactory than any of the others previously described. Some of the advantages of this substance have already been stated by Harris & Raymond [1939, 2].

The amount of colour developed in the cyanogen-amine reaction depends on many variable factors, which are not absolutely controllable, hence the use of a standard reference curve cannot be recommended. This difficulty was successfully circumvented by the procedure described by Harris & Raymond (see below).

Aniline and metol give less colour, as will be seen in Fig. 1. With increasing amounts of the aromatic amine the colour increases to a certain maximum.

[1] Communicated to the Biochemical Society, 9 December 1939 [Kodicek, 1939].

p-Aminoacetophenone developed an intense colour even with 10 μg. nicotinic acid in 15 ml., and when as little as 0·2 ml. of amine solution (10 %) was used. The same intensity was reached as a maximum only with as much as 5 ml. of aniline (2 %), and the maximum intensity of colour with 10 ml. of *ca.* 5 % metol was even lower. Also because of their relative insolubilities a larger volume of the reagents has to be taken when aniline or metol are employed for work with animal extracts, and this unavoidable dilution further diminishes the sensitivity. It was quite obvious therefore that *p*-aminoacetophenone was more suitable for our purpose than other amines, many of which had already been examined by Harris & Raymond.

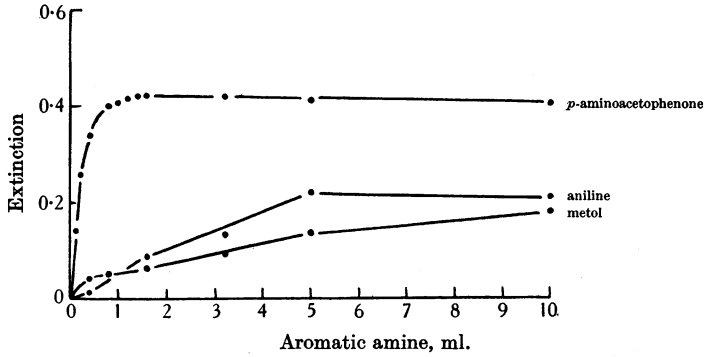

Fig. 1. Comparison of colour intensity of different aromatic amines (10 μg. of nicotinic acid used).

In the work described in this paper the various factors influencing the reaction have been checked step by step, so as to eliminate possible errors as far as possible, and quantitative studies have been made of the conditions affecting the accuracy of the results.

Principle of the method used

The material is first extracted or hydrolysed as it stands with 8 % NaOH or H_2SO_4 for 1 hr. By this treatment derivatives of nicotinic acid such as amides, pyridine nucleotides and possibly others, are converted into free nicotinic acid. Other substances are next precipitated by the addition of an excess of 96 % ethyl alcohol, leaving the nicotinic acid in solution. The reaction is cautiously brought to *p*H 6 with conc. HCl, with the use of an external indicator and with $NaHCO_3$ as a buffer. The solution is made up to a fixed volume. Three samples are taken to circumvent the use of a standard reference curve, one for the unknown, and two for the unknown to which have been added known amounts of nicotinic acid; there is also a blank. These are heated with cyanogen bromide and later treated with acidified *p*-aminoacetophenone. From the 3 samples 3 colour readings are taken in the Pulfrich photometer, and as the reaction has been shown to obey Beer's law the amount of nicotinic acid in the unknown can be calculated, all 3 results lying on a straight line [see Harris & Raymond, 1939, 2; Kodicek, 1939].

Quantitative study of factors influencing the reaction

(*a*) *Methods of extraction.* For animal tissues, three methods of extraction, called *a*, *b* and *c* in Table 1, can be used, all of which give, so far as has been found, the same final result for the total amount of nicotinic acid present. Extraction

Table 1. *Alternative methods of extraction*

Extraction method ...	Nicotinic acid found, μg. per g. fresh weight				
	a	*b*	*c*	*d*	*e*
Liver (ox)	186	187	182	—	90
Muscle (ox)	40	40	40	—	11
Goldberger diet*	17	26	6	20	—
Yellow maize meal (sample D)	41	45	8	37	—
,, (sample C)	12	27	6	—	—
,, (sample B)	18	33	—	—	—

Extraction *a*. Minced tissue suspended in water and hydrolysed with NaOH or H_2SO_4.

Extraction *b*. Minced tissue suspended in EtOH and hydrolysed with NaOH or H_2SO_4.

Extraction *c*. Extracted with water at 100°; extract subsequently hydrolysed with NaOH.

Extraction *d*. Residuum after water extraction *c* hydrolysed with NaOH and estimated for nicotinic acid.

Extraction *e*. Extracted with 96% EtOH and extract subsequently hydrolysed with NaOH.

* Goldberger diet; yellow maize meal 4000, pea meal 500, extracted casein A/E (Glaxo) 600, $CaCO_3$ 30, NaCl 100, cottonseed oil 300.

during hydrolysis with NaOH has been the method actually used in this and the following paper. For animal tissues extraction by boiling water is equally effective, indicating that all the nicotinic acid present is soluble in water, or rapidly liberated by boiling. For cereals the question of extraction is more complicated. It will be seen from Table 1 that extraction of cereals with water gave distinctly lower results than the other methods. It is not yet certain which type of extraction gives the real content of biologically active nicotinic acid. Some varieties of yellow maize meal, as in Table 1, apparently contained very high concentrations of nicotinic acid. However, L. J. Harris and I (unpublished) found that these samples of maize were as inactive in blacktongue experiments as white maize which gave a lower colour value in the chemical test. It seems probable therefore that the watery extract (method *c*) contains the "true" or "active" nicotinic acid, the concentration of which would thus be very low. It would in this respect behave like the active nicotinic acid in animal tissues which is readily extractable by water. Thus the total concentration of substances derived from cereals which give the cyanogen-amine reaction when estimated by direct hydrolysis either in water (method *a*) or in alcohol (method *b*), does not presumably represent the "true" or "active" nicotinic acid. This point has to be left open for investigation in further work which is now in progress; it will be mentioned again in the following paper [Kodicek, 1940].

Hydrolysis in ethyl alcohol (method *b*, Table 1) was of some help on occasions when it was preferable to use water as little as possible in order to obtain clear, non-turbid solutions. The fourth method of extraction (*e*)—precipitation with ethyl alcohol before hydrolysis—yielded lower results, which are not due to imperfect extraction, but which make it possible to distinguish an "alcohol-soluble fraction". Whether this fraction can be explained as a partial precipitation of certain nicotinic acid derivatives or as a selective precipitation of some special fraction has not yet been determined.

(*b*) *Time necessary for hydrolysis with 8% NaOH.* As shown in Table 2, unhydrolysed nicotinamide gives only 20% of the colour of the equivalent amount of nicotinic acid. But after hydrolysis for only 5 min. it reaches the maximum colour, being converted into nicotinic acid. The same is true of the nicotinic acid derivatives in liver and muscle; the coenzymes are very easily

Table 2. *Time necessary for hydrolysis with 8% NaOH*

	Nicotinic acid, μg./g. fresh weight				
		Hydrolysed			
	Unhydrolysed	5 min.	30 min.	90 min.	180 min.
Liver, ox (sample B)	0	113	—	97	—
,, (sample B)	20	—	160	120	150
Muscle, beef	10	—	40	40	40
Nicotinamide: % recovery	20	104	—	104	93
Nicotinamide boiled in water for 15 min.: % recovery	38	—	—	—	—

hydrolysed. The "unhydrolysed alcoholic fraction" of liver and muscle was obtained by precipitating the watery extract with excess of ethyl alcohol (see Table 2, and Table 8 in the following paper), and the solution was estimated without preceding hydrolysis. It is assumed that by this procedure the free nicotinic acid and one-fifth of the free amide are estimated.

(*c*) *Concentration of NaOH.* Results obtained after hydrolysis for 1 hr. are summarized in Table 3 For animal tissues 4% NaOH was effective; nevertheless, for the work described in this paper a final concentration of 8% NaOH has been used. As nicotinic acid is very stable, even to prolonged treatment with alkali, there is no objection to the use of the more concentrated solution of NaOH. The recovery of cozymase was calculated from the nicotinic acid found after hydrolysis compared with figures obtained from the direct spectrographic estimation of the cozymase itself.[1]

Table 3. *Hydrolysis with NaOH for 1 hr.*

	Nicotinic acid, μg./g. fresh weight Final concentration of NaOH	
	4%	8%
Muscle, beef	40	42
Cod	30	28
Salmon	95	82
Whole wheat	26	30
Whole flour	13	15
Cozymase (Green): % recovery	82	89 impure (11% purity)

Table 4. *Hydrolysis with H_2SO_4 for 1 hr.*

	Nicotinic acid, μg./g. fresh weight		
	Final concentration of H_2SO_4		Hydrolysis with 8% NaOH
	4%	8%	
Muscle, beef	10	40	40
Whole meal	26	42	30
White flour	—	15	15

(*d*) *Concentration of H_2SO_4 for hydrolysis.* Table 4 gives the results obtained in hydrolyses with different concentrations of H_2SO_4. A final concentration of 4% gives low results. A concentration of 8%, however, gives similar results to

[1] I am indebted to Sir Charles Martin for placing at my disposal a preparation of the cozymase which he had received from Prof. v. Euler, and to Dr D. E. Green for other specimens and also for the spectrographic estimation.

those obtained by hydrolysis with 8% NaOH. As trigonelline, according to Karrer [1938], may be converted by hydrolysis with acid into nicotinic acid, it seemed to be preferable, for the sake of specificity, to hydrolyse with NaOH.

(*e*) *Precipitation of other substances.* Trichloroacetic acid, 20%, was tried for the preparation of an extract (see Table 5). Nicotinic acid in water, or added to

Table 5. *Precipitation with 20% trichloroacetic acid*

	Total nicotinic acid found	Recovery of added nicotinic acid	
	μg.	μg.	%
10 ml. serum + 5 ml. trichloroacetic acid	0·0	—	—
+ 20 μg. nicotinic acid	20·0	20·0	100
+ 150 μg. nicotinic acid	146·0	146·0	97
25 ml. urine	148·0	—	—
+ 5 ml. trichloroacetic acid + 30 μg. nicotinic acid	180·0	32·0	107
+ 100 μg. nicotinic acid	243·0	95·0	95
20 μg. nicotinic acid in 4% trichloroacetic acid	20·0	20·0	100

serum and urine, could be recovered quantitatively. Serum itself was found to contain no nicotinic acid. In one experiment 10 ml. of serum (horse) plus added nicotinic acid were treated with 5 ml. of 20% trichloroacetic acid; 10 ml. distilled water were added, the product was centrifuged and the supernatant liquid then hydrolysed. The colour faded slowly however and was less intense (cf. also Table 5*a*); trichloroacetic acid used after hydrolysis was also unsatisfactory.

Table 5*a*. *Stability of colour in trichloroacetic acid*

	Time min.	Extinction
20 μg. nicotinic acid in 4% trichloroacetic acid	0	0·33
	10	0·29
	20	0·28
	40	0·27
	60	0·26
	150	0·16

On the other hand, precipitation with excess of 96% ethyl alcohol (9 parts), acetone or amyl alcohol gave better results. With ethyl alcohol, nicotinic acid could be recovered quantitatively either in pure solution (e.g. Table 6) or added

Table 6. *Precipitation with 96% ethyl alcohol*

	Amount taken	Nicotinic acid found, μg.	
	μg.	In water	In ethyl alcohol
Nicotinic acid	100	99 (±2·0)	103 (±1·3)
Nicotinamide	100	102 (±3·2)	105 (±3·5)
Urine (dog)	—	1·2 μg. per ml.	1·2 μg. per ml.
Urine (human)	—	1·6 μg. per ml.	1·8 μg. per ml.

Figures in brackets give the standard error of the mean.

to various tissue extracts (Table 10). Clear urine yielded the same results whether ethyl alcohol was used or not. The intensity and stability of colour were the same as in water. Thus with ethyl alcohol no interfering substances are introduced and the other difficulties alluded to above are obviated. The final con-

centration of alcohol must be above 80 %. Below this some tissue extracts often get turbid. The following results were noted with extracts from liver tissues.

Concentration of ethyl alcohol %	Result
78	Solution turbid
81	Solution clear
84	Solution clear

(*f*) *Influence of* p*H during treatment with CNBr.* As Harris & Raymond [1939, 2] have explained, the concentration of H ions is most important; this was found also by other authors who used aniline. If the *p*H, however, is adjusted with or without buffer within the range of 5·5–7·5, a reproducible, non-varying colour of maximum intensity may be obtained (Fig. 2).

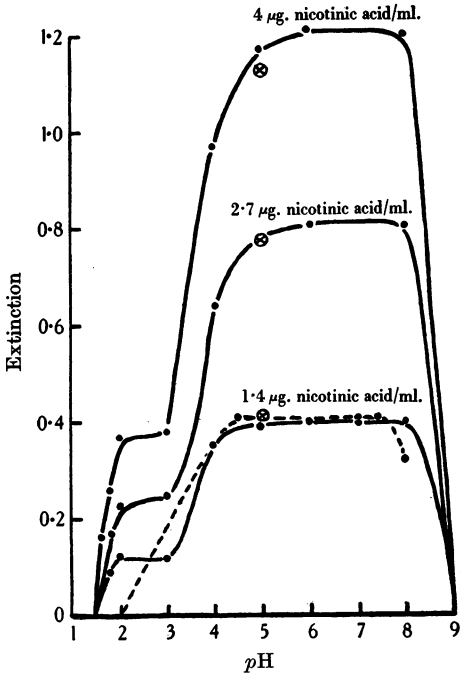

Fig. 2.

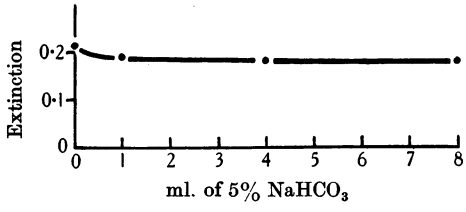

Fig. 3.

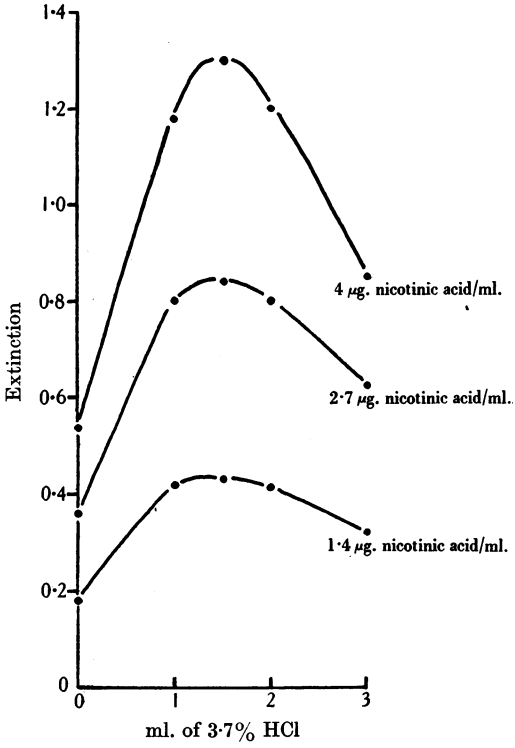

Fig. 4.

Fig. 2. Influence of *p*H. 3 cm. cells in step-photometer; —— *p*H adjusted with HCl; - - - - *p*H adjusted with phosphate buffer, 0·1 *M*; ⊗ *p*H adjusted with acetate buffer, 0·1 *M* at *p*H 5.

Fig. 3. Influence of salts. Nicotinic acid 1 μg. per ml. Increasing amount of 5% $NaHCO_3$.

Fig. 4. Influence of acidification after addition of 0·2 ml. of *p*-aminoacetophenone.

(*g*) *Influence of salts.* The influence of salts is less marked. $NaHCO_3$ in increasing concentration causes a very slight fading (Fig. 3). Acetate buffer (Fig. 2) 0·1*M* at *p*H 5 did not interfere with the development of the colour, as was found by v. Euler *et al.* [1938] to be the case when other aromatic amines were used. Glacial acetic acid in high concentration, however, when used for preservation of urine, prevented the development of any colour at all.

(*h*) *Use of cyanogen bromide.* (1) *Preparation of CNBr.* It was found to be of importance to prepare the substance in ice-cold solutions [Kulikow & Krestowosdwigenskaja, 1930]. The reproducibility of the results was thereby increased.

(2) The heating to 70–80° is necessary for the reaction. Without heating no colour developed. If CNBr were added together with *p*-aminoacetophenone and the mixture then heated, no colour was produced.

(3) *Different amounts of CNBr.* There was no change in the intensity of the colour when CNBr was used in amounts varying from 0·5 ml. to 4 ml. in 15 ml. of solution (e.g. Table 7).

Table 7. *Different amounts of CNBr added to 10 μg. nicotinic acid and 0·2 ml. 10% p-aminoacetophenone*

Amount of CNBr, ml.	Extinction	Amount of CNBr, ml.	Extinction
0·0	0·00	2·0	0·20
0·5	0·20	4·0	0·20
1·0	0·20	8·0	0·18

(*i*) *Development of colour. Effect of acidification on colour after adding* p-*aminoacetophenone.* Acidification upon the addition of the *p*-aminoacetophenone is another important factor (Fig. 4). The optimum amount under the conditions described for Fig. 4 was found to be 1·2 ml. of 3·7% HCl. The *p*-aminoacetophenone was prepared as follows: to 5 g. *p*-aminoacetophenone 14 ml. of 10% HCl were added, and the volume was made up to 50 ml. with water. Later, however, it was found more convenient to add the *p*-aminoacetophenone and the optimum amount of HCl together in the same solution, and the procedure then adopted was to use 0·4 ml. of an acidified solution of *p*-aminoacetophenone. The mixed solution of *p*-aminoacetophenone and HCl was prepared as follows: to 5 g. *p*-aminoacetophenone 30 ml. of 32% HCl were added and the volume was made up to 100 ml. with water. There was no difference in the intensity of the colour whether the HCl was added with the *p*-aminoacetophenone or after it.

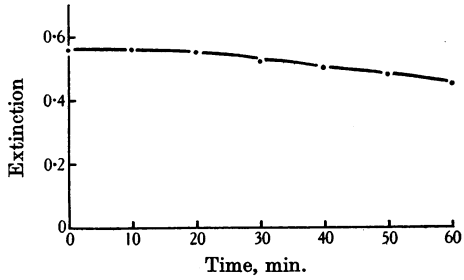

Fig. 5. Time curve after adding acidified *p*-aminoacetophenone (0·4 ml.) to 1·4 μg. of nicotinic acid per ml.

(*j*) *Time after addition of acidified* p-*aminoacetophenone.* The colour is fairly stable for 20–30 min. if kept in the dark (Fig. 5). The estimation was actually performed during the first 15 min. after the addition of the amine reagent.

Colour curve

The extinction curve of the coloured solution as determined in the step-photometer declines from violet to red (Fig. 6). For the estimations of nicotinic acid the blue filter No. 47 of the step-photometer has been used. Filter No. 43 was found to be too dark.

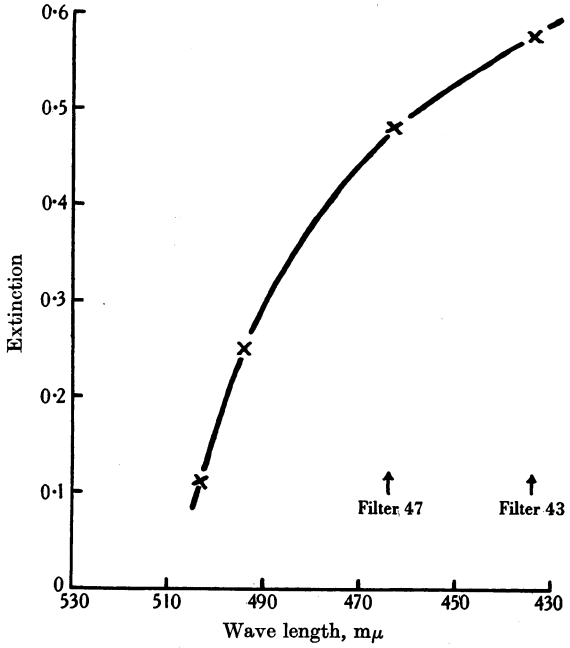

Fig. 6. Extinction curve. Nicotinic acid, 2 μg. per ml.; 1 cm. cells in step-photometer.

Specificity

Numerous amino-acids and other substances unrelated to pyridine were investigated and none gave a colour reaction (Table 8). Among the pyridine derivatives which were available at the time, adermin, trigonelline and quinolinic acid were found to give no colour. Pyridine, α-aminopyridine, nipecotic acid and nicotinic acid-*N*-diethylamide (coramine) gave some colour. But if no more than 20 μg. in 15 ml. of each substance were used, only with nicotinic acid-*N*-diethylamide was the colour measurable, being of the same intensity as that of nicotinic acid, into which it is converted upon hydrolysis. This substance is also biologically active, whereas the others are said to be inactive [Woolley *et al.* 1938]. It is interesting to notice how these substances differ, according as to whether they are estimated before or after hydrolysis. Nicotinamide gives only 20 % of its maximum colour if unhydrolysed; but, in contrast, nicotinic acid-*N*-diethylamide gives a more intense colour without hydrolysis. It seems that the side chain in the β-position is of some importance for the reaction. The unoccupied neighbouring carbon atoms in the α-position must be free to take part in the opening of the pyridine ring. It would appear from this suggestion that some pyridine derivatives fulfilling these conditions might give the reaction without being active in blacktongue or pellagra. This point has to be considered in the estimation of nicotinic acid in cereals.

Table 8. *Specificity of reaction*

(1) Miscellaneous substances

Negative. *l*-Cystine, cysteine, glutathione, glycine, *d*-alanine, *d*-lysine hydrochloride, *d*-arginine carbonate, *l*-leucine, *iso*leucine, histidine dichloride, adenine, guanidine hydrochloride, *d*-*l*-valine, *l*-asparagine, glutamic acid, aspartic acid, guanylic acid, urea, β-hydroxyproline, *l*-proline, nucleic acid, uric acid, adenosine, guanosine, inulin, inositol, xylose, protocatechuic acid, 2-amino-4-methylthiazole-5-carboxylic ester, 3-benzyl-2:3-dimethylthiazoloniumbromide, aneurin, tyrosine, thyroxine, creatinine, isoxazolecarboxylic acid, and pyrrole if hydrolysed.

(2) Pyridine derivatives

Negative. Adermin, trigonelline, quinolinic acid.

Colour developed. Pyridine, α-aminopyridine, nipecotic acid, nicotinic acid-*N*-diethylamide.

Extinction values with 20 μg. of pyridine derivatives

	Extinction	
	Hydrolysed	Unhydrolysed
Nicotinic acid	0·48	0·48
Nicotinamide	0·48	0·10
Nicotinic acid-*N*-diethylamide	0·47	0·70
Pyridine	0·0	0·0
α-Aminopyridine	0·0	0·0
Nipecotic acid	0·0	0·0
Adermin	0·0	0·0
Trigonelline	0·0	0·0
Quinolinic acid	0·0	0·0

(3) Solvents

Negative. Ethyl alcohol, chloroform, sulphur-free toluene, trichloroacetic acid, acetone, ethyl acetone, amyl alcohol.

Slight colour (due to impurities present). Toluene, *iso*butyl alcohol, benzene, propyl alcohol.

Some of the solvents, if impure, may give a colour. Impure toluene gives a positive reaction but repurified "sulphur-free" toluene is negative and can therefore be used for the preservation of urine or other biological material.

Sensitivity

As little as 1–2 μg. of nicotinic acid per g. of material can generally be detected with *p*-aminoacetophenone. This appears to be about 3–5 times as sensitive as aniline and metol.

Standard reference curve

Such a curve (Fig. 7) can probably be used only for any one given material, and then only when the conditions already described are regularly fulfilled. The standard reference curve has the equation:

$$\mu g. \text{ nicotinic acid in aliquot sample} = 47A,$$

where A is the extinction value of the unknown. The Pulfrich step-photometer was used, with 3 cm. cells and filter No. 47. For a given substance, for example muscle (Table 9), the agreement is good. If, however, a different material is taken the results from the standard reference curve and from the experimentally extrapolated curve (see above) may differ appreciably from one another. In Fig. 8 readings obtained on a great variety of material have been collected for comparison. The values obtained from the extrapolated curve, in the manner suggested by Harris & Raymond [1939, 2], using additions of known amounts of

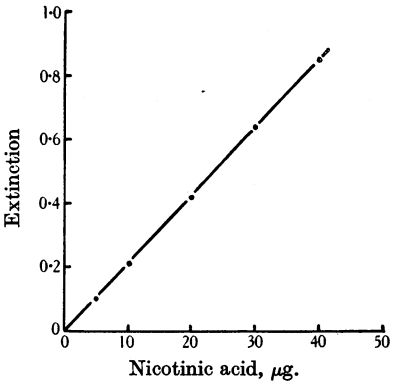

Fig. 7. Standard reference curve for nicotinic acid. 3 cm. cells in step-photometer, 0·4 ml. of *p*-aminoacetophenone.

Table 9. *Reproducibility of results*

Nicotinic acid found per g. of muscle in separate determinations on same specimen

(1) Method of extrapolation	(2) Comparison against standard reference curve for each single reading
μg.	μg.
40·0	40·0
40·0	40·0
40·0	42·5
35·0	32·5
45·0	47·5
40·0	45·0
52·5	58·5
42·5	47·5
40·0	45·0
45·0	47·5
47·5	47·5
42·5	45·0
42·5 (± 1·3 standard error of the mean)	44·8 (± 1·8 standard error of the mean)
Standard error of an individual result ±4·52	±6·24

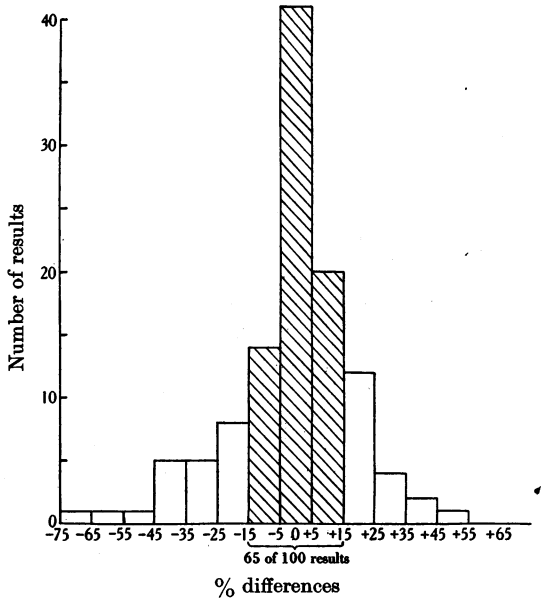

Fig. 8. Distribution of % differences between results from extrapolated curve and standard reference curve.

nicotinic acid to obtain a straight line, were accepted as the true results. The percentage differences of the results from those given by the standard reference curve were plotted as a histogram (Fig. 8). Out of 115 results 75 (65 %) lay within the range of ± 15 % difference. One of three results would have a greater error than this range. Therefore it seems preferable to use the extrapolated curve. The standard reference curve can be used only as a check on the reliability of the result.

Statistical accuracy of the method

Table 9 shows the results of repeated determinations on the same material (muscle). It will be seen that there is little variation. The standard error of an individual observation by the method of extrapolation is 4·52 mg. or 10·6 %.

Recovery of nicotinic acid and amide

The recovery of nicotinamide and nicotinic acid is complete either in solution in ethyl alcohol (with a standard error of ±3·5 % and ±1·3 % respectively), or when added to various substances (Table 10).

Table 10. *Recovery of added nicotinic acid and amide*

Description	Amount taken μg.	No. of esti-mations	Amount found μg.	Recovery %
Nicotinic acid in ethyl alcohol	50·0	15	51·5 (Mean value)	103 (±1·3 standard error)
Nicotinamide in ethyl alcohol, hydrolysed	50·0	16	52·5 (Mean value)	105 (±3·5 standard error)
Nicotinamide in ethyl alcohol, unhydrolysed	50·0	3	10·0 (Mean value)	20
Nicotinic acid added to:				
Serum	50·0	2	48·5 (Mean value)	97
Yeastrel	50·0	3	49·5 (Mean value)	99
Egg white	50·0	1	50·0	100
Marmite	50·0	2	57·5 (Mean value)	115
Muscle	50·0	2	54·0 (Mean value)	108
Milk	50·0	2	55·0 (Mean value)	105

SUMMARY

The procedure is discussed for the estimation of nicotinic acid in biological material by means of the cyanogen-*p*-aminoacetophenone method of Harris & Raymond. A quantitative study has been made of the various factors influencing the accuracy of the results: e.g. alternative methods of extraction, rate of hydrolysis, removal of interfering substances by means of ethyl alcohol, effects of changes in *p*H and of the final acidification. The specificity of the colour reaction is discussed. As little as 1–2 μg. of nicotinic acid can be detected in 1 g. of material, and in control tests quantitative recovery of added nicotinic acid was effected. This method is more sensitive than those in which metol and aniline are used.

In certain animal tissues nicotinic acid seems to be present bound to substances which are easily extracted by boiling water but which are partly precipitated by ethyl alcohol. In some cereals only a small fraction of the chromogen behaves like the nicotinic acid of animal tissues, i.e. is readily extracted by boiling water. It is believed that only this fraction (or a portion of it) is the "true" or "active" nicotinic acid; otherwise yellow maize which is known to be deficient would have a high concentration of nicotinic acid.

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