CCLXXIII. THE WATER-SOLUBLE B-VITAMINS
X. NICOTINAMIDE AND OTHER PYRIDINE DERIVATIVES IN THE NUTRITION OF THE RAT

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IN a recent publication [1937] we demonstrated that rats require for maximum growth four distinct factors of the vitamin B complex, one more heat-labile and three relatively heat-stable. These are (1) vitamin B_1, (2) lactoflavin, (3) an unidentified factor present in an autoclaved aqueous yeast extract after extraction with fuller's earth (yeast filtrate factor), (4) an unidentified factor present in the barium hydroxide eluate of a fuller's earth adsorbate from an autoclaved aqueous yeast extract (yeast eluate factor). Nos. (2), (3) and (4) compose what has previously been called vitamin B_2.

Warburg & Christian [1935] found that nicotinamide was contained in the molecule of codehydrogenase II, prepared from red blood cells. Codehydrogenase II is the coenzyme required in the dehydrogenations in which the lactoflavin-bearing yellow oxidation enzyme also plays a part. Nicotinamide has also been found present in the cozymase molecule [Euler *et al.* 1936]. Knight [1937] has more recently proved that nicotinic acid is one of the essential growth factors contained in the high-vacuum distillate from yeast extracts, required for the growth of *Staphylococcus aureus*. Since the two identified members of the vitamin B complex required by rats, vitamin B_1 and lactoflavin, have been proved to be essential growth factors for certain micro-organisms and since the close relationship between certain vitamins and enzymes is well established, it seemed possible that one of the two unidentified factors of the vitamin B complex required by rats might be identical with nicotinamide or some related compound.

We have investigated nicotinic acid, nicotinamide and codehydrogenase II, the last kindly supplied by Prof. Warburg, to find if these compounds could replace either the yeast filtrate factor or the yeast eluate factor in rat-growth experiments. While these experiments were in progress, Euler & Malmberg [1936] have reported that, although cozymase is inactive, nicotinamide has some growth-promoting action for rats, but details of these experiments have not appeared. Elvehjem *et al.* [1937] recently made the important discovery that canine blacktongue is cured by nicotinic acid and that curative concentrates from liver have yielded biologically active crystals identified as nicotinamide.

METHODS AND RESULTS

Growth of young rats was the criterion employed. The basal diet used was that usually employed in this laboratory, deprived of all B-vitamins [Chick & Roscoe, 1928; Chick *et al.* 1935]. Vitamin B_1 was supplied as the crystalline

synthetic vitamin (aneurin), except in a few of the earlier experiments, in which Peters's concentrate was used. The rats also received crystalline lactoflavin, either the synthetic product or the natural, prepared from liver extract.

The nicotinic acid used in the following experiments was prepared by oxidation of nicotine [Pictet & Sussdorff, 1898], M.P. 232°. Nicotinamide was obtained by the method of Pollak [1895] and was crystallized from benzene containing a trace of absolute ethyl alcohol, M.P. 127°. The sample of codehydrogenase II used in these experiments had 60% of the enzymic activity of pure codehydrogenase II. (Private communication from Prof. Warburg.)

The nicotinic acid and other pyridine derivatives were investigated for vitamin activity by the following three types of experiment. The compounds were fed to rats receiving the basal, vitamin B-deficient diet and (*a*) only vitamin B_1 and lactoflavin of the vitamin B complex, (*b*) vitamin B_1, lactoflavin and our yeast filtrate factor, (*c*) vitamin B_1, lactoflavin and our yeast eluate factor.

(*a*) *Effects of nicotinic acid, nicotinamide and codehydrogenase II on the growth of rats receiving only vitamin B_1 and lactoflavin of the vitamin B complex*

The animals at weaning received the basal diet supplemented by vitamin B_1. When growth had ceased, after approximately 10 days, the animals were given 12γ daily of lactoflavin supplemented by varying doses of the pyridine derivatives; control animals received 12γ daily of lactoflavin only. The weights of the animals were recorded over a 4-week period. Codehydrogenase II was further tested by feeding it to two animals which had received the basal diet supplemented by vitamin B_1 and lactoflavin for 3 weeks.

Comparison of the growth rates (Table I) reveals that no significant increase in the growth rate was effected by addition of any of the above pyridine derivatives. In the case of nicotinic acid a slightly increased growth rate was

Table I. *Effects of nicotinic acid, nicotinamide and codehydrogenase II on the growth of rats receiving vitamin B_1 and lactoflavin (12γ daily) of the vitamin B complex*

No. of rats	Vitamin B_1 given as (daily)	Pyridine derivatives given (daily dose)	Av. weekly gain in wt. over a 4-week period g.	No. of rats in control group receiving no pyridine derivatives	Av. weekly gain in wt. over a 4-week period g.
2	Peters's conc. = 0·3–0·6 g. dry yeast	1 mg. nic. acid	8·1	2	7·1
9	,,	5 mg. nic. acid	6·7	10	5·5
5	Crystalline vitamin (5γ)	5 mg. nic. acid	4·0	3	3·2
2	Peters's conc. = 0·3–0·6 g. dry yeast	1 mg. nic. amide	7·4	2	7·1
4	,,	5 mg. nic. amide	5·8	6	5·3
2	Crystalline vitamin (5γ)	40γ codehy.	2·25	4	4·75
2	,,	100γ ,,	2·5	4	4·75
2	,,	500γ ,,	1·0*	—	—

* These 2 rats, prior to dosing with the codehydrogenase II, had received vitamin B_1 and lactoflavin for 3 weeks, the average weekly gain being 2·7 g. The figure 1·0 g. weekly entered in the table was the average weekly weight increase during 2 subsequent weeks while receiving the codehydrogenase in addition to vitamin B_1 and lactoflavin.

noted in rats receiving 1 or 5 mg. daily, but the difference between these animals and the controls was so slight that the increase can hardly be regarded as significant. Feeding of 1 and 5 mg. daily of nicotinamide also caused no

significant increase in the growth rate, while the rats which received 40 or 100γ daily of the codehydrogenase II preparation actually grew at a slower rate than the control animals. In the case of two rats, which had received lactoflavin and vitamin B_1 only of the B-vitamins for 3 weeks previously, the addition of 500γ daily of the codehydrogenase II preparation was followed by a decrease in the growth rate. All the rates of weight increase shown in Table I are markedly subnormal.

The addition of the yeast filtrate factor to the diet of rats receiving only vitamin B_1 and lactoflavin of the vitamin B complex causes a marked increase in growth rate [Edgar *et al.* 1937; Edgar & Macrae, 1937], while the addition of the yeast eluate factor under similar circumstances causes a smaller, but still significant, increase. It is, therefore, clear that neither the yeast filtrate factor nor the eluate factor can be replaced by any of the above pyridine derivatives.

(*b*) *Effects of nicotinic acid, nicotinamide and codehydrogenase II on the growth of rats receiving vitamin B_1, lactoflavin and the yeast filtrate factor of the vitamin B complex*

Two types of experiment were employed.

In the first series, A, the rats at weaning received the basal diet supplemented by vitamin B_1 until all weight increase had ceased; they then received in addition daily 50γ of lactoflavin, yeast filtrate factor corresponding to 1 g. dry yeast and 1 mg. of nicotinic acid or nicotinamide while the control animals received no added pyridine derivative. The growth rates of the animals in the three groups over a 4-week period were nearly identical (Table II A, Fig. 1). The addition of the yeast eluate factor to the diet of the rats receiving nicotinic acid, however, resulted in an immediate increase in the growth rate.

Table II. *Effects of nicotinic acid, nicotinamide and codehydrogenase II on the growth of rats receiving daily crystalline vitamin B_1 (10–15γ), lactoflavin (50γ) and yeast filtrate fraction (= 1 g. dry yeast) as sources of the B-vitamins*

Series	No. of rats	Pyridine derivatives (daily dose)	Av. weekly wt. increase of the group during 3 weeks subsequent to dosing g.	Additional supplement (*S*) given later, daily	Av. wt. increase of group during the week after giving *S* g.
A	3	1 mg. nic. acid	27, 21, 20·6	Yeast eluate factor (= 1 g. dry yeast)	36·5 (2 rats)
	3	1 mg. nic. amide	24·7, 21, 19·3	—	—
	3	—	25, 20, 18·7	Yeast eluate factor (= 1 g. dry yeast)	30 (2 rats)

	No. of rats	Av. weekly wt. increase in group during 2 preliminary weeks (g.)	Additional supplement (*S'*) (daily dose)	Av. weekly wt. increase of group after receiving *S'* g.
B	3	18·7, 15·3	2 mg. nic. acid	16, 15·3
	3	20, 18	2 mg. nic. amide	15, 11·7
	2	17·5, 15	500γ codehyd.	20, 14·5
	3	19·3, 16	None	15·3, 13·7
	3	19, 19·7	Yeast eluate factor (= 1 g. dry yeast)	28, 23·7

In the second series of experiments, B, the rats received the basal diet supplemented by vitamin B_1, lactoflavin and yeast filtrate factor for 2 weeks; they then received daily in addition 2 mg. of nicotinic acid or 2 mg. of nicotinamide

or 0·5 mg. of the codehydrogenase II preparation for a further 2-week period. As controls, 3 animals were maintained on the basal diet supplemented by the above three vitamins of the B complex for the whole of the 4-week period,

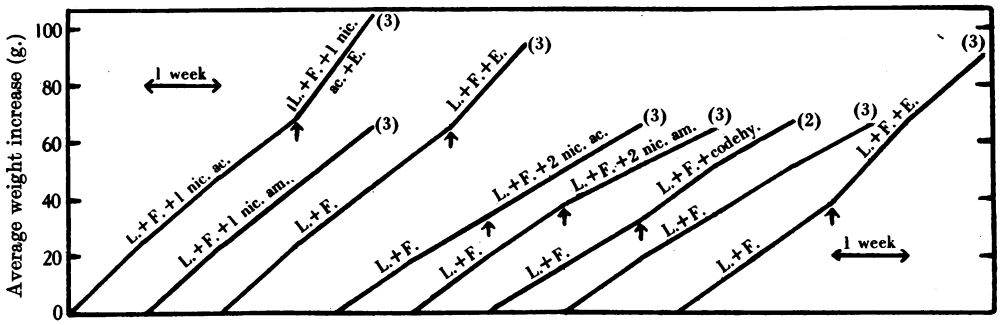

Fig. 1. Effects of nicotinic acid (1 mg. daily = 1 nic. ac.; 2 mg. daily = 2 nic. ac.), nicotinamide (1 mg. daily = 1 nic. am.; 2 mg. daily = 2 nic. am.) or codehydrogenase II (0·5 mg. daily = codehy.) compared with that of yeast eluate factor (corresponding to 1 g. dry yeast daily = E.) on the growth rate of young rats receiving vitamin B_1 (10–15γ daily), lactoflavin (50γ daily = L.) and yeast filtrate factor (corresponding to 1 g. dry yeast daily = F.). The arrows indicate the points at which the doses were changed. The figures in brackets indicate the number of rats from which the growth curves were derived.

while others received, in addition to the three vitamins, the yeast eluate factor at the period when the experimental animals received the pyridine bases. The addition of nicotinic acid, nicotinamide or codehydrogenase II to the diets of the above animals did not cause any significant increase in the growth rate, while the addition of yeast eluate factor caused the usual increase previously recorded by us (Table II B, Fig. 1).

The yeast eluate factor therefore cannot be replaced by nicotinic acid, nicotinamide or codehydrogenase II.

(*c*) *Effect of nicotinic acid and nicotinamide on the growth of rats receiving vitamin B_1, lactoflavin and yeast eluate factor of the vitamin B complex*

Rats received the basal diet supplemented by vitamin B_1 from time of weaning until they ceased to grow, when the diet was supplemented by the daily addition of 50γ lactoflavin and of yeast eluate factor corresponding to 1 g. dry yeast. After 2 weeks certain animals were given a supplement of 2 mg. nicotinic acid and others a supplement of 2 mg. of nicotinamide while the remainder served as controls and received no further addition to the diet. All the animals were observed for a further period of 2 weeks, and at the end of this period, in addition to all previous supplements they received yeast filtrate factor corresponding to 1 g. dry yeast. No significant increase in the growth rate resulted on the addition of the pyridine derivatives, while the subsequent addition of the yeast filtrate factor caused the usual growth response (see Table III, Fig. 2; cf. Edgar & Macrae [1937]).

Therefore neither nicotinic acid nor nicotinamide can replace the yeast filtrate factor.

Although it is shown above that neither nicotinic acid, nicotinamide nor codehydrogenase II can replace either our yeast eluate factor or our yeast filtrate factor it cannot be assumed that these pyridine derivatives may not,

Table III. *Effects of nicotinic acid, nicotinamide and codehydrogenase II on the growth of rats receiving daily, crystalline vitamin B_1 (10–15γ), lactoflavin (50γ) and yeast eluate fraction (=1 g. dry yeast) as sources of the vitamin B complex*

No. of rats	Av. weekly wt. increase of group for 2 preliminary weeks (g.)	Pyridine derivatives (daily dose)	Av. weekly wt. increase of group for 2 weeks after giving the pyridine derivatives (g.)	Av. wt. increase of the group for the weeks after giving yeast filtrate factor (=1 g. dry yeast) g.
3	14, 8·7	2 mg. nic. acid	4·7, 5·3	16
3	14, 6·7	2 mg. nic. amide	4·3, 3·3	17·3
3	16·5, 8	—	5, 6	15

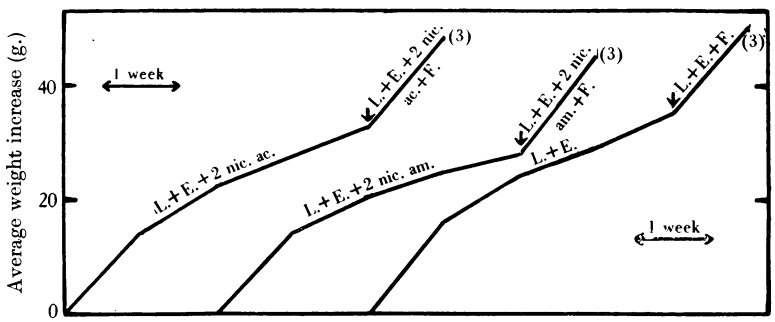

Fig. 2. Effect of nicotinic acid (2 mg. daily = 2 nic. ac.) or nicotinamide (2 mg. daily = 2 nic. am.) compared with that of yeast filtrate factor (corresponding to 1 g. dry yeast daily = F.) on the growth rate of young rats receiving vitamin B_1 (10–15γ daily), lactoflavin (50γ daily = L.) and yeast eluate factor (corresponding to 1 g. dry yeast daily = E.). Other details as in Fig. 1.

nevertheless, be dietary factors for rats, for it is possible that either or both the yeast eluate factor and the yeast filtrate factor are complex in nature and may contain one of these pyridine derivatives as an essential constituent. Since yeast contains cozymase and other pyridine derivatives it seems likely that one or both of the yeast fractions containing these dietary factors will contain nicotinamide or other pyridine derivatives.[1]

The presence of a growth factor for rats distinct from nicotinamide in an amyl alcohol extract of liver extract

Elvehjem & Koehn [1935] and Koehn & Elvehjem [1937] have reported that an amyl alcohol extract of liver extract, previously treated with fuller's earth, prevented dermatitis in chicks and cured canine blacktongue. After further purification, the amyl alcohol extract retained its activity both for chicks and dogs. These authors therefore suggested that these physiological effects are probably due to the same factor. A growth-promoting factor for rats present in liver extract after exhaustive extraction with fuller's earth has been described by Lepkovsky *et al.* [1936]. They presume this factor to be identical with the anti-chickdermatitis factor which, as already stated, in turn is regarded by Koehn & Elvehjem [1937] as probably identical with the anti-blacktongue factor.

[1] Since submitting this paper for publication we have isolated nicotinamide from the yeast eluate fraction. (Found: C, 59·4; H, 5·0; N, 22·1%. $C_6H_6ON_2$ requires: C, 59·0; H, 4·9; N, 22·9%.) M.P. 127°; mixed M.P. with authentic specimen of nicotinamide, 127°. M.P. of chloroaurate, 234–236° (decomp.); M.P. of chloroaurate of authentic specimen of nicotinamide, 235–237° (decomp.).

Recently Elvehjem *et al.* [1937] have isolated from liver concentrates a crystalline compound which cured canine blacktongue and was identified as nicotinamide.

The experiments reported in the present paper, however, show that nicotinamide is not one of the dietary factors recognized as essential for rats, while the following experiment definitely proves the existence in liver extracts of a factor, extractable by amyl alcohol, which does promote the growth of rats, and may be identical with our yeast filtrate factor.

500 ml. of a deproteinized liver extract (1 ml. corresponding to 2 g. dry liver), from which the pernicious anaemia factor had been removed by treatment with charcoal, was adjusted to *p*H 1·4 with sulphuric acid and extracted six times with 1 litre portions of amyl alcohol. The combined amyl alcohol extracts were shaken three times with 500 ml. portions of dilute NaOH. The alkali extracts were mixed and, after neutralizing with hydrochloric acid, the amyl alcohol was removed by distillation *in vacuo*.

The striking effect of feeding this extract (corresponding to 1 g. dry liver daily) to rats receiving adequate amounts of vitamin B_1, lactoflavin and our yeast eluate fraction is shown in Table IV and Fig. 3. There was an immediate

Table IV. *Growth-promoting action of an amyl alcohol extract prepared from aqueous liver extracts for rats receiving vitamin B_1 (10–15γ), lactoflavin (50γ) and yeast eluate fraction (=1 g. dry yeast)*

Rat	Weekly wt. increase during 2 preliminary weeks (g.)	Weekly wt. increase during 2 weeks after receiving the extract from liver (g.)
822 ♂	15, 9	32, 21
828 ♀	15, 10	24, 15
829 ♀	13, 10	26, 17

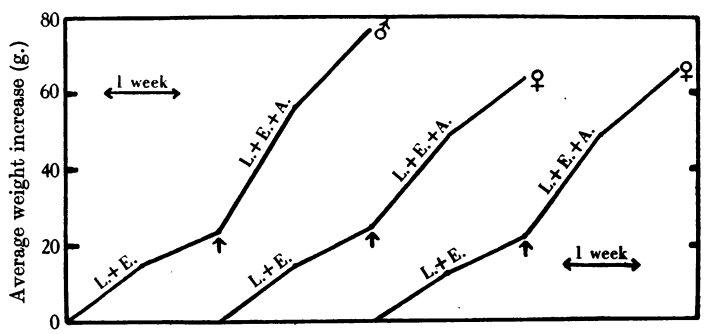

Fig. 3. The effect of amyl alcohol extract from liver extract on the growth rate of three rats receiving crystalline vitamin B_1 (10–15γ daily), lactoflavin (50γ daily = L.) and yeast eluate factor (corresponding to 1 g. dry yeast daily = E.). The arrows indicate the points at which the amyl alcohol extract (corresponding to 1 g. dry liver daily = A.) was given.

increase in the growth rate, similar to the effect produced by feeding the yeast filtrate fraction to rats receiving the basal diet with similar supplements [cf. Edgar & Macrae, 1937]. Further investigation will decide whether the substance thus prepared from liver extract is identical with our yeast filtrate factor. Since in our experiments neither nicotinamide nor the other pyridine derivatives had the biological activity for rats exhibited by this amyl alcohol extract from liver, it follows that there is present in this extract a growth factor for rats distinct from these pyridine derivatives. The essential dietary factors for the rat and

the dog present in amyl alcohol extracts from liver must therefore be regarded as distinct. This is not surprising for it has long been recognized, in this and other laboratories [Birch *et al.* 1935; Rhoads & Miller, 1935; Chick, unpublished experiments], that rats will thrive on diets which induce the development of blacktongue in dogs. Whether the chick factor contained in the amyl alcohol extract from liver extract is the same as the rat or the dog factor, or is distinct from both, must be decided by further experiment.

Summary

1. Neither nicotinic acid, nicotinamide nor codehydrogenase II could replace either the yeast filtrate factor or the yeast eluate factor in the diet of the rat, as tested by observations on growth.

2. Amyl alcohol extracts prepared from liver extracts contain a growth factor for rats which is distinct from the anti-blacktongue factor contained in such preparations, identified by Elvehjem *et al.* [1937] as nicotinamide.

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