CLXVI. THE SPECIFICITY OF ANEURIN AND NICOTINAMIDE IN THE GROWTH OF *STAPH. AUREUS*

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It has been shown [Knight, 1937, 1, 2, 3] that nicotinamide and aneurin (or certain closely related derivatives corresponding to these two compounds) are necessary for the growth of typical strains of *Staph. aureus* in a medium of known chemical composition.

The activities of several compounds closely related to nicotinamide and aneurin which were previously tested [Knight, 1937, 3] showed that only a limited departure from these two chemical structures was compatible with the maintenance of growth activity. It was further found that a mixture of a pyrimidine and a thiazole, both having substituents corresponding to those of the component pyrimidine and thiazole rings of aneurin, could be used in place of aneurin itself. The behaviour of the further series of compounds recorded in the present paper emphasizes the specificity of both the nicotinamide and the aneurin structures in the growth requirements of *Staph. aureus*.

Technique

The methods used for the growth tests were the same as those previously described [Fildes *et al.* 1936; Fildes & Richardson, 1937]. The test organism was a typical strain of *Staph. aureus* which could not grow on the basal amino-acid medium without the further addition of nicotinamide and aneurin (or the equivalent pyrimidine + thiazole; see below).

The substances to be tested fell into four categories:

- (1) Aneurin analogues: as substitutes for the complete aneurin molecule.
- (2) Pyrimidine series: as substitutes for the pyrimidine component of aneurin.
- (3) Thiazole series: as substitutes for the thiazole component of aneurin.
- (4) Pyridine series: as substitutes for nicotinamide.

The compound to be tested was added in serial dilution to the basal medium which was nutritionally adequate in all respects except for that component, the substitute for which was under test. Thus of the complete group of nutrients required for growth only one component was varied at a time. Pyridine derivatives (as substitutes for nicotinamide) were always tested in presence of an excess of aneurin ($1 \times 10^{-7} M$). Similarly aneurin analogues or derivatives (pyrimidines and thiazoles) were tested in the presence of an excess of nicotinamide ($1 \times 10^{-6} M$). When the component parts of the aneurin molecule were being investigated separately, i.e. either the pyrimidine or the thiazole moiety

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was being varied, then an excess of the corresponding thiazole or pyrimidine was added. Thus when testing thiazoles, 2-methyl-4-amino-5-aminomethylpyrimidine was used as the complementary active pyrimidine; conversely when testing pyrimidines 4-methyl-5- β -hydroxyethylthiazole was used.

Growth was aerobic and incubation was continued for 3 days to allow time for slow utilization, if it occurred, to become apparent. In general the picture of activity shown within 24 hr. was not altered, qualitatively or quantitatively, on further incubation. Only in two cases have compounds apparently inactive at 24 hr. been found to permit growth on longer incubation; these compounds were methyl nicotinate [Knight, 1937, 3 and Table IV] and 4-methyl-5-vinylthiazole (see No. 14, Table III).

The results obtained are collected in Tables I-IV; for completeness and convenience of comparison the chief results previously obtained are also included. Fig. 1 shows the structural formulae of aneurin and its components for convenience of reference.

Fig. 1.

Aneurin analogues

In all this work where the typical active substances (e.g. aneurin) are of high potency it is necessary when testing closely related substances to be certain that apparent activities are not due to contamination by active impurities. In these circumstances failure to obtain growth is acceptable evidence of lack of activity, whereas an apparently lowered potency must be regarded sceptically, until it can be shown, either by repeated purification leading to no alteration of activity, or by the method of synthesis, that an active impurity is not the cause of the observed growth.

The much lowered potency of the "isoaneurin", about 1/10,000th of the activity of aneurin, is probably real. From the method of preparation [Andersag & Westphal, 1937, pp. 2050-1] no contamination with aneurin itself seems

Table I. *Aneurin analogues*

	Conc. <i>M</i>	Growth 24 hr.
Aneurin chloride HCl	1×10^{-8}	+++
"	2×10^{-9}	+++
"	4×10^{-10}	++
"	8×10^{-11}	?
<i>iso</i> Aneurin	1×10^{-5}	+++
"	1×10^{-6}	?
" + correct pyrimidine $10^{-7} M$	1×10^{-7}	+++
" + correct pyrimidine $10^{-8} M$	1×10^{-8}	?
Chloroaneurin	1×10^{-5}	+++
"	1×10^{-6}	?
"	1×10^{-7}	0
" + correct pyrimidine $10^{-7} M$	1×10^{-8}	++++
" + correct pyrimidine $10^{-7} M$	1×10^{-9}	+++
Aneurin minus hydroxyethyl group	1×10^{-5}	0
" " " + correct thiazole $2 \times 10^{-7} M$	1×10^{-8}	++++
2-Methylanaurin	1×10^{-5}	0
" + correct thiazole $1.0 \times 10^{-7} M$	1×10^{-8}	+++

Aneurin: synthetic specimen (Merck).

*iso*Aneurin: having CH₃ at 6 instead of 2 in the pyrimidine. (I.G. Farbenindustrie; transmitted by Dr A. Lwoff.)

Chloroaneurin: having Cl instead of CH₃ at 2 and CH₃ at 6 in the pyrimidine. (Drs Bergel & Todd.)

Aneurin minus hydroxyethyl group: lacking the 5-β-hydroxyethyl group of the thiazole ring. (Bergel & Todd.)

2-Methylanaurin: having an additional CH₃ at 2 in the thiazole. (Bergel & Todd.)

In this and in subsequent tables:

++++, ++, etc.; = number of + signs proportional to related mass of growth.

tr. = trace of growth just visible to the naked eye.

? = just detectable effect visible to naked eye.

0 = no detectable effect at concentration shown.

possible. Using the "*iso*aneurin" as a source of the thiazole only, that is, adding also 2-methyl-4-amino-5-aminomethylpyrimidine, there was an enhancement of activity, although not reaching the level expected from equivalent active pyrimidine + thiazole mixtures.

Similarly the much reduced potency of the "chloroaneurin" is probably real and not due to active impurity. Bergel & Todd [1937] reported that this compound apparently possessed some vitamin activity on one occasion, but further examination of the material failed to confirm this. The chloro compound was a potent source of the thiazole for *Staph. aureus* when tested in the presence of the correct pyrimidine.

The other two aneurin analogues tested have both altered substituents in the thiazole ring and their lack of activity is confirmed by the inactivity of the corresponding thiazoles in thiazole-pyrimidine pairs (Nos. 6 and 12, Table III). These two analogues were found by Bergel & Todd [1937] to be devoid of vitamin activity for rats.

Pyrimidines

The results with the new pyrimidines tested confirm the conclusions of the previous work [Knight, 1937, 3]. The apparent activity of 2-methyl-4-methoxy-5-aminoethylpyrimidine (No. 4, Table II) was undoubtedly due to contamination with the corresponding active 4-amino compound (No. 1, Table II). This is clear from the mode of preparation of the 4-methoxy compound [Andersag & Westphal, 1937, pp. 2046-7] which involves the separation of a 4-methoxy compound from the corresponding 4-amino compound by different solubilities

in methanol. This might easily permit sufficient contamination of the former by the latter to account for the apparent activity; contamination of less than 0.1 % would suffice.

The observed effects with all the pyrimidines tested show that the essential substituents of the ring for activity are: a CH_3 group at position 2; an NH_2 group at 4; and at position 5 a CH_3 group substituted in certain ways. Thus at 5, $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{OH}$ and $-\text{CH}_2\text{NH.CSH}$ permit growth; but $-\text{CH}_2\text{CO.NH}_2$ does not, nor does an unsubstituted $-\text{CH}_3$.

These results have a bearing on the question of whether *Staph. aureus* requires the complete molecule for its growth activity and whether it does in fact join an active pyrimidine and thiazole together to make aneurin when given the two components. The $-\text{CH}_2-$ at position 5 is that which forms the link between the pyrimidine and thiazole rings in aneurin. Since the results in Table II show that the nature of the groups attached to this 5- CH_2- group

Table II. *Pyrimidines*

	Conc. <i>M</i>	Growth 24 hr.
1. 2-Methyl-4-amino-5-aminomethylpyrimidine	1×10^{-8} 2×10^{-9} 4×10^{-10} 8×10^{-11}	+ + + + + + + ?
2. 2-Methyl-4-amino-5-hydroxymethylpyrimidine	1×10^{-8} 1×10^{-9} 1×10^{-10}	+ + + + + + +
3. 2-Methyl-4-amino-5-thioformamidomethylpyrimidine	1×10^{-6} 1×10^{-7} 1×10^{-8}	+ + + + + 0
4. 2-Methyl-4-methoxy-5-aminomethylpyrimidine	1×10^{-7} 2×10^{-8} 4×10^{-9}	+ + * + 0
5. (2-Methyl-4-aminopyrimidyl (5))-acetamide	1×10^{-5}	0
6. 2-Methyl-4-hydroxy-5-aminomethylpyrimidine	2×10^{-6}	0
7. 2-Methyl-4-hydroxy-5-hydroxymethylpyrimidine	2×10^{-6}	0
8. 2:5-Dimethyl-4-aminopyrimidine	1×10^{-5}	0
9. 2:6-Dimethyl-4-hydroxy-5-aminopyrimidine	1×10^{-5}	0
10. 2-Methyl-4-hydroxy-6-aminopyrimidine	1×10^{-5}	0
11. 2-Methyl-4-mercaptopyrimidine	1×10^{-5}	0
12. 2-Hydroxy-4-aminopyrimidine (cytosine)	2×10^{-5}	0

* The apparent activity of No. 4, 2-methyl-4-methoxy-5-aminomethylpyrimidine, was undoubtedly due to contamination with the corresponding 4-amino compound (No. 1, see p. 1243). The specimen was an I.G. Farbenindustrie product.

Nos. 1, 3, 6, 7: obtained from Drs A. R. Todd & F. Bergel.

Nos. 2, 4, 5, 9: obtained from I.G. Farbenindustrie and made by Andersag & Westphal [1937]; transmitted by Dr A. Lwoff (Institut Pasteur, Paris).

Nos. 8, 10, 11: obtained from Hoffmann-La Roche; transmitted by Dr A. Lwoff.

No. 12: synthesized by Dr G. M. Richardson [1936].

does affect the growth activity, this is evidence that the 5- CH_2- group is concerned in whatever function the pyrimidine has. The relative effects of the differently substituted 5- CH_2- group and the unsubstituted 5- CH_3 group probably indicate that the pyrimidine and thiazole are joined by *Staph. aureus*, this being possible with $-\text{CH}_2\text{NH}_2$, $-\text{CH}_2\text{OH}$, more difficult with $-\text{CH}_2\text{NH.CSH}$, but not possible with $-\text{CH}_2\text{CO.NH}_2$ or $-\text{CH}_3$. On chemical grounds the formation of the thiazolium compound is likely with the first three groupings; the corresponding 5- CH_2Br -pyrimidine is used in the Williams & Cline [1936] synthesis. It is not likely with $-\text{CH}_3$ and hydrolysis of

$-\text{CH}_2\text{CO}.\text{NH}_2$ would yield $-\text{CH}_2\text{COOH}$, having one carbon atom too many for the link.

Hypotheses suggesting that an active pyrimidine and thiazole are used by *Staph. aureus* separately for different reactions, or for different parts of the same process, with the corollary that aneurin itself is split into its two components seem improbable in the light of these findings. Hills [1938], who showed that aneurin or the corresponding pyrimidine and thiazole together were necessary for the normal metabolism (aerobic or anaerobic) of pyruvate by *Staph. aureus*, found that the maximal effect of adding the two components simultaneously appears slightly later than the full effect of adding aneurin in a parallel experiment, suggesting a short lag while aneurin was made by linking the components. Other reasons for believing that the complete molecule or derivatives of it, are the active substances required for essential metabolic reactions in *Staph. aureus*, are discussed later.

Thiazoles

The most interesting new features shown by the thiazoles now examined are the reduced but real potencies of the β - and γ -hydroxypropyl compounds (Nos. 9 and 10, Table III), the delayed effect with the vinyl compound (No. 14, Table III), and the activity of 4-methyl-5- β -acetoxyethylthiazole (No. 2, Table III). Together

Table III. *Thiazoles*

	Conc. <i>M</i>	Growth 24 hr.			
1. 4-Methyl-5- β -hydroxyethylthiazole	5×10^{-8} 1×10^{-8} 2×10^{-9} 4×10^{-10}	+++ +++ ++ tr.			
2. 4-Methyl-5- β -acetoxyethylthiazole	1×10^{-8}	+++			
3. 4-Methyl-5- α -hydroxyethylthiazole	5×10^{-7}	0			
4. 3-Benzyl-4-methyl-5- β -hydroxyethylthiazole*	1×10^{-6} 1×10^{-7} 1×10^{-8}	++ ++ tr.			
5. 3-(4'(5')-Methylimidazole)-4-methyl-5- β -hydroxyethylthiazole*	1×10^{-6} 1×10^{-7} 1×10^{-8}	++ ++ +			
6. 2:4-Dimethyl-5- β -hydroxyethylthiazole	5×10^{-7}	0			
7. 2-Hydroxy-4-methyl-5- β -acetoxyethylthiazole	1×10^{-5}	0			
8. 4-Methyl-5-ethylthiazole	5×10^{-7}	0			
9. 4-Methyl-5- β -hydroxypropylthiazole	5×10^{-7} 5×10^{-8}	++ 0			
10. 4-Methyl-5- γ -hydroxypropylthiazole	5×10^{-7} 5×10^{-8}	+ 0			
11. 4:5-Dimethylthiazole	1×10^{-7}	0			
12. 4-Methylthiazole	1×10^{-5}	0			
13. 4-Methyl-5-mercaptothiazole	1×10^{-5}	0			
			22 hr.	43 hr.	90 hr.
14. 4-Methyl-5-vinylthiazole	$5 \times 10^{-7} M$ $5 \times 10^{-8} M$	0 0	tr. 0	+	0

* Nos. 4 and 5. The apparent activity of these two compounds was probably due to contamination with the correct thiazole (i.e. not substituted at 3) from which they would be prepared (see text, p. 1246).

Nos. 1 and 12. Synthesized by Drs A. R. Todd & F. Bergel.

Nos. 2 and 7: I.G. Farbenindustrie product [Andersag & Westphal, 1937]; transmitted by Dr Lwoff.

Nos. 4, 5, 11 and 13: Hoffmann-La Roche product; transmitted by Dr Lwoff.

Nos. 3, 6, 8, 9, 10 and 14: obtained from Dr E. R. Buchman (California Institute of Technology, Pasadena, U.S.A.).

these show that there is some, although limited, scope for variation in the structure of the thiazole which is an essential part of the growth requirements of *Staph. aureus*.

The activity of 4-methyl-5- β -acetoxyethylthiazole is not unexpected; conversion into the aneurin thiazole (5- β -hydroxyethyl) merely involves hydrolysis of the acetate. *Staph. aureus* is known to be able to hydrolyse an ester link, as in methyl nicotinate (see Table IV).

We are assured by Dr E. R. Buchman, who supplied us with the specimens of 4-methyl-5- β - and - γ -hydroxypropylthiazoles that the activities we observe are not due to traces of contaminating active impurity (the aneurin thiazole). He and his colleagues had occasion to examine this point with regard to *Phycomyces* growth activity (see below) and found that these hydroxypropyl derivatives plus the correct pyrimidine had activities equal to the corresponding aneurin analogues which had been recrystallized several times. The quantitative equivalence found could not therefore be explained by an active impurity in these two cases. We feel confident that tests of the corresponding aneurin analogues on *Staph. aureus* will confirm the real but much lower potency (e.g. 1/1000) of the 4-methyl-5- β - and - γ -hydroxypropylthiazoles found in the present tests. The hydroxypropyl aneurin analogues had no antineuritic activity in rats at a dose 1000 times the curative dose of aneurin (Dr E. R. Buchman; private communication).

The delayed activity of the 4-methyl-5-vinylthiazole (No. 14, Table III) is real; an active contamination would give an immediate response. It is reasonable to suppose that some of the active 5- β -hydroxyethyl compound is formed by addition of water to the $-\text{CH}=\text{CH}_2$ side chain. The effect with the vinyl compound is presumably delayed while this takes place.

The inactivity (no effect at 1 to $2 \times 10^{-7} M$) of the 4-methyl-5- α -hydroxyethyl-, 4-methyl-5-ethyl- and 4:5-dimethyl-thiazoles, and the lowered potency of the hydroxypropylthiazoles (an effect at $5 \times 10^{-7} M$) compared with the activity of the aneurin thiazole (4-methyl-5- β -hydroxyethylthiazole) which shows a detectable effect at $10^{-9} M$, illustrates the importance of the side chain at position 5.

It is also interesting that substitution of a $-\text{CH}_3$ or $-\text{OH}$ group at position 2 in the thiazole ring (Nos. 6 and 7, Table III) inactivates an otherwise active thiazole; the same holds for a corresponding aneurin analogue (2-methylaneurin). When aneurin is oxidized to thiochrome it is the C atom at 2 in the thiazole ring which links with the nitrogen in the 4-amino group in the pyrimidine. The inactivity of these 2-substituted thiazoles suggests the question whether oxidation to thiochrome is involved in any of the metabolic functions of aneurin.

The apparent activity of the two N-substituted compounds, Nos. 4 and 5, Table III, cannot be accepted as real without examination of further purified specimens, since the method of preparation, from the highly active aneurin thiazole itself, renders contamination by the latter very probable. Furthermore similar pure quaternary salts have been found inactive in the *Phycomyces* growth test, which in general parallels the growth effects with *Staph. aureus* (E. R. Buchman, private communication).

*Aneurin and its pyrimidine and thiazole components in the growth
of various organisms*

The observation that the pyrimidine and thiazole components of aneurin together were as effective in the growth of *Staph. aureus* as aneurin itself [Knight, 1937, 3] has been extended to other micro-organisms. It has been shown that the corresponding thiazole-pyrimidine pairs are effective in the growth of the

mould *Phycomyces blakesleeanus* [Schopfer & Jung, 1937; Schopfer, 1937; Sinclair, 1937; Robbins & Kavanagh, 1937; 1938]. Similarly Lwoff & Dusi [1937; 1938], Lwoff, A. & Lwoff, M. [1937; 1938] and Lwoff, M. [1937] have extended the field of action of aneurin or its components by showing that they are essential nutrients for certain unicellular protista [*Eucaryotes*; Lwoff, 1932].

A comparison of the need for aneurin or its components in a wide group of fungi has been made by Robbins [1938] and Robbins & Kavanagh [1938, 3], for species of the genus *Ustilago* by Schopfer & Blumer [1938], and for *Mucor Ramannianus* (Möhl) by Müller & Schopfer [1937].

These investigations have shown that there are some species which need be given only one or other of the aneurin components, while other species cannot utilize the two components but must be given the complete aneurin molecule. Of the latter type are the flagellate trypanosome *Strigomonas oncopelti* [Lwoff, M. 1937]; the ciliate *Glaucoma piriformis* [Lwoff, A. & Lwoff, M. 1937, 2; 1938] and *Ustilago scabiosae* (Sow.) [Schopfer & Blumer, 1938]. Aneurin analogues inactive for *Staph. aureus* were also inactive for *Glaucoma piriformis* [Lwoff, A. & Lwoff, M. 1938].

All the strains of *Staph. aureus* examined in our laboratory (now about 30 typical strains) have required both the pyrimidine and the thiazole. In no case has a strain been observed which needed only one of them, or which could utilize only aneurin and not the two components. It is possible that other species of bacteria may be found which exhibit these growth requirements.

We are indebted to Dr G. P. Gladstone for the unpublished observations that an examination of the filtrates from cultures of *Chromobact. prodigiosum*, *Pseudomonas pyocyanea*, *B. subtilis* and *Bact. typhosum*, has shown that all these bacteria, cultivated in media which do not contain aneurin, do in fact synthesize this substance (or its two components) since the filtrates can supply the aneurin requirements of *Staph. aureus*. This is an extension of the earlier observation of Hughes [1932] with *Bact. coli*. For these bacteria therefore the dispensability of aneurin (or its components) as a nutrient essential does not mean that this substance may not be involved in their metabolism; it is in fact synthesized by these organisms.

Robbins & Kavanagh [1938, 3] found that those species of fungi which required only one or other aneurin component for growth synthesized the complementary component, since the culture filtrates could supply both components to *Phycomyces blakesleeanus*. Analogous observations for *Mucor Ramannianus* and *Rhodotorula* were made by Müller & Schopfer [1937] each organism supplying one component to the other. In general therefore it is found for the organisms discussed here that a need for one or both aneurin components, or for aneurin itself, as nutrients, reflects the relative ability of the organism to synthesize aneurin. The latter appears to be required in the metabolism of all of them, and it or its components are nutrient essentials if they cannot be synthesized.

An examination of the specificity of pyrimidines and thiazoles which can be used by various fungi and unicellular protista in place of the aneurin which provides essential nutrient material for them, and a comparison with similar observations for *Staph. aureus*, supports this view. Generally speaking the specificity in the growth of *Staph. aureus* is paralleled by the effects shown by *Phycomyces* [Robbins & Kavanagh, 1938, 1, 2; Schopfer, 1937; Sinclair, 1937] and by the protista studied by Lwoff & Dusi [1938, 2, 3]. It will therefore be simplest to note only the points of difference.

(a) *2-Methyl-4-amino-5-thioformamidomethylpyrimidine*. Sinclair [1937] found for *Phycomyces blakesleeanus* that this compound was less potent than the

corresponding 5-aminomethyl derivatives, in agreement with the results with *Staph. aureus*. Robbins & Kavanagh [1938, 1] confirmed this, using a sample of the 5-thioformamidomethyl derivative from the same source as the specimens used by Sinclair and by Knight, namely Dr A. R. Todd. Further, Robbins & Kavanagh showed that the activity of the 5-thioformamidomethyl derivative decreased on autoclaving to sterilize, a filtered solution being the most active. This did not occur with the 5-hydroxymethyl or 5-bromomethyl derivatives. Presumably it would not occur with the 5-aminomethyl derivative which would be the probable active contaminant of the 5-thioformamidomethyl compound if one were present (this follows from the mode of synthesis [Todd & Bergel, 1937]). Schopfer [1937] with a different specimen of the 5-thioformamidomethyl derivative (Hoffmann-La Roche) recorded only very feeble activity for *Phycomyces*, but he used an autoclaved solution. Lwoff & Dusi [1938, 2] found that a specimen of the 5-thioformamidomethyl derivative (also from Hoffmann-La Roche) but not autoclaved, had activity for *Polytomella caeca* and *Chilomonas paramoecium*, but was less potent than the corresponding 5-aminomethyl compound. With the exception of Schopfer's results, probably explicable by loss of activity on autoclaving, all the observations are in agreement that the thioformamidomethyl derivative can be utilized by *Staph. aureus*, *Phycomyces blakesleeanus*, *Polytomella caeca* and *Chilomonas paramoecium*, but in all cases is less readily available than the corresponding 5-aminomethyl, bromomethyl or hydroxymethyl derivative. This suggests a common objective in the utilization of the pyrimidine by all these organisms.

(b) *Thiazoles*. The most striking difference of behaviour in the utilization of thiazoles by the different organisms considered here is that shown with the compound 2:4-dimethyl-5- β -hydroxyethylthiazole. This was inactive for *Staph. aureus* (No. 6, Table III) and for *Phycomyces blakesleeanus* [Robbins & Kavanagh, 1938, 2]; but Lwoff & Dusi [1938, 3] using the same specimen as we have used in the present work find that it can be utilized by the flagellates *Polytoma caudatum*, *P. ocellatum*, and *Polytomella caeca*. The corresponding 2-hydroxy-4-methyl-5- β -acetoxyethylthiazole was inactive for these flagellates as it was for *Staph. aureus* (No. 7, Table III).

With the exception of this difference the specificity of the pyrimidine and thiazole components of aneurin is the same for all the organisms discussed here. These results emphasize the high specificity of the aneurin structure in the physiology of all these varied types of organism and also harmonize with the results obtained by Bergel & Todd [1937] with aneurin analogues for anti-neuritic activity in the rat, suggesting a fundamental metabolic role for aneurin common to all these organisms.

Compounds related to nicotinamide

The results collected in Table IV emphasize the specificity of nicotinamide and two closely related compounds in the growth of *Staph. aureus*. One of the functions of the nicotinamide must be to contribute that molecule to the synthesis of codehydrogenase I or II (pyridine nucleotide phosphate compounds) which *Staph. aureus* is known to produce. This follows from the fact that the organism is known to synthesize the "V factor" for *Haemophilus parainfluenzae*, which Lwoff, A. & Lwoff, M. [1937, 1] have shown to be identical with either codehydrogenase I or II. Nicotinamide is more active than nicotinic acid, in agreement with the fact that it is the amide which is required in the codehydrogenase. *Staph. aureus* can make the amide from the acid if necessary. It appears therefore that *Staph. aureus* must find nicotinic acid preformed be-

Table IV. *Pyridine derivatives*

	Conc. <i>M</i>	Growth 24 hr.
1. Nicotinamide	2×10^{-7}	+++
	4×10^{-8}	++
	8×10^{-9}	?
2. Pyridine-3-carboxylic acid (nicotinic acid)	1×10^{-6}	+++
	2×10^{-7}	?
3. Methyl nicotinate	5×10^{-7}	+(3 days)
	1×10^{-7}	? (3 days)
4. Pyridine-3-carboxylic acid diethylamide ("Coramine")	1×10^{-5}	0
5. Pyridine-3-sulphonate	1×10^{-4}	0
6. Pyridine-3-nitrile	2×10^{-5}	0
7. 3-Methylpyridine (β -picoline)	1×10^{-4}	0
8. Trigonelline methyl sulphate	1×10^{-4}	0
9. Trigonelline chloride	1×10^{-4}	0
10. Pyridine-4-carboxylic acid (<i>isonicotinic acid</i>)	1×10^{-4}	0
11. Pyridine-2-carboxylic acid (<i>picolinic acid</i>)	1×10^{-4}	0
12. Pyridine-2:3-dicarboxylic acid (<i>quinolinic acid</i>)	1×10^{-4}	0
13. 2:4-Dimethylpyridine-3:5-dicarboxylic acid	1×10^{-4}	0
14. 2:4:6-Trimethylpyridine-3:5-dicarboxylic acid	1×10^{-4}	0
15. Nicotine	1×10^{-5}	0

None of the compounds which failed to permit growth was toxic, since growth ensued on subsequently adding nicotinamide. Failure of growth was therefore due to non-utilizability.

No. 4. "Coramine" was obtained from Messrs Ciba, Ltd., through the courtesy of Dr K. Miescher.

Nos. 6 and 9: obtained from Dr H. King.

Nos. 10 and 12: obtained from Prof. G. R. Clemo.

No. 2. A commercial specimen of nicotinic acid which even on repeated crystallization from water gave pale yellow warty clusters of needles, was obtained free from the persistently associated impurities by sublimation and recrystallization from water after digestion with norite charcoal. It then separated in long colourless flat needles, m.p. 232° (uncorr.).

No. 5. *Pyridine-3-sulphonic acid* was prepared by a method based on those given by Meyer & Ritter [1914] and Gastel & Weibaut [1934].

No. 8. *Trigonelline methyl sulphate*. Nicotinic acid (1.0 g.) and freshly distilled dimethyl sulphate (0.8 ml.) heated at 120° for 15 min., yielded a viscous gum which when crystallized from alcohol gave colourless prisms of the methosulphate, m.p. 210° . The alcoholic solution with picric acid yielded trigonelline picrate, m.p. 204° .

No. 13. *2:4-Dimethylpyridine-3-carboxylic acid hydrochloride* was prepared according to Michael [1885].

No. 14. *2:4:6-Trimethylpyridine-3:5-dicarboxylic acid (K salt)* was prepared according to Hantzsch [1882].

No. 11. The copper salt of pyridine-2-carboxylic acid was obtained from Prof. G. R. Clemo, and the free acid (m.p. 139°) was prepared from it by passing H_2S over the dry salt and extracting with water.

Nos. 7 and 15 were both commercial specimens.

cause it cannot synthesize it: the remainder of the codehydrogenase molecule (ribose, adenine) it can synthesize. The inactivity of the diethylamide ("coramine") indicates that the organism cannot remove the two ethyl groups. It can hydrolyse the methyl ester of nicotinic acid since methyl nicotinate is active, although growth is delayed. The other compounds in Table IV, all of which are inactive, emphasize how small an alteration from the structure appearing in the codehydrogenase molecules produces inactivity in growth.

Nicotinic acid has been found among the nutrient requirements of strains of *C. diphtheriae* by Mueller [1937] and of strains of dysentery bacilli by Koser *et al.* [1938]. Fildes [1937] has indicated a series of bacteria whose relative abilities to synthesize the whole or parts of the codehydrogenase I or II molecules are reflected in the nutrient requirements of the different species. It appears probable that the organisms studied by Mueller and by Koser *et al.* will be found to synthesize codehydrogenase I or II, or both.

SUMMARY

An examination of a further series of aneurin and pyridine derivatives emphasizes the previous conclusion that aneurin and nicotinamide are highly specific in the growth requirements of *Staph. aureus*. Only very limited departures from these structures are permissible if growth activity is to be maintained.

A comparison with similar observations made upon other types of organisms, in particular certain fungi and unicellular protista, suggests an underlying biochemical unity.

This work has been carried out under the direction of Dr P. Fildes who, with Dr G. P. Gladstone, has been responsible for the bacteriological side of the work.

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Note added 8 June 1938. Since the above was written Woolley, Strong & Madden (*J. biol. Chem.* 1938, **123**, *Proc. cxxxi*) have reported a similar high specificity of structure for pyridine derivatives in the cure of canine black-tongue. They had previously shown that nicotinic acid or its amide could effect the cure (Elvehjem, Madden, Strong & Woolley, *J. Amer. chem. Soc.* 1937, **59**, 1767; *J. biol. Chem.* 1938, **123**, 137). The non-utilisable derivatives agree with those found for *Staph. aureus* with the exception that β -picoline has a definite effect, and nicotinic acid *N*-methyleamide a slight effect with the dogs.