THE UTILIZATION OF NICOTINIC ACID AND RELATED PYRIDINE COMPOUNDS BY THE PROTEUS GROUP OF ORGANISMS

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The compound nicotinic acid has attracted considerable attention in connection with studies on bacterial nutrition since it has been identified as a "growth factor" for *Staphylococcus aureus* (Knight, 1937, a and b), certain strains of *Corynebacterium diphtheriae* (Mueller, 1937), some of the dysentery bacilli (Koser, Dorfman and Saunders, 1938) and for *Proteus* (Fildes, 1938, Lwoff and Querido, 1939). More recently this compound has become associated with the nutrition of *Brucella* (Kerbey, 1939). Kerbey observed that some species of this genus grew luxuriantly when nicotinic acid was added to a medium containing thiamin hydrochloride and Bacto-Tryptose.

The fate of nicotinic acid in the culture medium, at least in part, appears to be that of entering into the synthesis of some pyridine nucleotides, such as Warburg's coenzyme (triphosphopyridinenucleotide) or cozymase (diphosphopyridinenucleotide). Warburg, Christian and Griese (1935) demonstrated that nicotinamide constitutes a portion of the molecular structure of each of these compounds and that the pyridine ring is involved in reversible oxidation-reduction reactions. Further evidence indicative of nicotinic acid being built up into a coenzyme or cozymase molecule by bacteria is offered by the work of Lwoff and Lwoff (1937, a and b), Fildes (1938), and others.

The work done with *Staphylococcus aureus* suggests that the ability of an organism to utilize derivatives of nicotinic acid, its isomers or closely related compounds, as growth factors, is

limited. (Knight and McIlwain 1938, Landy 1938, a and b, and summarized by Lwoff 1938.)

In the present investigation an attempt has been made to determine whether all of a large stock collection of *Proteus* organisms would behave similarly in the chemically defined medium plus nicotinic acid as used by Fildes (1938), and, at the same time, to evaluate the biological activity of some 13 related pyridine compounds by substituting them individually for nicotinic acid.

EXPERIMENTAL

Preparation of medium

The medium employed throughout this study was that proposed by Fildes (1938), namely:

KH_2PO_4	4.5 grams
$(NH_4)_2SO_4$	0.5 grams
NH_4Cl	0.5 grams
CH_3COONa (M/2)	50 ml.
H_2O (distilled) to	900 ml.

The reaction of this mixture was adjusted to pH 7.6, tubed in 4.5 ml. quantities and autoclaved. Prior to inoculation with the test organism, the medium in each tube was supplemented with the following materials from sterile stock solutions:

	ml.
$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (M/500 in M/50 HCl)	0.12
$MgSO_4 \cdot 7H_2O$ (0.4 per cent in H_2O)	0.05
Nicotinic acid or other pyridine compounds (10^{-3}M in H_2O)	0.10
H_2O (distilled) to a final volume of	5.0

The addition of 0.1 ml. of a 10^{-3} M solution of the pyridine compounds gave a final concentration of the respective substances of 2×10^{-5} M in the cultural medium. That this amount is in excess of that actually required for growth was shown in previous quantitative experiments.

Cultures used

The *Proteus* strains studied in this investigation were obtained from various laboratories and type culture collections throughout this country as well as abroad. Those strains furnished by others

were grouped according to their designation when received and are listed as such in table 1. The two groups listed under *Proteus melanovogenes* were described by Miles and Halnan (1937) and Haines (1938). Although both groups were isolated from rots in hen's eggs, Haines has pointed out that certain differences exist with respect to their physiological characteristics. In addition, several strains were isolated from a variety of specimens received by this laboratory from the University Hospital, and for the present time this group is designated simply as "original isolations". In all, some 189 individual strains of *Proteus* were employed in these experiments.

Pyridine compounds tested

Nicotinic acid and 13 related compounds, as tabulated in table 1, were tested for their biological activity. The nicotinic acid was obtained from the Eastman Kodak Company while all other compounds came from the Research Division of the S. M. A. Corporation, Cleveland.

Technique used to determine biological activity of pyridine compounds

A 24-hour agar slant culture of the various strains was used as the source of inoculum, and a trace of the growth was transferred on the point of a wire needle into the medium under test. After 48 hours incubation at 37°C. those tubes showing evidence of growth (distinct turbidity) were transferred to fresh tubes of the same medium, using one loopful of the positive culture as the inoculum. Five successive transfers, with growth resulting each time, were considered as evidence that the medium satisfied the nutritive requirements of the organism, and this was termed a positive culture. A few strains showed no growth in 48 hours, and in these instances the cultures were incubated for four days before concluding that they were negative, i.e., failed to grow.

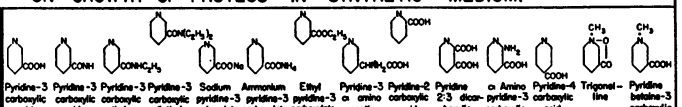
RESULTS

A complete summary of the observations made in this study is presented in table 1. These results show that the response of the

various strains employed was not uniform throughout so far as growth was concerned, although the majority grew in the basic medium plus nicotinic acid (pyridine-3-carboxylic acid) or amide (pyridine-3-carboxylic amide). Further analysis of the data indicates that the following compounds were biologically active to a similar degree: ethylnicotinamide (pyridine-3-carboxylic ethyl amide), diethylnicotinamide (pyridine-3-carboxylic diethyl amide), sodium nicotinate (sodium pyridine-3-carboxylate),

TABLE I

EFFECT OF NICOTINIC ACID & RELATED COMPOUNDS ON GROWTH OF PROTEUS IN SYNTHETIC MEDIUM.

ORGANISMS															
PROTEUS SPECIES OR STRAINS	NUMBER OF CULTURES	Pyridine-3 carboxylic acid	Pyridine-3 carboxylic amide	Pyridine-3 carboxylic ethyl amide	Pyridine-3 carboxylic diethyl amide	Sodium pyridine-3 carboxylate	Ammonium pyridine-3 carboxylate	Ethyl pyridine-3 carboxylate	Pyridine-3 α amino acetic acid	Pyridine-2 carboxylic acid	Pyridine 2:3 dicarboxylic acid	α Amino pyridine-3 carboxylic acid	Pyridine-4 carboxylic acid	Trigonelline	Pyridine betaine-3 carboxylic acid
		NUMBER OF CULTURES POSITIVE													
P. vulgaris, proteus, etc.	46	44	44	44	44	44	44	44	44	4*	4*	4*	4*	4*	4*
Original isolations (from urine, stools, blood cultures, etc.)	54	45	45	45	45	45	45	45	45	1*	1*	1*	1*	1*	1*
XI9, OXI9, HXI9	34	29	29	29	29	29	29	29	29	0	0	0	0	0	0
XL, OXL, HXL	5	5	5	5	5	5	5	5	5	0	0	0	0	0	0
XK, OXK, HXK	6	6	6	6	6	6	6	6	6	0	0	0	0	0	0
X_2, OX_2, HX_2	16	16	16	16	16	16	16	16	16	0	0	0	0	0	0
P. anindologenes	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
P. pseudovalerice	2	2	2	2	2	2	2	2	2	2*	2*	2*	2*	2*	2*
P. para-americanus	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
P. americanus	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
P. ammoniae	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0
P. melanovogenes	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	3	3	3	3	3	3	3	3*	3*	3*	3*	3*	3*
P. ichthyosmus	1	1	1	1	1	1	1	1	1	1*	1*	1*	1*	1*	1*
P. mirabilis	4	4	4	4	4	4	4	4	4	0	0	0	0	0	0
P. subtilis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. asiaticus	6	6	6	6	6	6	6	6	6	5*	5*	5*	5*	5*	5*
P. sphingidis	2	2	2	2	2	2	2	2	2	2*	2*	2*	2*	2*	2*
P. nocturnum	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0

*These cultures grew in the basic medium when the pyridine compounds were omitted.

ammonium nicotinate (ammonium pyridine-3-carboxylate), ethyl nicotinate (ethyl pyridine-3-carboxylate) and nicotinyl glycine (pyridine-3-alpha amino acetic acid). The remaining compounds, picolinic acid (pyridine-2-carboxylic acid), quinolinic acid (pyridine-2:3-dicarboxylic acid), alpha-amino pyridine-3-carboxylic acid, pyridine betaine-3-carboxylic acid, and trigonelline, were inactive. With one exception, these findings are in accord with results reported on similar investigations with *Staphylococcus aureus* strains as the test organisms. Diethyl nicotinamide

(pyridine-3-carboxylic diethyl amide), shown by Landy (1938) and Knight and McIlwain (1938) to be biologically inactive as a growth factor for *Staphylococcus aureus*, was demonstrated as active for *Proteus* in the present investigation.

Our observations also indicate that a few of the strains tested did not respond with growth in any of the media, while others (see footnote at the bottom of table 1) were capable of growing in the absence of any added pyridine compound. Previous investigations on the nutritional requirements of *Proteus* as summarized by Knight (1936) suggest the existence of "exacting" and "non-exacting" strains. Those organisms which failed to grow in the test media employed in this study may constitute the "exacting" group, and the degree of this "exacting nature" will be determined by further studies. Preliminary experiments indicate that the nutritional requirements of most of the cultures in this group can be satisfied by the addition of an amino acid mixture. As yet no "training" experiments have been conducted to determine whether the amino acids may be gradually dispensed with for these particular organisms.

Those strains of *Proteus* which were capable of growing in the basic medium without the addition of nicotinic acid or an equivalent active pyridine compound were evidently able to effect synthesis of such compounds from NH_3. In this respect they may behave similarly to *Escherichia coli*, which is capable of synthesizing cozymase from NH_3 (Fildes, 1938).

THE BEHAVIOR OF MORGAN'S BACILLUS IN THE BASIC MEDIUM PLUS NICOTINIC ACID AND RELATED COMPOUNDS

Since the organisms allocated to this group have been associated systematically with the genus *Proteus*, particularly since the work of Rauss (1936), it was of interest to determine their response when inoculated into the basic synthetic medium plus the various pyridine compounds. Some 37 strains of Morgan's bacillus were obtained from the National Type Culture Collection at the Lister Institute, Dr. K. F. Rauss of the State Hygienic Institute in Hungary and Dr. B. R. Sandiford of the Public Health Laboratories at Cairo. In brief, the results were con-

sistently negative; none of the strains grew on continued subculture in any of the test media employed in this study. The significance of this information with regard to their systematic position as well as their nutritional requirements is under investigation at this time.

SUMMARY

One hundred eighty-nine strains of *Proteus vulgaris* and related species were studied from the standpoint of their ability to grow on continued subculture in a simple chemically defined basic medium plus nicotinic acid or one of 13 other related pyridine compounds. The results indicate that the same pyridine compounds which were previously found to be biologically active for *Staphylococcus aureus* were likewise active for the majority of the *Proteus* strains investigated. In addition, diethylnicotinamide was likewise demonstrated as biologically active. The nutritional requirements of strains allocated to this genus did not appear to be uniform, since some strains were capable of growing in the absence of the nicotinic compounds while others failed to grow even when such compounds were present.

All 37 of Morgan's strains employed were unable to grow in the chemically defined media which supported the growth of the majority of the *Proteus* strains used in this study. Some additional supplement is undoubtedly essential to meet satisfactorily the nutritional requirements of this group.

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