Nutritionally Variant Streptococci

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INTRODUCTION

Clinical microbiologists occasionally observe the growth of small satellite colonies near larger colonies of "helper" bacteria. Diffusion of compounds required by the satellite organism into the agar medium surrounding the helper colony supports a halo of satellite growth. This graphic representation of the phenomenon of microbial commensalism can sometimes be seen on agar surfaces inoculated with specimens containing mixed bacterial flora. Alternatively, satelliting growth can be induced on agar media specifically seeded with a helper organism or with purified forms of the compounds that the latter bacteria produce.

Satelliting streptococci, called nutritionally variant streptococci (NVS) in this review, were first isolated by Frenkel and Hirsch in 1961 (25) from patients with endocarditis and otitis media. Within the first 15 years following their report, only a handful of papers devoted to NVS appeared in the literature. During the late 1970s and throughout the 1980s, however, approximately 50 studies dealing with various aspects of these organisms were published. Although our knowledge of NVS has increased greatly in the last 30 years, these bacteria (also known as nutritionally deficient, pyridoxal- or vitamin B₆-dependent, thiol-dependent, or symbiotic streptococci) still hold their share of secrets and challenges for both laboratorians and clinicians. This review presents information concerning the microbiological characteristics of NVS and their participation in infectious processes.

MORPHOLOGICAL OBSERVATIONS

The first report of the colonial morphology of NVS described their satellite zones as being narrower and with a sharper edge than satellite zones produced by *Haemophilus* species. While satellite growth can be observed after 24 h of

incubation, colonies at the outer edge of the zone become enlarged at 48 h, presumably because of the abundance of nutrients in the surrounding medium (25). Satelliting colonies are usually nonhemolytic or alpha-hemolytic, and a variety of gram-positive and gram-negative bacteria (staphylococci, streptococci, family *Enterobacteriaceae* strains) as well as yeasts will support their growth (13, 25, 39). However, strains of *Streptococcus pyogenes* (39) and *Pseudomonas aeruginosa* (11) were reported to be unable to serve as helper bacteria for NVS. NVS colonies are small, measuring 0.2 to 0.5 mm in diameter (13, 39).

The microscopic appearance of NVS cells can vary from that of a typical streptococcal isolate to swollen pleomorphic forms. This variable morphology is influenced by the nutritional state of the organisms. Under optimal nutritional conditions (i.e., in close proximity to growth of a helper strain or in an appropriately supplemented broth medium), gram-positive cocci or coccobacilli in chains are observed. Colonies growing farther away from the helper strain or on media with less than optimal concentrations of required nutrients contain cells that are pleomorphic, with globular and filamentous forms (16, 28, 39). NVS growing in blood cultures have been described as gram variable or even gram negative, with morphologies reminiscent of those of fungal cells (20, 39). Electron microscopy reveals normal streptococcal ultrastructure in NVS cells grown under optimal conditions. With insufficient nutritional supplementation, cell walls appear thickened, and filament formation seems to result from an inability to produce cross walls (6, 7, 16).

The aberrant cellular morphology displayed by NVS growing under suboptimal nutritional conditions led early authors to question the similarities of these organisms to L-forms. Cell wall-deficient L-forms arise spontaneously or as a result of the treatment of bacteria with penicillin or murelytic enzymes in a hypertonic medium. The resulting

spherical structures are completely or partially devoid of cell walls and may persist as L-forms or revert spontaneously to walled cells. L-forms are resistant to cell wall-active agents and form colonies with a "fried egg" appearance on osmotically supportive solid media, similar to those of mycoplasmas (38). Frenkel and Hirsch (25) found that two of their four NVS isolates produced typical L-form colonies, but other authors (13, 28) were unable to demonstrate L-form growth with any of their strains.

PHYSIOLOGICAL AND TAXONOMIC STUDIES

Nutritional Requirements

The original efforts to identify the factor(s) required for growth of NVS focused on sulfhydryl-containing compounds. Frenkel and Hirsch (25) reported that while cysteine, thioglycolate, reduced glutathione, and thiomalic acid were capable of supporting the growth of NVS, numerous other sulfur-containing compounds were ineffective. They hypothesized that their strains were unable to reduce disulfide bonds, a defect overcome by the addition of sulfhydryl compounds to the growth medium. Other studies (11, 13, 39) demonstrated that L-cysteine was an effective supplement for NVS growth. Cayeux and co-workers (13) used brain heart infusion as a basal medium and found that good growth was produced by either L-cysteine or glutathione in combination with either thioglycolate or dithiothreitol.

The possibility that these stimulatory compounds were encouraging growth by altering redox potential was addressed by a number of authors (13, 25, 28). Anaerobiosis alone would not allow growth of NVS; their nutritional requirements remained the same regardless of the growth atmosphere. Some interesting variations on the effect of growth atmosphere have, however, been noted. Some of the strains described by McCarthy and Bottone (39) grew only under anaerobic conditions with added L-cysteine. Koshi and Lalitha (36) reported an endocarditis isolate that grew readily in an anaerobic atmosphere but required cross-streaking with a helper organism for growth in air. Organisms displaying this behavior have been isolated in my laboratory, but these strains do not display the enzymatic profiles of typical NVS (52; see "Identifying Characteristics of NVS" below). Perhaps these organisms are atypical strains of NVS or variants of other streptococcal species.

A number of authors have documented the ability of vitamin B₆ analogs to support the nutritional requirements of NVS. George (28) found that pyridoxine (vitamin B₆) supplementation allowed for growth of NVS on horse blood agar. Carey and associates (11) and Roberts et al. (50) found that while pyridoxal and pyridoxamine (active forms of vitamin B₆) were effective, pyridoxine did not support growth of NVS. Sherman and Washington (54) reported that pyridoxine was inhibitory for an NVS strain they studied. The apparent discrepancies between these observations and those of George could have been caused by pyridoxine preparations containing other B₆ analogs or confusion over the nomenclature of vitamin B₆ compounds. The studies of Schiller and Roberts (53) suggested that pyridoxine is not assimilated by NVS and can inhibit their growth in media supplemented with other forms of vitamin B₆, presumably by competing for binding sites in a transport system for vitamin B₆ compounds.

It is now generally agreed that pyridoxal hydrochloride at a concentration of 0.001% or L-cysteine at a concentration of 0.01% will support the growth of typical NVS. The fact that

vitamin B₆ analogs function as coenzymes in the synthesis of cysteine and other sulfhydryl compounds explains their efficacy in substituting for sulfhydryl-containing molecules in the growth medium. The participation of vitamin B₆ in the conversion of L-alanine to D-alanine (necessary for peptidoglycan synthesis) may account for the gram variability and pleomorphism observed in NVS grown on nutritionally suboptimal media (1).

Bouvet et al. (7) investigated the growth of NVS on a chemically defined medium originally developed for the culture of group A streptococci. Good growth of NVS was obtained only when this medium was supplemented with a dialysate of Todd-Hewitt broth. Grown under these conditions, NVS strains exhibited normal streptococcal ultrastructure. Alteration of the pyridoxal and cysteine concentrations in the medium revealed variability in the nutritional requirements of individual strains.

Many commonly used laboratory media are incapable of supporting the growth of NVS unless supplemented with pyridoxal or some other suitable compound. Published studies have produced conflicting reports of the usefulness of some media for culturing NVS. These discrepant results are probably due to inconsistent levels of required nutrients in different formulations of the same medium (e.g., manufacturer-to-manufacturer variation) or variability in the levels of nutrients required by individual NVS strains. In general, unsupplemented tryptic soy agar with 5% sheep blood does not support NVS growth, and the behavior of chocolate agars is variable (43, 47). Different results have been obtained with brain heart infusion, Todd-Hewitt broth, and Columbia blood agar (18, 43, 61). Authors who examined thioglycolate or thiol broths reported successful culture of NVS with these media (18, 28, 31, 39, 47, 61).

Since NVS are etiologic agents of endocarditis, the ability of these organisms to grow in commonly used blood culture media is of interest. The addition of human blood to these media, in order to simulate blood culture conditions, was found to enhance the recovery of NVS, probably because of the pyridoxal contained in human erythrocytes (31, 47, 61). Although the results of such studies are influenced by medium, strain, and inoculum size variation, a few conclusions can be drawn. Thioglycolate or thiol broths are adequate for the growth of NVS in blood specimens. Other media, except for tryptic soy broth, seem to be satisfactory as long as they contain blood. Tryptic soy broth should be supplemented with pyridoxal, since the addition of human blood alone will not ensure recovery of all strains (31, 47, 61). Two studies (31, 61) suggest that early (within 48 h) subculture of bottles suspected of harboring NVS is required, since the viability of organisms begins to decrease with continued incubation. Gill and Williams (30) found that horse blood agar supplemented with pyridoxal was useful for recovery of NVS with the Dupont Isolator system.

Identifying Characteristics of NVS

In addition to studies on morphology and the unique nutritional requirements of NVS, numerous publications have dealt with the biochemical activities and other microbiological characteristics of these organisms. Early work led to the conclusion that NVS were nutritional mutants of species of viridans streptococci. In these studies, biochemical activities were usually determined in pyridoxal-supplemented media. Under those conditions, isolates of NVS were reported to resemble *Streptococcus mitis* (mitior), *Streptococcus salivarius*, *Streptococcus sanguis* II, *Strepto-*

coccus sanguis, *Streptococcus intermedius*, *Streptococcus anginosus*, *Streptococcus constellatus*, or *Streptococcus morbillorum* (11, 18–20, 33, 50, 68).

Further investigation suggested that many NVS isolates most closely resembled *S. mitis*. Roberts and colleagues (50) reported that NVS strains contain amounts of cell wall rhamnose similar to those found in *S. mitis*. In a more detailed examination of cell wall constituents, van de Rijn (62) found that NVS strains contain ribitol and phosphorus (perhaps constituents of a ribitol teichoic acid), small amounts of rhamnose, and, in some isolates, galactosamine. These compounds are distinguishing features of *S. mitis* cell walls.

Bouvet and colleagues (7) described a chromophore common to NVS and *S. mitis* cells. When these bacteria are subjected to acid and heat (boiling for 5 min in 2 N HCl [pH 2]), cell suspensions turn red, indicating either the release of preformed chromophore or the production and subsequent release of chromophore. Van de Rijn and Bouvet (65) found that the chromophore is localized in the cell wall and is not released by trypsin treatment. Stein and Libertin (56) demonstrated that the chromophore can be released by lysozyme more efficiently from stationary-phase cells than from exponentially growing cells and suggested that the chromophore was carbohydrate in nature. The chromophore's absorbance maximum occurs at 504 nm, and absorbance decreases when the pH rises above 2.5 (65). Production of chromophore by NVS is dependent on the growth medium (57, 65), and NVS, *S. mitis*, and *S. sanguis* II (considered in some identification schemes to be the same as *S. mitis*) seem to be the only streptococci that contain this compound (8).

Despite compelling evidence for the similarity between NVS and *S. mitis*, the work of Bouvet and colleagues (8) revealed differences in the enzymatic capabilities and penicillin-binding proteins of these two groups of bacteria. They also described three biotypes of NVS and suggested that these organisms were a separate group of streptococci rather than variants of *S. mitis*. One useful datum noted by Bouvet and co-workers was the elaboration of the enzyme pyrrolidonyl arylamidase by NVS but not by *S. mitis*. Since this enzyme is also absent in other species of viridans streptococci (22), it serves as a valuable marker for NVS identification. It should be remembered, however, that this enzyme is not unique to NVS. Other gram-positive coccoid bacteria (*S. pyogenes*, some coagulase-negative staphylococci, and members of the genera *Enterococcus*, *Aerococcus*, *Lactococcus*, and *Gemella*) also produce pyrrolidonyl arylamidase (51). In addition to the enzymatic and penicillin-binding protein differences noted by Bouvet et al. (8), Stein and Libertin (58) demonstrated discrete restriction endonuclease chromosomal digestion patterns in successive blood isolates of *S. sanguis* II, *S. mitis*, and an NVS strain from the same patient. Further evidence of the unique characteristics of NVS was provided by Pompei and colleagues (44). They found bacteriolytic activity against *Micrococcus luteus* in NVS isolates but not in strains of viridans streptococci.

Taxonomic data, including data from DNA-DNA hybridization studies, prompted Bouvet and co-workers to propose two new *Streptococcus* species to accommodate NVS (5). *Streptococcus defectivus* includes the NVS biotype 1 strains, and *Streptococcus adjacens* accounts for the biotype 2 and 3 strains described previously (8). Both species have the following characteristics in common: they are resistant to optochin but susceptible to vancomycin; pyrrolidonyl arylamidase and leucine aminopeptidase are produced, but alkaline phosphatase is not produced; hippurate and arginine

TABLE 1. Differential characteristics of *S. defectivus* and *S. adjacens*^a

Characteristics	Reaction ^b	
	<i>S. defectivus</i>	<i>S. adjacens</i>
Production of:		
α - and β -galactosidases	+	—
β -Glucuronidase	—	V
β -Glucosidase	—	V
Acidification of:		
Trehalose	+	—
Inulin	—	V
Lactose	V	—
D-Raffinose	V	—
Starch	+	—

^a Data are those of Bouvet et al. (5).

^b +, Positive; —, negative; V, variable.

are not hydrolyzed; no acidification of D-ribose, L-arabinose, D-mannitol, sorbitol, or glycogen occurs; and polysaccharides are not produced from sucrose (5). Differential characteristics of the two species are shown in Table 1. Strains belonging to both species produce lactic acid as the major end product of glucose catabolism, lending further support for inclusion of NVS in the genus *Streptococcus* (12).

SEROLOGICAL STUDIES

Lancefield Antigens

Many isolates of NVS have been examined for the presence of Lancefield antigens. While many strains were ungroupable, strains with group antigens A (14, 35, 45, 57), F (28), H (7, 11), L (7), and N (13) have been reported. Recently published species descriptions characterize *S. defectivus* as ungroupable or producing a weak reaction with group H antiserum and *S. adjacens* as ungroupable (5).

The nutritionally variant group A strains that have been described appear to be variants of *S. pyogenes* rather than typical NVS. These isolates (14, 35, 45) were beta-hemolytic, and some were typeable with M and T antisera, suggesting a close relationship to *S. pyogenes*. A group A NVS strain was recently found to be chromophore negative, unlike typical NVS (57). Chapman's study (14) of numerous group A strains isolated from skin lesions and upper respiratory tract specimens suggests that L-cysteine stimulated growth of the streptococci on sheep blood agar by reversing the effects of an inhibitory factor in sheep serum. These strains required nutritional supplementation only when grown under aerobic conditions (14). Organisms like these may be true variants of *S. pyogenes* instead of NVS as currently defined (5).

Antigens Unique to NVS

The inability to demonstrate a common Lancefield antigen among the NVS spurred research into the serological characteristics of these organisms. Van de Rijn and George (66) studied a large collection of NVS and found that although these organisms failed to produce a common group antigen, three serotype antigens not found in other streptococci could be identified. The distribution of these antigens in the strains studied allowed the delineation of three serotypes. The majority (about 75%) of the isolates could be placed into one

of the three serotypes, while most of the remaining strains contained more than one antigen. Only 4 of the 103 isolates studied failed to express any of the serotype antigens. Surface proteins specific for serotype I were detected, while serotypes II and III displayed one or more common surface proteins.

Further serological investigations of NVS by George and van de Rijn (26, 27) led to the purification and characterization of the serotype I antigen. This amphipathic molecule appears to be a lipid-substituted ribitol teichoic acid. The serotype I antigen was found in both intra- and extracellular locations. In addition to serving as a serotyping antigen, this molecule replaces lipoteichoic acid in NVS.

Host Response to NVS Antigens

Using a rabbit endocarditis model, van de Rijn (63) demonstrated that antibody to heat-killed cells of any of the three NVS serotypes protected against infection with the homologous strain. However, antibody against purified serotype I antigen was not protective, suggesting that some other surface molecule is probably involved in adherence of NVS to host tissue. Immunization with a given serotype followed by challenge with a heterologous serotype resulted in cross-protection between serotypes II and III, but serotype I antibodies were effective only against homologous strains. Passively transferred serotype I antibodies did not prevent infection, suggesting that additional components of the immune system are involved in protection against endocarditis (64).

An enzyme-linked immunosorbent assay for serotype I antibodies was positive with sera from approximately three-fourths of 31 human patients with NVS endocarditis. Although none of the healthy control patients had elevated titers of serotype I antibody, 2 of 30 patients with non-NVS streptococcal endocarditis did. Both patients had been infected with *S. mitis*, but elevated serotype I titers were not found in nine other *S. mitis* patients (67). Assay of anti-NVS antibodies could prove to be helpful in diagnosing endocarditis caused by NVS, especially when difficulty in recovering the organism is encountered.

ANTIMICROBIAL SUSCEPTIBILITY OF NVS

In Vitro Studies

A number of reports on the antimicrobial susceptibility of NVS have appeared in the literature (3, 11, 13, 17, 19, 20, 23, 28, 29, 37, 39, 40, 46, 50, 55). Many of these publications deal with small numbers of strains, and methods and results are variable. The largest studies are those of Cooksey and Swenson (17), Gephart and Washington (29), Roberts et al. (50), and Bosley and Facklam (3). These studies show that while many NVS strains are susceptible to penicillin, a fair number have penicillin MICs that would place them in the moderately susceptible category according to current guidelines from the National Committee for Clinical Laboratory Standards (41). In the recent study of Bosley and Facklam (3), a high percentage (65%) of the 43 strains examined were moderately susceptible to penicillin. High-dose penicillin or penicillin plus an aminoglycoside is recommended for treatment of serious infections caused by such organisms (41). Bosley and Facklam also noted that four of their strains had penicillin MICs of greater than 4 µg/ml, indicating resistance (3).

In studies by Gephart and Washington (29) and Cooksey

and Swenson (17), most strains were susceptible to clindamycin, chloramphenicol, erythromycin, and vancomycin. Variable results with aminoglycosides have been noted; gentamicin MIC ranges of 2 to 32 µg/ml (17) and 0.5 to 8 µg/ml (3) have been reported. Variable results also occur with cephalosporins. Gephart and Washington (29) found rifampin to be the most active antibiotic they tested, and Stein and Libertin (55) provided evidence for vancomycin-rifampin synergy against NVS in time-kill studies.

In Vivo Studies: Synergy

One of the first studies examining in vivo antimicrobial activity against NVS used mice into which bacteria contained in agar cylinders were implanted intraperitoneally. Using this technique, Cayeaux and associates (13) concluded that penicillin and streptomycin exerted no synergistic effect in vivo, even though synergy was observed in vitro. Three subsequent papers examined synergistic drug combinations in a rabbit endocarditis model, and all concluded that penicillin plus an aminoglycoside was more effective than penicillin alone (4, 10, 32). Henry and co-workers (32) demonstrated penicillin and high-dose streptomycin to be more effective than a penicillin-low-dose-streptomycin combination. The action of penicillin plus low-dose gentamicin was comparable to that of both penicillin-high-dose gentamicin and penicillin-high-dose streptomycin. Bouvet and co-workers (4) observed that vancomycin alone was as effective as penicillin plus an aminoglycoside or vancomycin plus an aminoglycoside. They noted that in vivo results differed from those observed in vitro and suggested that the differing physiological states of NVS cells growing under the two conditions might account for these results.

Tolerance

Antibiotic tolerance in NVS, defined as an MBC/MIC ratio of 32 or greater, has been observed with penicillin (4, 23) and vancomycin (23). Holloway and Dankert (34) studied tolerance in 11 strains of nutritionally deficient streptococci and found that tolerance to penicillin in all isolates could be demonstrated in the presence of pyridoxal, cysteine, penicillinase, and a staphylococcal streak on the subculture medium. None of the strains displayed tolerance when the subculture medium was supplemented with only pyridoxal and cysteine, and various numbers of isolates were tolerant in the presence of other combinations of supplements. This work emphasizes the impact of methodology in such studies. If similar phenomena occur in vivo, they might contribute to the difficulties encountered in treating NVS endocarditis (see Clinical Importance of NVS below).

CLINICAL IMPORTANCE OF NVS

Endocarditis

NVS are part of the normal oral flora (5, 28, 44) and, like other residents of the oral cavity, may cause endocarditis. The data of Roberts and co-workers (50) suggest that NVS account for 5 to 6% of microbial endocarditis cases and may be an important cause of culture-negative endocarditis. A number of studies referring to endocarditis caused by NVS have appeared in the literature (3, 13, 19–21, 23, 25, 36, 37, 39, 40, 46, 49, 50, 59, 68). Three cases in children (23, 40) and two cases of prosthetic-valve endocarditis (21, 59) are included among these reports.

Stein and Nelson (59) recently reviewed the clinical aspects of 30 cases of NVS endocarditis. They found that the mortality rate in their series was higher than that reported for endocarditis caused by enterococci or viridans streptococci. Although vegetations seemed to be comparatively small, embolization was common. These infections proved difficult to treat; a relapse rate of 17% and a bacteriologic failure rate of 41% were noted despite treatment with antibiotics that were effective in vitro in two-thirds of the cases reviewed. Stein and Nelson hypothesized that the slow growth rate of NVS may account for the difficulties encountered in treatment and suggested that longer courses of antimicrobial therapy may be required for successful cures. Frehel and co-workers (24) demonstrated increased structural abnormalities and exopolysaccharide production in later stages of rabbit endocarditis due to NVS. They suggested that nutrient limitation within vegetations could account for altered cell morphology and that exopolysaccharide might be involved in NVS pathogenicity.

Other Infections

In addition to their involvement with endocarditis, NVS have been implicated in a variety of other infections. Reports have documented the isolation of NVS from the blood of patients with postpartum or postabortal sepsis (39), cirrhosis (39), and pancreatic abscess (11). Isolations have also been made from patients with otitis media (25), otitis externa, wound infections, and vaginal discharge (28) and from a synovial biopsy sample from a patient with culture-negative endocarditis (69). NVS have been implicated in conjunctivitis (2) and infectious crystalline keratopathy (42). Specimens from various equine, bovine, and ovine infections (15), including corneal ulcers in horses (33), have yielded NVS. In some of the reports mentioned above, other bacteria were also isolated, making it difficult to assess the pathogenic role of NVS.

LABORATORY CONSIDERATIONS

NVS should be suspected when Gram stains of specimens reveal microbial cells but cultures are negative. Microbiologists should remember that NVS cells may exhibit variable morphology and staining characteristics (see Morphological Observations above). Supplementation of laboratory media is desirable and often necessary to encourage the growth of these organisms. Filter-sterilized pyridoxal hydrochloride solutions (added to produce a final concentration of 0.001%), pyridoxal-containing filter paper disks (commercially available), or cross-streaks of a suitable helper strain (*Staphylococcus aureus*) can be employed for culture of NVS. Reimer and Reller (48) studied the growth of non-NVS clinical isolates on pyridoxal-supplemented Trypticase soy-based sheep blood agar. They found that some strains of *S. pyogenes* were inhibited on this medium, and they therefore recommended against the routine use of pyridoxal-supplemented media.

Once a suspected NVS is cultured, its identity should be confirmed by establishing its requirement for pyridoxal. This test should be carried out on a medium that is incapable of supporting the organism's growth without pyridoxal supplementation. A positive pyrrolidonyl arylamidase test along with typical morphology (see Morphological Observations above) should further serve to identify an isolate as an NVS. Additional characteristics can be tested with the API Rapid Strep strip (called the API 20 Strep in Europe and the United

Kingdom). The method of Bouvet and co-workers (8), using pyridoxal supplementation, should be adhered to. Pompei and colleagues reported that this system allowed for species identification (*S. defectivus* or *S. adjacens*) in all 34 isolates they examined (44). Bosley and Facklam, however, found that approximately half of the 44 isolates they examined could not be identified to the species level with this product (3).

Reference to previously described treatment protocols (see Clinical Importance of NVS above) should guide the antimicrobial therapy of serious infections caused by NVS. If antibiotic susceptibility studies are to be attempted in the laboratory, supplemented media must be employed. Thornsberry et al. (60) recommended cation-supplemented Mueller-Hinton broth with 5% lysed horse blood and 0.001% pyridoxal for broth microdilution testing of NVS. Inocula should be prepared with growth taken from an agar plate, and susceptibility tests may be incubated in increased CO₂ if necessary. Two studies (3, 9) reported the use of commercially available microdilution trays for determining antimicrobial susceptibilities of NVS.

FUTURE PROSPECTS

Since their original description in 1961, the NVS have evolved from laboratory curiosities into bona fide streptococcal species. Although we know a fair amount about their microbiology, further studies will no doubt provide insights into the mechanisms by which they produce disease. This in turn should allow the development of better therapies for the treatment of endocarditis caused by these organisms. In clinical laboratories, awareness of NVS and the willingness to seek them out in apparently negative cultures will add to our understanding of the roles they play in other types of infections. The vast opportunities for basic and applied studies of the NVS are open to any microbiologist with access to a small amount of pyridoxal.

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