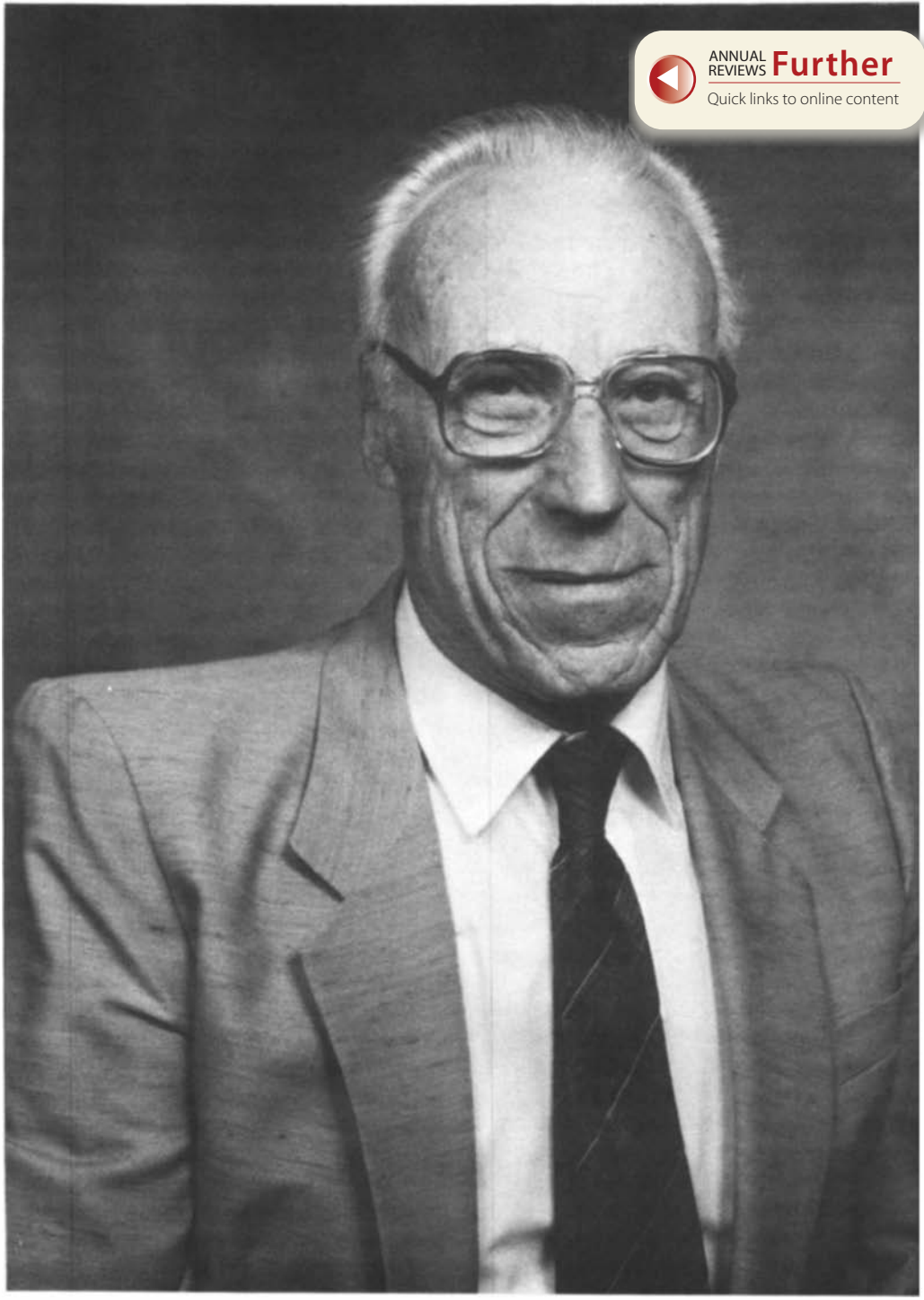

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NUTRITION RESEARCH WITH LACTIC ACID BACTERIA: A Retrospective View[1]

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CONTENTS

INTRODUCTION

From 1935 to 1955 an almost explosive growth occurred in knowledge of the nutritional requirements of animals and bacteria. Results in each of these

[1]This chapter cites only a few original articles from the relevant literature. It emphasizes work on the lactic acid bacteria during the period between about 1936 and 1952 and is an extension of previous accounts of this subject (75, 79). Reviews that cover nutritional requirements of both these and other microorganisms in greater detail are available (16, 18, 55, 77, 34). Reviews dealing extensively with development and use of microbiologic assays are also available (1, 3, 77). A useful anthology of papers in bacterial nutrition has appeared (35).

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fields contributed greatly to understanding the other. However, the extent to which studies of microbial nutrition contributed to recognition of the identity, distribution, and function of vitamins and related nutrients is not widely known today, and these are the contributions I emphasize here.

Today's biochemists have difficulty appreciating the many hurdles associated with the initial definition of the trace nutrients required by either animals or microorganisms. Imagine a time when the basis for nutritional requirements was unknown and their complexity unsuspected; when differences in the nutritional requirements of related species or even (in bacteria) different strains of one species also were unsuspected; when nutritionally important substances such as amino acids were mostly unavailable from supply houses; when present criteria of purity were lacking, and even supposedly pure compounds frequently contained sufficient impurities to obscure a given nutritional response; and when purification procedures—chromatography was barely beginning, ion exchange resins and molecular sieves were unknown—were primitive by today's standards. Small wonder that 27 years elapsed between the discovery in 1901 of "bios," a trace nutrient required for yeast growth (106), and identification of inositol as one of its components in 1928 (13); and that four years elapsed following the first isolation of thiamine by Jansen and Donath in 1926 and recognition of its identity with an unidentified growth factor for yeast (110).

One hotbed of research in nutritional biochemistry during this period was the University of Wisconsin. I was fortunate to receive a $400 Wisconsin Alumni Research Foundation Fellowship (the great Depression was still on) that permitted me to enroll there as a graduate student in the fall of 1935. There, to my own surprise, since I was an undergraduate chemistry major with no exposure to biochemistry, I chose biochemistry as a major field and W. H. Peterson, a fermentation biochemist, as my major professor. For a thesis problem, he suggested we explore the nutritional requirements of lactic acid bacteria. Neither he nor I suspected at that time the complexity of these requirements, which turned out to parallel to a remarkable extent those of animals and that required for their elucidation the efforts of many individuals and many more than the four years I spent under Peterson's guidance in Madison. I emphasize these findings in the following narrative, not because they were always the first or most important in characterizing a given nutrient, but rather because I am most familiar with them and because they illustrate the manifold contributions of such studies to animal nutrition. These contributions include (*a*) the initial or independent discovery and identification of several vitamins and related substances, (*b*) the provision of convenient, sensitive, and accurate methods for following purification of these substances and for determining their distribution in nature, and (*c*) the provision of clues to the metabolic origin, roles, and fate of several vitamins.

ACETATE, LIPOATE, RIBOFLAVIN, PANTOTHENATE, AND PANTETHEINE

Where were we to begin investigation of the lactic acid bacteria? Of the presently known B vitamins, only thiamine was available in pure form. Riboflavin had just been isolated in Europe, and Orla-Jensen et al reported in 1936 (51) that it and additional factors of unknown nature were required for growth of certain lactic acid bacteria in a charcoal-treated whey medium.

The experimental approach we chose was similar to that used in studies of animal nutrition, i.e. to use a crude medium (Medium A: peptone, 0.5%, glucose, 1%: inorganic salts) that did not permit good growth of our test organism (initially *Lactobacillus delbrueckii)* and to determine what supplements to the medium were necessary for growth. A potato extract was an excellent source of unidentified growth-promoting factors (Figure 1), one or more of which was not precipitated by mercuric acetate in sodium carbonate (Neuberg's reagent), could be extracted in part with ether from the acidified, dried extract, and was partially replaced by sodium acetate. These results demonstrated for the first time the peculiar efficacy of acetate in promoting growth of lactic acid bacteria and led to inclusion of acetate in essentially all later media designed for nutritional studies with these organisms. Clearly, however, acetate was present in insufficient amounts to account for the growth-promoting effects of the potato extract. Much later, in following up these observations, we partially purified a water-soluble, acetate-replacing factor that was far more active than acetate in promoting growth of organisms such as *Lactobacillus casei* (19) (Figure 2). By use of the bacterial assay thus provided, Reed et al (63, 64) isolated a crystalline compound that they named *lipoic acid* and that proved to be an essential cofactor in oxidation of pyruvate to acetate by the pyruvate dehydrogenase (49, 62). The same substance was

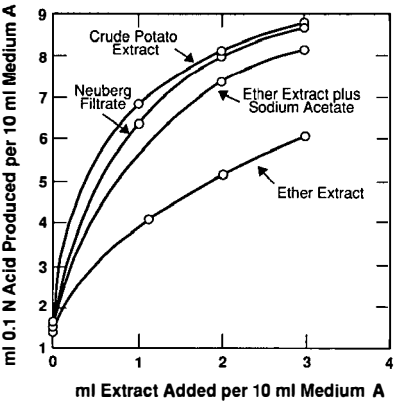

Figure 1 Effect of fractions of a potato extract and acetate on growth of *Lactobacillus delbrueckii* in Medium A. [From (93) with slight modifications.]

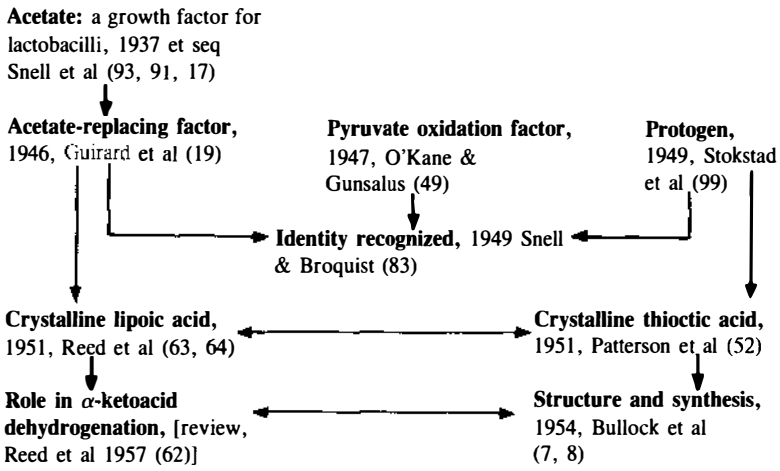

Figure 2 A partial chronology of findings related to the discovery, isolation, structure, and function of lipoic acid.

isolated independently at Lederle Laboratories as a growth factor for the protozoan *Tetrahymena geleii*. These studies eventually led to the synthesis of lipoic acid (7, 8).

When sodium acetate was added to Medium A, relatively good growth of *L. casei* was obtained. When the peptone was treated with alkali (1N NaOH) for 24 h at room temperature, however, no growth occurred unless small amounts of yeast or liver extracts were added. One of the substances necessary under these conditions was identified as riboflavin, which was known to be alkali labile and to be required by certain lactic acid bacteria (51). Addition of both riboflavin and acetate to the NaOH-treated peptone medium (Medium B), however, permitted growth only if an additonal, unidentified factor supplied by yeast or liver extracts was added. I spent more than two years as a graduate student purifying this substance. Toward the end of this period, apparent similarities in properties led us to exchange purified preparations with Professor R. J. Williams. We found his concentrates of an unidentified growth factor for yeast (named *pantothenic acid* although they, too, were not pure) were highly active for our test organisms and vice versa. The chronology of microbiologic studies in this area is shown in Figure 3. Williams's group had discovered pantothenic acid before we did and was substantially ahead of us in activity of their concentrates. They also found that β-alanine substituted for pantothenate in promoting yeast growth under some conditions. This observation led them to the discovery that pantothenic acid was an amide of an unidentified hydroxy acid with β-alanine. The Merck group later isolated and characterized this hydroxy acid as its lactone (97) from hydrolysates of pantothenic acid concentrates in work that led to the initial synthesis of pure pantothenic acid (96).

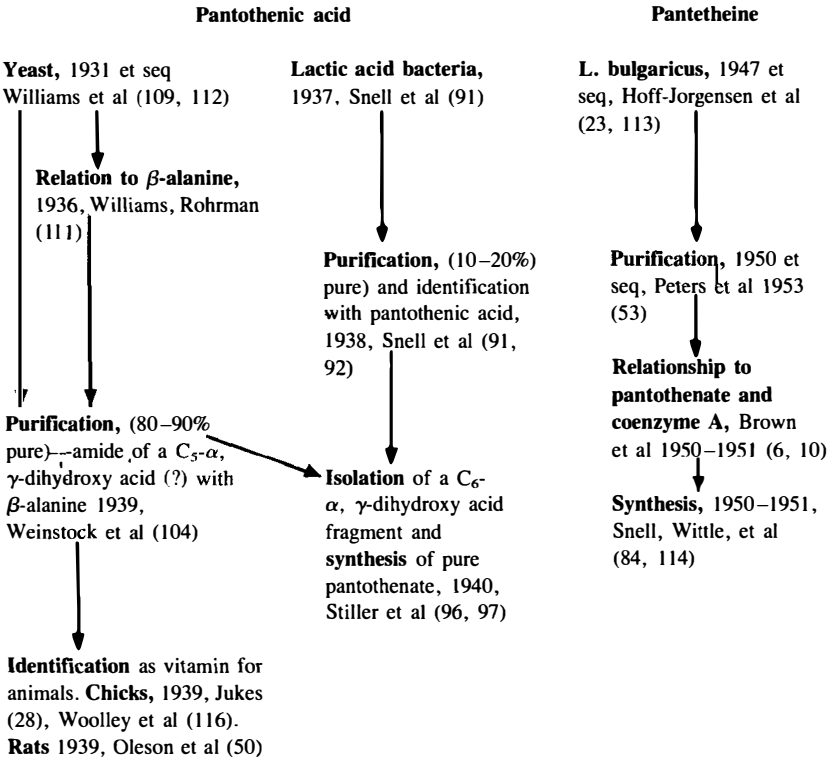

Figure 3 A partial chronology of studies leading from the discovery of pantothenic acid and pantetheine as microbial growth factors to their synthesis.

Some nine years later, we discovered another unidentified factor necessary for growth of *Lactobacillus bulgaricus* in a medium that contained pantothenate (Figure 3). Large amounts of pantothenate replaced this factor, however, which indicated a close relationship of the two substances. We eventually showed that the growth factor was an amide of pantothenic acid with the then-unidentified fragment of coenzyme A, β-mercaptoethylamine (cysteamine), a substance not previously known to occur in nature (6). We named the -SH form of the synthetic compound *pantetheine* and the disulfide form *pantethine* by euphoniously combining segments of the names *pan*tothenic acid, β-mercapto*eth*ylamine, and cyste*ine*-cyst*ine* (84).

MICROBIOLOGIC ASSAYS

I have jumped ahead of events, however. To return to 1938, we had a medium in which excellent growth responses of *L. casei* to either pantothenate (if riboflavin were added) or to riboflavin (if pantothenate were added) could be obtained. The nutritional significance of riboflavin and its distribution in

foods were topics of considerable interest at that time; we believed that our procedure for its determination with *L. casei* would be far superior to other methods then available. Since pantothenic acid was not yet commercially available, we supplied it (and perhaps other stimulatory, though nonessential, growth factors) by adding to Medium B a yeast extract (0.2%) from which riboflavin had been removed by adsorption on lead sulfide and photolysis (Medium C). The growth response of *L. casei* to riboflavin in this medium was excellent (Figure 4), whether measured acidimetrically or turbidimetrically, and values obtained for the riboflavin content of a series of standard samples (Table 1) were in excellent agreement with those obtained by the much more lengthy, cumbersome, and expensive rat assay. Recoveries of added riboflavin were excellent, and the organism responded to a series of riboflavin analogs in much the same way as rats. As a result, this assay was rapidly accepted, was widely used, and served as a prototype for similar methods developed later for each of the other B vitamins [for reviews see (1, 3, 77)].

We found later that the idea of using microorganisms for vitamin assay was not new. For example, R. J. Williams (107) had suggested use of yeast for assay of "vitamine" as early as 1919, before "vitamine" was known to be a complex and before any components of that complex had been identified. West & Wilson (105) had described use of *Staphylococcus aureus* for thiamine assay in 1938. That the latter method failed to gain wide recognition I believe stems from several factors: (*a*) other reasonably satisfactory traditional methods (e.g. the thiochrome method for thiamine) were available, (*b*) *S. aureus* is a potential pathogen, and (*c*) the method was not validated by careful recovery experiments or cross-checking against other available

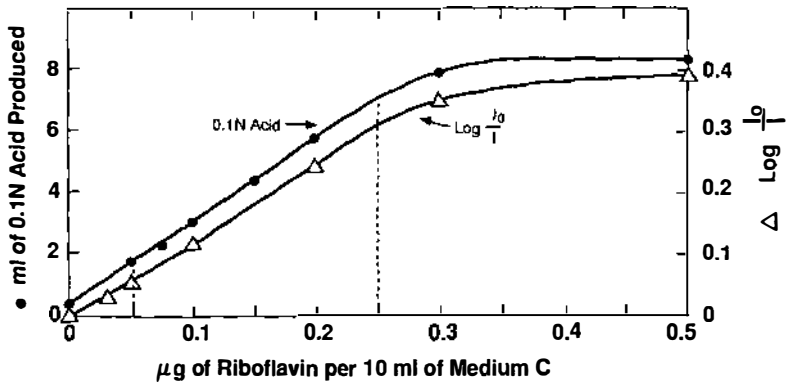

Figure 4 Response of *Lactobacillus casei* to pure riboflavin in the riboflavin-free assay Medium C. [Redrawn from (90).]

Table 1 Riboflavin content of crude reference samples as determined with *Lactobacillus casei* or with rats. From (90)

Material	Microbiologic assay		Rat assay	
	Direct (μg/g)	Extract (μg/g)	Direct (μg/g)	Bourquin-Sherman units[a]
Dried grasses				
Sample I	—	24.4	20	—
Sample II	—	24.1	25	—
Sample III	—	22.6	22	—
Sample IV	—	23.9	20	—
Dried oat plant	30.3	31.2	35	—
Skim milk powder	17.1	—	17	—
Debittered yeast *a*	39.2	38.5	37	—
Debittered yeast *b*	—	36.5	(38.8)	17.7
Debittered yeast *c*	34.7	33.6	(34.4)	15.7
Yeast extract	149.6	—	(145.6)	66.5
Nondebittered yeast	31.8	32.8	(32.0)	14.6

[a] A value of 2.19 μg riboflavin/unit was used for conversion of these values to those in parentheses.

methods. In contrast, the lactic acid bacteria used for assay of riboflavin and many other vitamins are nonpathogens, and their growth can be followed either turbidimetrically or by titration of the lactic acid produced. The latter feature was important, since some samples were highly colored or even turbid; furthermore, appropriate photoelectric colorimeters or nephelometers for turbidimetric measurements of growth were expensive and not widely available at that time.

The complexity of the medium required for growth of lactic acid bacteria, sometimes cited as a disadvantage of these methods, actually is an advantage. Because such media contain many different physiologically important materials that could stimulate (or inhibit) response to an essential vitamin, their use minimizes aberrant responses that might result from addition of these same materials with the sample being assayed. Such aberrant responses were often observed in minimal media such as those used for the initial detection of the vitamins or in simple "synthetic" media such as those later used for demonstrating the response of auxotrophic mutants to a limiting nutrient. Assays with such mutants or with wild-type bacteria in minimal media have been very useful in limited areas, such as in purification of a growth factor or in studies of its biosynthesis or degradation. For quantitative assay of crude samples, however, complex assay media are preferable. The problems in use of minimal media with crude samples are akin to those in determining the pH of

unbuffered samples; one gets a number, but its quantitative significance is doubtful.

NICOTINIC ACID, PYRIDOXINE, BIOTIN, AND AVIDIN

At this point, we could begin to analyze the unknown substances supplied by the peptone component of the medium. For this purpose, the NaOH-treated peptone of Medium B was replaced by an acid hydrolysate of purified casein plus tryptophan, and riboflavin and a pantothenic acid concentrate were added. Under these conditions a distinct growth response of several lactic acid bacteria to nicotinic acid was obtained (92). We tried nicotinic acid because it had already been established as an essential nutrient for some bacteria in reports that appeared during the spring of 1937 [for *Staphylococcus aureus* by Knight (32) and for *Corynebacterium diphtheriae* by Mueller (45)]. These reports followed the discovery in 1936 (38) that NAD^+ was essential for growth of *Hemophilus parainfluenzae*. I well remember Bob Madden (one of Professor Elvehjem's graduate students), Wayne Woolley, and myself discussing these discoveries in a late evening laboratory "bull session." Very shortly thereafter Madden gave a nicotinic acid supplement to his black-tongue-afflicted dogs. Forty-eight hours later the answer was in: it worked! Their important report (14) and subsequent reports of the effectiveness of nicotinic acid in human pellagra [see Bean (4)] appeared that same fall.

Although nicotinic acid stimulated growth and acid production on our hydrolyzed casein medium, this stimulation was not evident upon subculture, apparently because of exhaustion of additional, unidentified essential nutrients carried over with the inoculum. We therefore added nicotinic acid to our basal medium and examined the additional crude supplements necessary for growth. Unidentified factors present in both the Norite[2] eluate and the Norite filtrate of a liver extract proved essential for growth (87, 88). We first added a preparation of the Norite eluate factor to our basal medium and examined the nature of the Norite filtrate.

Immediately following the isolation of pyridoxine in 1938 as a vitamin for animals, Moeller (43) showed that it acted as an essential growth factor for some lactic acid bacteria. Following his lead, we found that pyridoxine partially replaced the Norite filtrate for *L. casei* but that another substance(s) also was required (88). Moeller & Schwarz (44), in a report we learned of only later because of war in Europe, had also shown that biotin, a substance originally discovered and isolated as a yeast growth factor (33), was required by lactic acid bacteria. We later confirmed this report (95) and found that biotin and pyridoxine together completely replaced the Norite filtrate for *L.*

[2]"Norite" is a trade name for a widely used activated charcoal.

casei. The resulting medium, with nicotinic acid omitted, was used in a successful assay for nicotinic acid with *L. arabinosus* (95).

I left Madison in 1939 for my first job as a postdoctoral research associate with R. J. Williams at the University of Texas. In Austin, I was to purify an unidentified growth factor for yeast that Williams called "biotic acid." These plans changed when we received a few micrograms of pure biotin from F. Kögl. It completely replaced "biotic acid" for yeast; it also replaced an unidentified growth factor required by *Clostridium butylicum* (94). The work with yeast was not a loss, however, for it provided a sensitive microbiologic assay for biotin (85) that we used in the first purification and characterization of avidin (11), the egg white injury factor studied earlier by Parsons and others. The assay was also used by du Vigneaud and co-workers (10a) in their reisolation of biotin from liver prefatory to their initial proof of its structure and synthesis.

THE NORITE ELUATE FACTOR, FOLIC ACID

Since biotin was the last of the new growth factors for yeast, Williams suggested that I return to investigation of unidentified growth factors for lactic acid bacteria. This change presented difficulties, since Professor Peterson planned to continue work on the Norite eluate factor in Madison, and we had agreed that I would not use *L. casei* as a test organism for this purpose in Austin. Little was then known about nutrition of the lactic acid–producing *Streptococci,* however, so I turned my efforts to this field with *Streptococcus faecalis (S. lactis R)* as the test organism. Within the year, however, we realized that an unidentified factor we were fractionating for this organism, which we extensively purified (41) and called "folic acid" (because spinach leaves were our source material), was the same as the Norite eluate factor required by *L. casei*. The two organisms thus introduced have continued to be those most widely used in study of this vitamin and its derivatives.

The chronology in elucidation of the nature of folic acid is shown (in part) in Table 2. Work on the Norite eluate factor for *L. casei* was continued in Peterson's laboratory by Hutchings & Bohonos (26), who later participated at Lederle Laboratories in isolation of two crystalline forms of this substance, pteroylglutamic acid and pteroyltriglutamate (27, 100). An interesting review that describes early work and early confusion in this field is that of Pfiffner & Hogan (58).

About the time most investigators thought that enough forms of folic acid were already known, Sauberlich & Baumann (69) discovered a new essential growth factor for *Leuconostoc citrovorum* related to folic acid. A closely related substance(s) was discovered by Shive and colleagues by studying growth inhibition of *S. faecalis* by folate analogs and its reversal by natural

Table 2 Assay organisms used in discovery and for monitoring purification of folic acid and some related compounds

Discovery and partial purification[a]
1. *Lactobacillus casei* (Norite eluate factor, etc.) 1939 et seq (87, 88, 26, 98)
2. Chicks (vitamin B_c) 1939 et seq (24)
Chicks, *L. casei* (vitamin B_c conjugate) 1947 (57)
3. *Streptococcus faecalis* (folic acid, etc.) 1941 et seq (41, 42)
4. *S. faecalis* (Rhizopterin[b]) (29)
5. *Leuconostoc citrovorum, S. faecalis* (Citrovorum factor, folinic acid, etc.) 1948 et seq (69, 5)
Crystallization of active compounds
1. From Liver: 1943–1947[c] (2, 100, 56, 57)
2. From *Corynebacterium* fermentation: 1946[d] (27)
Structure and synthesis: 1946 et seq
1. Folic acid (pteroylglutamic acid)[c] (2, 103)
2. Vitamin Bc conjugated[e] (57)
3. Formyltetrahydrofolate[f] (5)

[a] A more complete account of the involved early history of these substances is given in several reviews (e.g. 65, 102, 108).
[b] N^{10}formyl pteroylglutamic hydroxide.
[c] Pteroylglutamic acid, PteGlu.
[d] $Pte(Glu)_3$.
[e] $Pte(Glu)_7$.
[f] N^{10}–H_4PteGlu and related compounds.

extracts (5, 15). One of the effective compounds was identified as a formylated reduced folic acid, N^{10}-formyltetrahydrofolate. This work provided one key to elucidation of the role played by folic acid coenzymes in one-carbon metabolism (15, 25).

PYRIDOXAL AND PYRIDOXAMINE

By omitting pyridoxine and supplementing our basal medium for *L. casei* or *S. faecalis* with folic acid and biotin, we hoped to have a medium suitable for quantitative assay of pyridoxine. This medium did indeed permit excellent growth of these organisms when supplemented with pyridoxine. Much to our surprise, however, the values we obtained upon assay of tissues for pyridoxine were far higher than those obtained by assay of the same samples with a vitamin B_6–requiring yeast; evidently, something other than pyridoxine was contributing to growth of *S. faecalis* and *L. casei* under these conditions. We were able to prove (86) that the growth response of these organisms to pyridoxine was due not to pyridoxine per se but rather to one or more substances formed from it in minute yield during heat sterilization of the growth medium. Similar products were formed and partially excreted when pyridoxine was fed to rats or other animals. We provisionally termed this

substance *pseudopyridoxine* (86) and showed (73, 86) that its activity resulted from an aldehyde and an amine formed by oxidation or amination, respectively, of pyridoxine. By appropriate tests we reduced the number of possible structures to two for the aldehyde and two for the amine (73, 21). With this information we enlisted the expertise of Folkers and his group at Merck, who had previously synthesized pyridoxine, and now synthesized our candidate compounds (21). Two of them, which we named *pyridoxal* and *pyridoxamine,* were in fact thousands of times more active than pyridoxine in supporting growth of *S. faecalis;* only pyridoxal showed such activity for *L. casei* (74).

The chronology of events in this field (Table 3) illustrates a point of general validity with respect to priority in scientific investigation. If methods, however primitive, become available, for studying a topic of interest, that topic will attract multiple investigators and its clarification becomes only a matter of time. One should note, for example, that following development of an appropriate animal assay for vitamin B_6, five different groups reported isolation of pyridoxine within a few weeks of each other (see 81, 102). Similarly, if we had not identified pyridoxal in 1944, Carpenter & Strong (9), who independently found that partial oxidation of pyridoxine increased its activity for *L. casei*, would probably have done so. Had they by chance failed, Gunsalus et al (20), who knew that the coenzyme for tyrosine decarboxylase was related to vitamin B_6, were in an excellent position to do so. Subsequent related work showed (*a*) that the two new forms of vitamin B_6 were as active as pyridoxine for animals, (*b*) that pyridoxal-5'-phosphate (PLP) was the coenzyme of the amino acid decarboxylases (20, 22), (*c*) that pyridoxamine-5'-phosphate (PMP) also occurs naturally (60), and (*d*) that PMP (PLP is less active) is itself an essential growth factor for some bacteria (40) that apparently cannot synthesize it even when supplied with an external source of pyridoxamine.

Table 3 A partial chronology of discovery of vitamin B_6 (pyridoxine, pyridoxal, and pyridoxamine)

1934:	Rat assay for *vitamin B_6* reported by György [reviews (81, 102)]
1938:	*Pyridoxine* (PN) isolated in 5 different laboratories [reviews (81, 82, 102)]
1938–1939:	PN reported as an essential growth factor for lactic acid bacteria (43) and yeasts (12, 70)
1939:	Synthesis of PN (see 102)
1942–1944:	Discovery of *pyridoxal* (PL) and *pyridoxamine* (PM) ("pseudopyridoxine"), formed from pyridoxine by oxidation or amination, as actual growth factors for lactic acid bacteria; pyridoxine inactive (86, 73)
1944:	Synthesis (21) and activity (74) of PL and PM as vitamins for bacteria and yeasts
1945–1951:	PL, PM occur naturally (76) and are about equally as active as PN for rats (89, 36, 67), chicks (37), and dogs (68)

Vitamin B_6 is thus a complex of three compounds (and their 5'-phosphates) that vary in their chemical properties and in their ability to support growth of different vitamin B_6–dependent organisms. *Lactobacillus casei* responds only to pyridoxal; *S. faecalis* responds about equally to pyridoxal and pyridoxamine but not to pyridoxine, whereas yeast (like animals) responds about equally to all three. For many years differential microbiologic assays with these three organisms (cf 60a) supplied the only quantitative estimates of the individual forms of the vitamin in natural products (81).

Although pyridoxal and pyridoxamine have now been known for more than 40 years, the multiple nature of vitamin B_6 frequently is not reflected in articles dealing with this vitamin, despite the recommendations of nomenclature committees (see 82). *Pyridoxine* is commonly (and incorrectly) used both as a synonym for vitamin B_6, and (correctly) to designate the single compound first characterized and still sold under that name. Imprecise nomenclature promotes imprecise thought. I've always believed that the unfortunate incident, in which a commercial milk formula produced vitamin B_6 insufficiency with attendant convulsions in infants, resulted in part from the belief that vitamin B_6 in milk should show stability characteristics of pyridoxine, rather than those of the much less stable pyridoxal and pyridoxamine, which are the predominant forms of vitamin B_6 in milk and many other foods. Many examples of similar confusion could be cited. A striking recent example is provided by an article (48) that describes the "pyridoxine" content of some archaebacteria, determined by assay with *L. casei* against a pyridoxine standard. Since both pyridoxine and pyridoxamine are inactive in promoting growth of this organism (their apparent activity depends upon the time and conditions of heating during sterilization of the medium), the assay values obtained suffice to show that vitamin B_6 is present in these organisms, but they are quantitatively meaningless.

The changes in activity for various bacteria that occur upon sterilization of pyridoxal, pyridoxamine, or pyridoxine with the growth medium result from a variety of reactions. Most interesting among these is the reversible reaction of pyridoxal with amino acids to form pyridoxamine and keto acids (76, 76a). Observation of this reaction led to the initial suggestion of the role of vitamin B_6 in enzymatic transamination (74), and eventually to formulation (40a) of a general mechanism that explains catalysis of vitamin B_6-dependent reactions of amino acids in terms of the chemical properties of pyridoxal.

THIAMINE AND VITAMIN B_{12}

Thiamine, although not essential for the lactobacilli used in the foregoing work, is required for many of the lactic acid–producing enterococci (47) and

some lactobacilli, e.g. *Lactobacillus fermenti* (66). The same is true for vitamin B_{12}; in this case, two observations of Shorb (72), first, that injectable liver concentrates prepared for control of pernicious anemia in man contained an essential growth factor for *Lactobacillus lactis* Dorner, and second, that their microbial activity paralleled their activity in man, provided an assay procedure that greatly facilitated isolation of vitamin B_{12} in the Merck laboratories. Isolation of this substance in England, in contrast, was guided by assay with pernicious anemia patients alone.

AMINO ACID AND PEPTIDE REQUIREMENTS OF LACTIC ACID BACTERIA

Once all of the vitamins required by the lactic acid bacteria had been identified and became commercially available, they could be added to the basal medium, and the hydrolyzed casein could be replaced with a complete assortment of amino acids. By single omissions, individual amino acids required for growth could be identified, and organisms that required them could then be used for their quantitative determination. Such microbiologic methods for amino acids were widely used (review 71) until the automated ion exchange procedures, first developed by Spackman, Moore, & Stein (95a), were introduced. Even now, for repetitive assays of amino acids of special interest, e.g. of essential amino acids in foodstuffs during nutritional survey work, I believe the microbiologic methods would be simpler, more convenient, sufficiently accurate, and much cheaper, especially in undeveloped areas, than the chromatographic methods.

Quantitative comparison showed that peptides of a given amino acid were sometimes more active than the free amino acid in promoting growth of these bacteria. We identified three circumstances under which this phenomenon occurred (see 80): (*a*) a free amino acid, but not its peptides, may be partially degraded by cellular enzymes [e.g. by decarboxylases (30)]; (*b*) uptake of an amino acid may be blocked by antagonistic amino acids, while uptake of its peptides is not (59); and (*c*) a simple deficiency in ability to transport a given amino acid into the cell may be present (54). Once inside the cell, the peptides seem to release the limiting amino acid by simple hydrolysis. Whether similar phenomena occur in animals is unknown. An untested possibility is that the requirement of animal cell cultures for some of the numerous peptide growth factors now being reported may find its explanation in similar terms. A widely studied peptide growth factor for bacteria, strepogenin, was identified and its activity explained in these terms (31).

THE STATUS QUO

By 1955, the vitamin, amino acid, and fatty acid requirements of most lactic acid bacteria were known. Similarly, the basis for the highly varied peptide requirements of some of these bacteria had been mostly established, together with some of their requirements for inorganic ions (39). Consequently, most of them can now be grown in media of known composition, and dehydrated media deficient in single vitamins suitable for vitamin assay can be purchased commercially. As a result, my own interests turned to more biochemically oriented investigations, e.g. of the mechanism of catalysis by pyridoxal (78), by various pyridoxal phosphate-dependent enzymes, and by the functionally related pyruvoyl enzymes (61), and to associated problems of vitamin function, metabolism, and degradation (46).

Many important problems in bacterial nutrition remain, however. Some pathogens cannot yet be grown in media of known composition. The problems of commensal and symbiotic growth and of parasitic relationships have scarcely been touched. While the pace of discovery of new substances of nutritional importance has slackened markedly, recent discoveries of new and distinctive coenzymes in the archaebacteria (115) suggest that this rewarding chapter in the history of nutrition may not yet be closed.

SUMMARY

From the above discussion, it is apparent that one or another of the lactic acid bacteria requires each of the B vitamins required by animals and that assay methods developed during study of nutrition of bacteria and yeasts have played a large role in the initial or independent discovery, isolation, and characterization of vitamins, vitamin derivatives, and functionally similar substances. Clear examples include biotin, biocytin, lipoic acid, nicotinic acid, pantothenic acid, pantetheine, folic acid and tetrahydrofolic acid (and their derivatives), pyridoxal, pyridoxamine, and pyridoxamine phosphate. Improved assay methods that use these organisms also have provided much of the currently available information concerning distribution and stability of the vitamins in natural products, while quantitative inconsistencies between assays, when traced to their origin, have frequently revealed previously unknown metabolic precursors, products, or functions of the vitamins and have provided explanations of the mechanisms by which certain peptide growth factors act. Extension of such studies to organisms that cannot yet be grown in media of known composition should provide additional insights into currently obscure areas of nutrition.

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