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NEWER LABORATORY METHODS FOR ASSESSING NUTRITURE OF SELECTED B-COMPLEX VITAMINS

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INTRODUCTION

Marked progress has been made in recent years in the development of new approaches and modified biochemical techniques for assessing nutritional status of humans of all ages. Biochemical measurements furnish objective assessments of nutriture that often provide preclinical or subclinical information. There are now microtechniques and automated procedures that are applicable to the small blood samples obtainable from infants and children. With the advent of automated procedures, radioassays, and other methodologies, practical nutritional assessment services have become more widely available in clinical laboratories and no longer are restricted to the researcher.

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This review is limited to discussion of the laboratory methods that have been developed since 1974 for assessment of thiamin, riboflavin, niacin, vitamin B_6, and folic acid nutriture. Most developments prior to that date have been reviewed elsewhere (187). The literature cited is focused on reports that have direct or indirect applications in nutritional assessment or have the potential to be developed into techniques useful for this purpose.

THIAMIN (VITAMIN B_1)

The most commonly used procedure for assessing thiamin nutriture has been the measurement of erythrocyte transketolase (EC 2.2.1.1) activity and its stimulation in vitro by the addition of thiamin pyrophosphate (TPP effect) (234). Although its usefulness has been demonstrated, the measurement of erythrocyte transketolase activity is difficult. Recently, Waring et al (233) published a detailed description of a semiautomated, continuous-flow procedure for erythrocyte transketolase activity. The procedure provides values for both unstimulated and TPP-stimulated transketolase activity that are expressed in international units of enzymic activity. The incorporation of dialyzers into the system eliminates hemoglobin interference and thereby increases the sensitivity, reliability, and precision of the method. The procedure uses an NADH-coupled indicator reaction and measures the glyceradehyde-3-phosphate produced by the transketolase enzyme reaction. This also permits the use of glyceradehyde-3-phosphate to standardize the system and enables improvements in quality control between laboratories.

Duffy et al (59) used an Abbott ABA-100 biochromatic analyzer to provide semiautomated measurement of the stimulation of erythrocyte transketolase by thiamin pyrophosphate. The system, based on the manual method of Smeets et al (207), can analyze 30 samples per hour. This procedure yields results only in terms of the TPP effect.

During the past decade, numerous other modifications or evaluations of measurement of erythrocyte transketolase activity as a means of assessing thiamin nutritional status have been reported (11, 13, 21, 29, 59, 107, 113, 115, 117, 135, 172, 188, 229, 251). Particularly significant is the report of Bayoumi & Rosalki (13), which describes in detail a manual optimized, ultraviolet spectrophotometric procedure for determining erythrocyte transketolase activity. Several studies have demonstrated a relationship between erythrocyte transketolase activity and urinary excretion of thiamin (107, 188, 251).

Basu et al (11) described a micromethod for the measurement of transketolase activity in human blood that can be readily applied to children because only a finger prick is necessary to provide the required 50 μl of whole blood. The sedoheptulose formed during the transketolase reaction is measured col-

orimetrically. Boni et al (21) evaluated a modification of the method of Brin (25) with various human subjects. In patients suffering from Wernicke's encephalopathy, the TPP effect varied from 28–67%. Several other modified or simplified procedures have been reported to measure erythrocyte transketose activity (128, 247). Buttery et al (29) reviewed these methods and noted several shortcomings that could result in erroneous erythrocyte transketolase activity values.

Reijnierse et al (172) described a novel radiochemical method for measuring transketolase activity in erythrocytes. Hemolysates of erythrocytes were incubated with radioactive [1–^{14}C]ribose-5-phosphate. The radioactive sedoheptulose-7-phosphate formed during the reaction was isolated on anion-exchange columns and eluted, and the radioactivity measured. The procedure compared favorably with colorimetric methods in terms of sensitivity and analytical time. At present a serious disadvantage of the method is the high cost of the radioactive ribose-5-phosphate required as a substrate.

Kimura & Itokawa (113) published a preliminary report on the use of high-performance liquid chromatography (HPLC) to measure the amount of transketolase enzyme in blood. Transketolase and other thiamin-binding proteins in blood were treated with potassium hexacyanoferrate to form fluorophores. The reacted proteins were separated by HPLC and detected with a spectrofluorimeter. Since the procedure measures the amount of transketolase enzyme present rather than enzyme activity, its usefulness as a nutritional evaluation technique will require further development and validation.

As an alternative to erythrocyte transketolase assay for assessing thiamin nutritional status, Cheng et al (39) investigated leukocyte transketolase activity in the rat. The results indicated that leukocyte transketolase activity responded to dietary intakes of thiamin and appeared to be a sensitive and specific indicator of thiamin status in the rat. Recent simplified procedures for the isolation of leukocytes may permit the assay of leukocyte transketolase activity to become a useful procedure for humans.

Leukocyte pyruvate decarboxylation rates were studied in thiamin-deficient rats as another possible method to evaluate thiamin status (91). Pyruvate decarboxylation rates were determined by assaying $^{14}CO_2$ production by leukocytes acting on [1–^{14}C]pyruvic acid in vitro. Although leukocyte pyruvate decarboxylation rates were depressed after 21 days of thiamin deficiency, only 7 days of deficiency were required to produce an effect on eythrocyte transketolase activity. Thus, leukocyte pyruvate decarboxylase activity measurements will not likely prove useful for assessing thiamin status in humans.

Some disease conditions may influence erythrocyte transketolase activity independent of thiamin status (115). Patients with diabetes mellitus had low erythrocyte transketolase activity values, apparently because of a reduced apoenzyme level that results from the disease. Polyneuritis patients also had

low erythrocyte transketolase activity values. In contrast, patients with pernicious anemia all had highly significant elevations in erythrocyte transketolase activity values. Recently, it was demonstrated that human erythrocyte transketolase enzyme is heterogeneous (106). The basis for using the erythrocyte transketolase assay to evaluate thiamin status is the assumption that the affinity of thiamin pyrophosphate for erythrocyte transketolase is constant. Whether this heterogeneity of the enzyme influences the TPP effect and the interpretation of thiamin nutritional status is a subject for future research.

Thiamin levels in serum, erythrocytes, whole blood, and urine have been used to evaluate thiamin nutritional status. However, the blood thiamin determinations have not been satisfactory primarily because of limitations in methodology, as the decreases in blood thiamin during deficiency are not great, e.g. 6.96 ± 1.26 vs 2.29 ± 1.06 μg thiamin/dl of blood for normal subjects vs beriberi patients, respectively (108). In the past few years, numerous attempts have been made to improve the methods used to measure thiamin and its phosphorylated forms in food and biological materials (56, 58, 61, 62, 88, 154, 155, 158, 159, 163, 169, 174, 175, 181, 186, 232, 235); more recently, HPLC has been used to provide more specific, rapid, and sensitive methods for measuring thiamin and related compounds (87, 97, 101, 111, 112, 178, 183, 191). However, in only a few instances have the modified methods been applied to human serum, blood, or urine samples or to the assessment of thiamin nutriture (58, 111, 163, 178, 191). Most methods reported are variations of the classical fluorimetric thiochrome procedure.

Roser et al (178) determined thiamin in urine with the use of HPLC. Some of the interfering compounds in urine were removed by Decalso cation-exchange column treatment. Thiamin eluted from the column was converted to the fluorescent thiochrome derivative with alkaline potassium ferricyanide (58). The isobutanol extract of the reaction mixture was measured for its thiochrome content with an HPLC system that incorporated fluorescent detection. In a comparison study using a similar HPLC system, the values obtained with human urine samples were comparable to those obtained by applying *Lactobacillus viridescens* microbiological assay for measuring thiamin; (M. H. Dong, J. H. Skala, H. E. Sauberlich, unpublished data).

Hilker & Clifford (97) separated thiamin, thiamin monophosphate, thiamin diphosphate, and thiamin triphosphate using HPLC. The procedure was applied to a breakfast food item and to a urine sample. The samples required a cleanup treatment prior to analysis. Disposable C_{18} reversed-phase columns were used for this purpose. Because ultraviolet detection is used, the sensitivity of this method is low.

Schrijver et al (191) developed a semiautomated HPLC procedure for measuring total thiamin in plasma and whole blood. Thiamin was converted to thiochrome and detected fluorometrically. Approximately four hours were

required to complete one analysis; this included sample preparation and assay time. Reproducibility and recovery of added thiamin pyrophosphate were good. Erythrocytes contained approximately 80% of the thiamin present in whole blood.

Kimura et al (111) also used HPLC for the measurement of total thiamin in whole blood. Blood was deproteinized, and the thiamin phosphate esters were converted to free thiamin by treatment with Takadiastase (EC 3.4.23.6; Aspergillus oryzae carboxyl proteinase). The thiamin in a sample aliquot was separated by HPLC, and thiamin in the effluent was converted on-line to thiochrome, which was measured with a spectrofluorometer. This is a sensitive and highly reproducible method for determining thiamin in as little as 0.1 ml of blood.

Kimura et al (112) also described an HPLC procedure for separating and measuring thiamin, thiamin monophosphate, thiamin diphosphate, and thiamin triphosphate. The compounds were separated with HPLC, postcolumn converted to fluorophores with alkaline hexacyanoferrate, and measured with a spectrofluorimeter. The chromatogram could be completed within 15 min at a sensitivity of 0.05 pmol for each thiamin compound, but it is not known whether the procedure was successfully applied to biological samples.

Sanemori and associates (100, 101, 183) used HPLC to separate thiamin and the thiamin phosphate esters. The thiamin compounds were converted to their thiochrome derivatives, extracted, and subjected to HPLC separation. Initially, straight-phase HPLC was used (100) and applied to measure thiamin, thiamin monophosphate, thiamin pyrophosphate, and thiamin triphosphate in rat tissues (101). However, difficulties were encountered in the measurement of thiamin pyrophosphate. Sanemori et al (183) subsequently used reversed-phase HPLC for an improved method. With some rat tissue samples, however, an interfering compound was present in the thiamin pyrophosphate peak that required blank peak correction.

Few studies have correlated blood thiamin pyrophosphate with erythrocyte transketolase activity. Kuriyama et al (122) observed that total thiamin level in whole blood and erythrocyte transketolase activity were significantly lower in beriberi patients than in normal subjects; however, TPP effect on erythrocyte transketolase activity was the most useful parameter in distinguishing beriberi patients from normal subjects.

Warnock et al (235) observed that erythrocyte thiamin pyrophosphate levels in thiamin-deficient rats fell before changes in erythrocyte transketolase activity occurred. Erythrocyte thiamin pyrophosphate was measured by a sensitive procedure employing the thiamin pyrophosphate–dependent yeast pyruvic decarboxylase apoenzyme. Reaction mixtures containing 0.2–1.5 ng of thiamin pyrophosphate could be measured. Normal human erythrocytes contain 50–150 ng of thiamin pyrophosphate per ml of packed cells. With the availability

of more sensitive and reliable methods for measuring erythrocyte thiamin pyrophosphate, new attention should be given to this approach for evaluating thiamin nutritional status.

In recent years it has become difficult to use the Official Analytical Chemists' procedure and similar procedures for measuring thiamin in foods and urine because of the discontinuation of commercial preparations of Decalso, used in purifying samples, and of Clarase and Mylase P, used in converting the phosphorylated forms of thiamin to free thiamin (62, 169). Takadiastase remains available for the treatment of the phosphorylated forms of thiamin, although not all batches are active. Bio-Rex 70 ion exchange and Amberlite CG50 cation exchange resins appear to be possible replacements for Decalso (62, 174).

Attempts to use gas chromatography to measure thiamin have had limited success (60). The low sensitivity obtained even with a nitrogen-phosphorus detector limits the applicability of this technique to such items as pharmaceutical preparations.

A fluorometric reaction rate method was described for the determination of thiamin (181). The procedure is not practical for use with biological preparations, but it may have some applicability to pharmaceutical or multivitamin preparations. Similarly, colorimetric and fluorometric determinations of thiamin that use an automated discrete-sampling techniques are applicable only to pharmaceutical preparations (154).

Electrophoretic fluorometric methods have been described for measuring thiamin and its phosphorylated forms in plasma and tissues (155, 158, 160, 163). The procedures are rather involved and time-consuming, and poor sensitivity limits their use to measurements of plasma and blood.

RIBOFLAVIN

The procedure most commonly used to evaluate riboflavin nutriture is still the measurement of erythrocyte glutathione reductase (EGR) activity and the stimulation of this activity by flavin adenine dinucleotide (FAD) added in vitro. Numerous recent reports have but further validated the usefulness of the procedure for this purpose (13, 22, 107, 118, 165, 166, 189, 217, 247). Readers planning to undertake EGR assays may find the papers of Bayoumi & Rosalki (13) and Thurnham & Rathakette (217) instructive. Although EGR activity is usually measured by means of enzyme-coupled kinetic assays, colorimetric methods have been described and applied in studies of humans (70, 121). In attempts to simplify the assay procedure, whole blood has been used instead of erythrocytes (70, 74). Garry & Owen (70) developed an automated procedure to measure glutathione reductase activity colorimetrically in whole blood that uses the reaction of 5,5'-dithiobis-2-nitrobenzoic acid with

glutathione that has been regenerated from oxidized glutathione by the enzyme reaction. Samples from more than 600 children from 6 to 60 months old were examined using this procedure.

The EGR activity is commonly expressed in terms of "activity coefficient" or in terms of per cent stimulation, both of which are derived from the stimulating effect of FAD added in vitro to the enzyme reaction. The validity of expressing results in these terms may need further evaluation. Although it has been reported that a chronic deficiency of riboflavin in the human does not change the level of apo-erythrocyte glutathione reductase, some animal studies suggest that certain deficiencies, such as vitamin B_6, may reduce the apo-enzyme level (198, 230). Age of subjects appears to influence the glutathione reductase activity of the erythrocyte. Age of the erythrocyte may have an influence, because in studies with fractionated erythrocytes, EGR activities declined with the age of the cells (12, 164). The validity of measuring EGR activity to evaluate riboflavin status in subjects with low erythrocyte glucose-6-phosphate dehydrogenase (EC 1.1.1.49) activity has been questioned, and additional investigation is needed (167).

Little information is available concerning the glutathione reductase activity of the human white cell. A study with riboflavin-deficient rats suggests that the measurement of the activation coefficient of white cell glutathione reductase activities may be a useful indicator of riboflavin status (150a). With the recent advent of more simple and efficient means of isolating leukocytes, this approach may receive attention as a possible technique for assessing riboflavin nutriture.

Attempts have been made to relate riboflavin levels in erythrocytes or blood to riboflavin status. Some laboratories have observed no relationship (9), while others report a significant correlation (74). Recently, new analytical approaches for measuring riboflavin in blood, urine, and other samples have been reported (71, 116, 136, 209, 219). Knobloch et al (116) described and used a microspectrofluorimetric method to measure riboflavin in the blood of newborn babies and their mothers. The method required the treatment of 0.5–1.0 ml of blood with trichloracetic acid; the treated blood was then purified on a Florisil column. The procedure had a sensitivity of 0.01 μg riboflavin/ml, which is fully adequate for measuring riboflavin in blood. Newborn babies showed an average riboflavin value of 17.1± 2.4 μg/dl of blood.

Riboflavin levels in urine have generally been determined by microbiological assay or by somewhat tedious fluorometric methods. Recently, several new procedures have been developed that should enhance the efficacy of measuring riboflavin in urine. Smith (209) used HPLC with a fixed-wavelength spectrofluorometer for detection. Untreated urine samples were injected directly onto a reversed-phase microparticulate C_{18} column. The limit of sensitivity was 0.05 μg riboflavin/ml of sample. Subsequently, Gatautis & Naito (71) reported on

an improved HPLC procedure to measure riboflavin in urine. Urine was injected directly into the system and quantitated by fluorometric detection (450 nm excitation and 530 nm emission). Concentrations of riboflavin as low as 0.01 μg/ml were readily detected. Both of the above HPLC procedures require further evaluation to ensure that urine specimens do not contain spurious compounds that may give falsely high riboflavin values.

Riboflavin has also been measured in urine and other samples by means of a competitive binding protein procedure. Bashor & Tillotson (219) isolated riboflavin-binding protein from chicken egg white. Using the purified apoprotein, they developed a method for quantitatively determining urinary riboflavin levels by measuring the change in fluorescence in the sample or standards before and after titration with the apoprotein. The method was rapid and sensitive, and no treatment of the urine samples was required prior to analysis. Values obtained by microbiological assay, however, were 15–25% lower than those obtained by the fluorometric apoprotein titration method. The nature of this discrepancy requires further investigation.

Lotter et al (136) also used the riboflavin-binding protein prepared from chicken egg white in their radioisotopic competitive binding assay. The procedure is based on the observation that the riboflavin-binding protein binds tightly to DEAE-cellulose but free riboflavin does not. A solution of [2–^{14}C]riboflavin was diluted with varying amounts of a standard unlabeled riboflavin solution or an unknown sample, mixed with a quantity of the aporiboflavin binding protein, and then washed through a small DEAE-cellulose column. The protein-bound riboflavin was eluted from the column into scintillation vials and counted, and the unknown samples were compared to a standard curve. The procedure suffered from a lack of sensitivity that could be improved by the use of radioactive riboflavin with a higher specific activity.

NIACIN (NICOTINIC ACID)

Niacin deficiency, which results in pellagra, continues to occur in certain maize-eating populations, such as those of South Africa and Egypt, and in those parts of India where jowar is a dietary staple. In the United States, clinical cases of pellagra may occur in association with alcoholism.

Few laboratory procedures are available for assessing niacin nutriture in humans. The general procedure is to measure one or more urinary excretion products of niacin metabolism; metabolites most commonly measured are N^1-methylnicotinamide (N^1-MN) and 2-pyridone (N^1-methyl-2-pyridone-5-carboxylamide). The excretion ratio of 2-pyridone to N^1-methylnicotinamide is the most reliable indicator of niacin nutritional status (187). Unfortunately, technical problems in the measurement of these metabolites have resulted in time-consuming procedures and variable results.

Recently, HPLC techniques have been applied to plasma and urine samples to measure nicotinic acid, nicotinamide, and their metabolites. This has led to simple, sensitive, and accurate methods of measuring 2-pyridone and N^1-methylnicotinamide.

A number of the HPLC procedures measure only nicotinic acid, nicotinamide, and nicotinuric acid in plasma and urine (57, 94, 212). Measurements of these compounds have not proven useful or reliable for assessing niacin nutritional status.

Mrocheck et al (150) used liquid chromatography to separate and quantitate nicotinuric acid, N^1-methyl-2-pyridone-5-carboxamide, N^1-methyl-4-pyridone-3-carboxamide, N^1-methylnicotinamide, and nicotinamide-N^1-oxide in human urine. The procedure involved a long elution time and the use of a special high-resolution liquid-chromatographic ultraviolet-analyzer.

Shaikh et al (195) used HPLC in determining N^1-methylnicotinamide in urine. However, the column chromatographic step required to purify the sample prior to HPLC analysis resulted in an overall procedure that took seven to eight hours per urine sample. Subsequently, Shaikh & Pontzer (194) reported a simplified method of measuring N^1-methylnicotinamide in urine. Urine samples, without pretreatment, were injected directly onto a reversed-phase HPLC column operating in the soap chromatography phase. The system used a hydrophilic eluent containing methanol as an organic modifier and a small concentration of a detergent (sodium dodecyl sulfate), which forms an ion-pair with an ionized form of a solute. The procedure appears to provide a simple, rapid, and specific method for the direct assay of N^1-methylnicotinamide in urine. However, it was used on only a few rat and human urine samples, and further verification is desirable.

Kutnink et al (123) have also described a simple HPLC procedure for determining N^1-methylnicotinamide in urine samples. Human or rat urine samples can be analyzed directly without need for prior extraction or ion exchange cleanup. The procedure was used during a three-month period to conduct over 1000 determinations, and there was no reduction in column performance or efficiency.

Recently, several HPLC procedures for determining both N^1-methylnicotinamide and 2-pyridone have been described (31, 143, 182, 214). Sandhu & Fraser (182) extracted the metabolites of niacin from the urine specimen into chloroform after adding isonicotinic acid to the urine as an internal standard. The chloroform was removed by evaporation, and the residue was dissolved in methanol and injected into the HPLC system with ultraviolet detection. Each chromatographic run required 30 min to perform. The procedure was sensitive to as little as 10 ng of either metabolite.

Carter (31) quantified 2-pyridone and N^1-methylnicotinamide in urine using isocratic reversed-phase HPLC with ultraviolet detection. The procedure used

simple anion-exchange column cleanup of the samples. The prepared samples were then analyzed for N^1-methylnicotinamide followed by a repeat analysis for 2-pyridone. The two analyses were performed in approximately 20 min. This reviewer and associates have successfully used the method to measure the two niacin metabolites in numerous human and rat urine samples.

The procedure of Terry & Simon (214) required extensive ion-exchange preparation of the urine samples prior to HPLC quantitation. The purified samples could undergo simultaneous HPLC quantitation of 2-pyridone and N^1-methylnicotinamide. The authors reported an average 2-pyridone:N^1-methylnicotinamide ratio of 3.60 ± 1.06 (range 1.76–5.90) for 50 normal adults.

McKee et al (143) utilized HPLC for the quantitation of nicotinamide, nicotinic acid, nicotinuric acid, N^1-methylnicotinamide, and 2-pyridone in rat and human urine samples. The untreated urine samples were injected directly into the HPLC system. The system employed a linear ion-pair mobile-phase gradient. Temperature of the system was critical for satisfactory separation of the compounds. It appears that the procedure must be applied with care in the quantitation of niacin metabolites in urine; human urine frequently contains unknown compounds that may interfere in the separation of niacin metabolites. The investigators noted that "often there was overlapping of peaks of unknown components with one or more of the five compounds studied."

Automated methods have been proposed for the measurement of N^1-methylnicotinamide in urine (156) and for nicotinic acid in serum (176). The procedures appear to have limitations, and hence have received little application in other laboratories.

There have been several reports on the use of modified microbiological assay procedures for determining the biologically active forms of niacin in plasma and blood (76, 110). The radiometric microbiologic assay of Kertcher et al (110) requires use of tracer amounts of a radioisotope and a commercially available item of equipment, but it is unique because colored or turbid materials do not interfere with the assay.

For laboratories lacking HPLC equipment, an exceedingly sensitive fluorimetric procedure has been reported for the measurement of N^1-methylnicotinamide and nicotinamide in serum (40, 41). The procedure permits detection of as little as 5 pmol N^1-methylnicotinamide/ml of the final fluorescent solution.

Recently, Arienti et al (5) observed an elevation in the activity of nicotinic acid mononucleotide pyrophosphorylase in erythrocytes of niacin-deficient pigs. They suggested that this measurement might serve as a functional test for evaluating niacin nutritional status. However, the activity of nicotinic acid mononucleotide pyrophosphorylase was not elevated in the erythrocytes of niacin-deficient rats (H. Vannucchi, M. A. Kutnink, and H. E. Sauberlich,

unpublished data). Whether the enzyme is elevated in the erythrocytes of human pellagrins remains to be evaluated.

In practice, laboratory assessment of niacin nutritional status is still limited to the measurement of niacin metabolites in urine. The availability of HPLC simplifies the process and enhances the speed, accuracy, and sensitivity of determining 2-pyridone and N^1-methylnicotinamide in urine and thus more reliable 2-pyridone:N^1-methylnicotinamide ratios.

VITAMIN B_6

In the past decade, the interest in vitamin B_6 nutrition and metabolism has increased greatly, as is reflected by the number of publications concerned with the nutrient. Vitamin B_6 nutriture has been assessed primarily by plasma pyridoxal phosphate levels, erythrocyte aminotransferase measurements, tryptophan load tests, and urinary excretion of 4-pyridoxic acid or of intact vitamin B_6 (185). Of the numerous reports on vitamin B_6 assessment methodology, most have been concerned with improvement, validation, or application of existing techniques. Few new procedures have appeared.

The newest approach has involved attempts to separate and quantitate vitamin B_6 compounds using HPLC (77, 79, 80, 129, 192, 220, 222–224, 226, 246). The initial procedures were of limited practical use because of low sensitivity (222, 246). Subsequently, Vanderslice et al (223) improved the sensitivity of their earlier HPLC method by adding semicarbazide to the buffer system, whereby pyridoxal and pyridoxal-5-phosphate were converted to their highly fluorescent oximes. The somewhat complicated system required the use of two different columns to enhance separation of the vitamers and employed changes in excitation and emission wavelengths to enhance sensitivities. The investigators used their method to analyze several samples of human plasma and milk for vitamin B_6 levels (221, 223). However, even with appropriate pretreatment of samples, a 3-ml plasma quantity and approximately 90 min were required to complete a single chromatographic run.

Coburn & Mahuren (43) recently attempted to provide a simpler procedure for measuring vitamin B_6 forms in plasma and other biological materials. A single column and one fluorometer setting were used to measure pyridoxal phosphate, pyridoxic acid, pyridoxamine phosphate, pyridoxal, pyridoxine, pyridoxamine, and pyridoxine phosphate in 0.25 ml of serum. The procedure required 40 min. Undoubtedly further investigations will yield a simpler and more versatile chromatographic procedure for analyzing the various forms of vitamin B_6 in biological fluids (43, 224).

Gregory & Kirk have described HPLC procedures for measuring pyridoxal-5-phosphate (77), 4-pyridoxic acid (79), and other vitamin B_6 compounds (80). Pyridoxal-5-phosphate was extracted from samples with perchloric acid and

then converted to the pyridoxal-5-phosphate-semicarbazone and quantitated with the use of HPLC (77). Although the procedure was adequate to measure pyridoxal-5-phosphate in liver, it was not sensitive enough for use on plasma.

Schrijver et al (192) successfully measured pyridoxal-5-phosphate in whole blood with an automated HPLC procedure. The pyridoxal-5-phosphate was extracted from blood samples with trichloroacetic acid and purified by HPLC. The pyridoxal-5-phosphate was reacted on-line with semicarbazide to form the semicarbazone derivative and was quantitated by fluorescence measurement. Use of a higher pH increased the sensitivity of the procedure to as low as 5 nmol/liter. The procedure could also measure pyridoxal during a run, but the concentration of this form of vitamin B_6 is very low in blood. Each run required approximately 20 min, but with automation, considerable numbers of samples could be analyzed in 24 hours. With normal adult subjects, pyridoxal-5-phosphate ranged from 50 to 120 nmol/liter of whole blood, with a mean of 80 nmol/liter.

Lim et al (129) readily separated pyridoxine, pyridoxamine, and pyridoxal by HPLC, but the procedure was applied to only a single sample of milk. Further evaluation will be needed to determine the usefulness of this method for use on other biological samples and its freedom from the effects of interfering compounds.

The analysis of 4-pyridoxic acid in urine by HPLC has been particularly successful (4, 79, 226). Urine samples merely require treatment with trichloroacetic acid and a brief centrifugation to remove proteins. Aliquots are then injected directly onto the HPLC system and fluorometrically detected. With the mininal sample preparation and short elution time (<6 min), numerous samples can be analyzed readily. The amount of 4-pyridoxic acid present in the urine correlates with the dietary intake of vitamin B_6 (203, 204, 226). In urine samples stored at $-20°C$, 4-pyridoxic acid remained stable for at least 20 months (4). Simon et al (204) also found 4-pyridoxic acid in urine samples to be stable.

Lumeng et al (137) described a microassay for pyridoxal phosphate using tyrosine apodecarboxylase. The procedure, actually a modification of previously available methods, has resulted in a more sensitive and reliable method. Based on experiments with rats, Lumeng et al (138) considered plasma pyridoxal-5-phosphate levels to be a sensitive, reliable indicator of vitamin B_6 nutritional status.

An exceedingly sensitive procedure for measuring pyridoxal-5-phosphate was described by Yang et al (253). It is based on the activation effect of pyridoxal-5-phosphate on apoasparate aminotransferase purified from pig heart. Aspartate aminotransferase activity was measured, through coupling to malate dehydrogenase, by spectrophotometrically monitoring the disappearance of NADH at 340 nm.

Shin-Buehring et al (199, 200) described an exceedingly sensitive enzymatic method for the determination of pyridoxal-5-phosphate in blood, urine, and tissues. The method involved the reaction of tyrosine apodecarboxylase and [^{3}H]tyrosine to form [^{3}H]tyramine. A simplified sample extraction procedure reduced the destruction of pyridoxal-5-phosphate. Pyridoxal-5-phosphate was observed to be stable in whole blood samples stored at −20°C for at least several months; plasma stored at −20°C, however, lost over 50% of its pyroxidal-5-phosphate within a week. Pyridoxal-5-phosphate levels were high in infants and fell with age. An effect of age on plasma pyridoxal-5-phosphate levels was observed also by Rose et al (177).

Bhagavan et al (19) have improved their previously reported methods for measuring pyridoxal-5-phosphate in plasma and other biological materials. Their microenzymatic method, which also utilizes tryosine apodecarboxylase and L-[1–^{14}C]tyrosine, is rapid, highly sensitive, and specific.

Camp et al (30) described a further modification of the tyrosine apodecarboxylase procedure for measuring pyridoxal-5-phosphate in plasma. The [^{3}H]tyramine formed from the pyridoxal-5-phosphate–dependent enzyme reaction on [^{3}H]tyrosine was isolated by extraction into ethyl acetate rather than by column chromatography (199, 200), and this was followed by scintillation counting. The procedure avoided the need to remove proteins from plasma samples before analysis. These investigators found pyridoxal-5-phosphate to be stable for at least 10 days in plasma stored at −80°C; this is in contrast to the losses reported when plasma was stored at −20°C (199).

Meadows et al (146) utilized the pyridoxal-5-phosphate–dependent apoenzyme tyrosine phenol-lyase purified from *Erwinia herbicola*. Pyridoxal-5-phosphate was quantitated spectrophotometrically in a coupled reaction with lactate dehydrogenase and NADH. An analysis could be completed within 20 min. However, with an assay range of 0.1–1.0 μg pyridoxal-5-phosphate/ml, the procedure is less sensitive than other available methods.

Chauhan & Dakshinamurti (36, 48) separated pyridoxal, pyridoxamine, pyridoxine, pyridoxal-5-phosphate, and pyridoxamine-5-phosphate by ion-exchange chromatography. The separated compounds were quantitated fluorometrically as cyanohydrin derivatives, which permitted a detection range of 10–50 ng/ml. Five milliliters of serum were required per analysis, and one analysis required six hours to perform. However, with a battery of columns, analysis of 10–15 samples could be accomplished daily.

Yasumoto et al (254), also described an ion-exchange system for measuring pyridoxal, pyridoxine, and pyridoxamine. However, the colorimetric detection procedure lacks sensitivity, which limits the applicability of the method with biological materials.

Smith et al (208) recently measured pyridoxal and pyridoxal-5-phosphate in human leukocytes and plasma by using a cation-exchange chromatography

isolation procedure. The procedure involved enzymatic conversion of pyridoxal-5-phosphate to pyridoxal. The isolated pyridoxal was converted to the 4-pyridoxolactone, which was measured by fluorimetric assay. Although the method was sensitive for determining pyridoxal-5-phosphate, it seems more time-consuming than other available methods.

As noted in this review, there are numerous procedures available for measuring pyridoxal-5-phosphate in plasma. Although some of the procedures appear to have advantages, no direct comparison of methods has been made.

The use of the radioimmunoassay, or enzyme-linked immunosorbant assay, is a novel approach to measuring phosphorylated forms of vitamin B_6 in biological samples (225, 228). Although as described, the procedure is not an acceptable assay procedure for forms of vitamin B_6, the investigators have demonstrated the production of the necessary antibodies. Further development could provide a highly specific, sensitive procedure for measuring the individual forms of vitamin B_6.

Guilarte and associates (82–86) described a radiometric-microbiological assay using *Kloekera brevis* as the assay organism for the measurement of total vitamin B_6 in plasma and food samples. An advantage of the procedure is that colored, turbid, or precipitated material did not interfere. Although the assay may be performed manually, special, commercially available item of equipment is required for efficient use of the procedure. Using this relatively expensive instrument, this reviewer has found the procedure suitable for the assay of vitamin B_6 in plasma, urine, and food samples. Guilarte et al (84, 86) reported that the *K. brevis* yeast responded equally to pyridoxine, pyridoxamine, and pyridoxal; significantly different growth responses to these forms were obtained with *Saccharomyces uvarum,* the yeast commonly used for vitamin B_6 assays. In contrast, Gregory (78) found unequal growth responses of *K. brevis* to equivalent molar concentrations of pyridoxine, pyridoxamine, or pyridoxal. In the experience of others including this reviewer, *S. uvarum* has been observed to respond equally to these three forms of vitamin B_6. Strain differences may exist, but more importantly, specific culture maintenance is required to obtain the equivalent growth response in this organism.

Some workers have considered the tryptophan load test inappropriate for evaluating vitamin B_6 status in women receiving oral contraceptives (14, 27). Alternative procedures are the use of the methionine load test (132) and the more expensive kynurenine load test (15, 27, 250). A vitamin B_6 deficiency in the human results in a substantial increase in the urinary excretion of cystathionine following a methionine load (127, 132, 147).

Measurements of erythrocyte alanine aminotransferase (ALT; EC 2.6.1.2) and asparate aminotransferase (AST; EC 2.6.1.1) activities, along with the in

vitro stimulation effect of pyridoxal-5-phosphate, have been commonly used to evaluate vitamin B_6 status (185). Colorimetric methods or coupled-enzyme spectrophotometric procedures are generally employed. These methods have lacked in specificity and sensitivity or have suffered from matrix effects of the hemoglobin present in the reaction mixture (206). In recent years, optimization of analytical conditions for measuring aminotransferases in serum has been emphasized (16–18, 28, 105, 168). Serum samples are free of the problems associated with erythrocyte preparations, but measurements of aminotransferases in serum have not proven useful for evaluating vitamin B_6 nutritional status.

Skala et al (206) have studied in detail the conditions necessary for automatically measuring both stimulated and unstimulated erythrocyte aspartate aminotransferase and alanine aminotransferase activities through the use of a centrifugal analyzer. The sample requirements were exceedingly small and an analysis could be completed within 10 min, but some difficulties were still encountered in measuring erythrocyte alanine aminotransferase activity due to the sample matrix. The same authors described a procedure that uses an automated continuous flow analyzer to measure erythrocyte aspartate aminotransferase activities (206). An important feature of the system was the incorporation of a dialyzer to minimize the matrix effects. Approximately 50 samples could be analyzed per hour. Further modification has produced an automated continuous flow procedure for simultaneous determination of alanine aminotransferase and aspartate aminotransferase enzyme activities in hemolysates or plasma (205). Pyridoxal-5-phosphate cofactor-stimulated or -unstimulated activities could be measured. Erythrocyte asparatate aminotransferase in samples stored at −70°C was stable for at least 76 days. Erythrocyte alanine aminotransferase in samples stored at −70° was stable for at least 60 days (206). Kishi & Folkers (67) found erythrocyte asparate aminotransferase in lyophilized erythrocyte hemolysates stable for up to 7 days.

Others have evaluated or modified the methods used to measure erythrocyte aspartate aminotransferase (13, 67, 114, 125, 126). Leinert et al (125, 126) reported in detail a semiautomatic method to determine the stimulation effect of in vitro addition of pyridoxal-5-phosphate on erythrocyte aspartate aminotransferase activity. The investigators found no indication of differences in erythrocyte aminotransferase activity between men and women. Oral contraceptives and riboflavin status have little if any influence on enzyme activity. However, various other drugs and disease conditions have been reported to influence serum aminotransferases (68, 255), and little is known about their effects on erythrocyte aminotransferases. Erythrocyte alanine aminotransferase enzyme activities were higher in the young cells and decreased with the age of the cells (99).

FOLIC ACID (FOLACIN, FOLATE)

Folic acid deficiency is relatively common as a result of poor dietary intakes, excessive alcohol consumption, pregnancy, and other influences such as oral contraceptive agents and medication (3, 6–8, 23, 46, 63, 72, 95, 140, 141, 153, 161, 162, 184, 202). Consequently, measurements to assess folic acid status are performed in many clinical laboratories (3). When macrocytic anemia is encountered, measurements for serum and red cell folic acid and serum vitamin B_{12} are essential to evaluate the cause (201). If a vitamin B_{12} deficiency is indicated, a Schilling test or plasma vitamin B_{12} uptake test is necessary for evaluation of the cause of the anemia (34).

A number of investigators have recently used HPLC to separate folate compounds (2, 3, 24, 32, 35, 42, 55, 64–66, 119, 124, 144, 145, 170, 171, 173, 186, 196, 210, 211). Most of the reports have discussed the use of HPLC to purify folic acid reference standards or to measure folic acid in pharmaceutical preparations or known mixtures. Poor limits of sensitivity in detecting folate derivatives restrict the applicability of HPLC with respect to biological specimens and food samples (35, 42, 55, 64, 65, 119, 124, 144, 196), which may contain more than 100 different forms of folic acid. Nevertheless, HPLC permits effective separation of various folate derivatives (2, 24, 170, 173) and of the glutamate-conjugated forms (32, 64, 65, 119, 196, 210). Use of radioactively labeled folate forms and special derivatizations has led to unique applications of HPLC in folate metabolic studies (64, 65, 119, 144, 196). In some instances, the folate forms have been separated by HPLC and then quantitated by microbiological assay (144). Application of HPLC has provided preliminary information on folate profiles in foods (42, 55, 173).

Lankelma et al (124) used HPLC to measure 5-methyltetrahydrofolic acid in plasma and spinal fluid. The procedure involved the deproteinization of 0.9 ml of plasma in the presence of ascorbic acid to prevent oxidation. The sample was centrifuged, and 1 ml of supernatant was injected onto a two-column HPLC system. The first column removed the antioxidant and concentrated the 5-methyltetrahydrofolic acid; it also permitted the use of a large sample injection to increase sensitivity. A commercially available electrochemical detector was used to quantitate the folate compounds; electrochemical detection provides greater sensitivity than ultraviolet spectrometry for measuring 5-methyltetrahydrofolic acid. The limit of detection for 5-methyltetrahydrofolic acid was 2×10^{-9}M. However, the sensitivity of the technique to folic acid (pteroylglutamic acid) and leucovorin (N^5-formyltetrahydrofolic acid) was low. Other forms of the nutrient were not studied.

Chapman et al (35) attempted to use HPLC to measure folate levels in serum. Their system separated folic acid, 5-methyltetrahydrofolic acid, dihydrofolic acid, and N^5-formyltetrahydrofolic acid. Ultraviolet detection was used, which

resulted in low sensitivity. The detection limit was 3 ng/50 μl of injected sample, which is inadequate for normal serum samples.

Krumdieck and associates (64, 65, 119) have isolated the folate forms present in tissues into three groups. The compounds present in each group were derivatized and their glutamate chain lengths studied by HPLC. Using azo dye derivatization and HPLC, Shane (196) was also able to study glutamate chain lengths of folates present in mammalian tissue and bacterial extracts. However, the procedures did not provide information on the one-carbon-substitution of the folates present. Others have converted the folate forms to their monoglutamate state and then applied HPLC to separate and quantitate the various monoglutamates (144). The monoglutamates were separated on a μBondapak C_{18} reversed-phase column, which permitted the separation of tetrahydrofolic acid, pteroylglutamic acid, dihydroglutamic acid, 5-CH_3-tetrahydrofolic acid, 5-CHO-tetrahydrofolic acid, 10-CHO-tetrahydrofolic acid, and 5,10-methylene-tetrahydrofolic acid. The separated forms were quantitated by microbiological assays, which were five times as sensitive as UV spectrophotometric detection.

Some of the difficulties with folate analytical studies are the potential interconversion of the folate forms, folate losses, and changes in glutamate chain length during sample preparation and extraction or even during the actual conduct of the analysis. Currently, few of the HPLC applications for measuring folates have the potential to serve as practical means of assessing folic acid nutritional status.

Microbiological assays remain the standard procedure for measuring total folic acid activity in serum, blood, tissues, and foods. Normally, *Lactobacillus casei* is used as the growth organism because of its nearly equal response to all of the monoglutamate forms of folic acid. It has been important to simplify the microbiological assay for laboratory technologists who have minimal experience in this area. Scott et al (193) described in detail a simplified assay for folic acid in erythrocytes and serum. To further minimize concern over sterile conditions and to decrease contamination problems, chloramphenicol may be added to the growth medium and the assay may be conducted with a chloramphenicol-resistant strain of *L. casei*. Because chloramphenicol has potential to produce aplastic anemia, its use in such assays has been criticized (248). Nevertheless, the procedure has received acceptance in numerous laboratories.

A continuous-flow automated folacin assay system that accommodates a large number of serum samples has been described (213). The system utilizes *L. casei* as the test organism, and the incubation period is approximately four hours. The growth response of the organism to folate is estimated by measuring the rate of reduction of 2,3,5-triphenyl tetrazolium chloride. The procedure compared well with a standard test-tube microbiological assay for folic acid.

Magnus (139) also briefly described an automated microbiological assay for folic acid that employed a commercially available instrument.

Use of glycerol-cryoprotected cultures to minimize contamination of maintenance cultures of *L. casei* and to provide a uniform assay inoculum has been reported (81, 249). The procedure was quite simple and eliminated the need to prepare inoculum cultures for each assay. The *L. casei* was grown in a batch quantity of medium and supplemented with a low concentration of folic acid (0.3 μg/liter). Following incubation, the growth medium was diluted with an equal volume of glycerol. Aliquots of the diluted growth medium were placed in sterile screw-cap vials and stored at −20°C for use as an assay inoculum. Chen et al (37) used lyophilized cultures to reduce routine maintenance and inoculum preparation time.

Chen et al (37) developed a radiometric microbiological assay procedure for determining folic acid in plasma, blood, foods, and other materials. The procedure involves the measurement of the $^{14}CO_2$ evolved from the metabolism by *L. casei* of [1–^{14}C]gluconate through the hexose monophosphate shunt. The production of $^{14}CO_2$ is related to the amount of folate present in the system. The sensitivity of the procedure was comparable to the standard folate microbiological assay, but had the advantage that any color or turbidity present in samples did not interfere in the assay. However, the procedure was subject to two common problems: bacterial contamination and the presence of antibiotics or antifolacins in the samples. Cost is a consideration in using the radiometric microbiological method, because it requires the use of a relatively expensive, specialized item of equipment in addition to the radioisotopes.

Various radioassays (radiometric binding assays) have been developed to overcome some of the problems encountered in microbiological assays for folic acid (26, 73, 90, 92, 102, 103, 109, 130, 134, 148, 149, 179, 180, 190, 216, 236–240). A number of commercial radioassay kits are available for measuring folic acid levels in serum and erythrocytes. Although the kits are convenient and easy to use, some uncertainties exist about the validity of the folate values obtained. Hence, a number of studies have compared radioassay and microbiological methods for determining folates in serum and erythrocytes (1, 10, 45, 54, 89, 98, 104, 120, 142, 180, 197, 231). Most of the radioassays provide serum folate values comparable to those obtained with the microbiological assay (26, 104, 134, 231, 239). In some instances, however, radioassay yielded abnormal values or values that were consistently either lower or higher than those obtained by microbiological assay (10, 54, 98, 120, 142, 197, 216). Radioassay methods have been less reliable when applied to erythrocytes or whole blood (142, 149); whether this is related to sample treatment (109, 130), folate standards used (102, 148, 216), pH (73, 89), sources of protein binders (237), or other factors remains unclear.

The most common standards used in the radioassays are N^5-methyl-

tetrahydrofolic acid and pteroyglutamic acid. In the radioassays, N^5-methyltetrahydrofolic acid has been reported to have a somewhat greater displacement ability than pteroylgutamic acid (148, 216); hence, serum or erythrocyte folate values would be lower when pteroylglutamic acid was used as the standard. Other investigators, however, observed no difference between the two folate standards when the radioassay was conducted at pH 9.3 with a milk folate binder (1, 73, 134). Differences may exist in the binding affinities of folate monoglutamates and folate polyglutamates. Although Schreiber & Waxman (190) reported none, more recent observations indicate a significant increase in binding activity for polyglutamates (45, 197).

Those contemplating the use of radioassay to measure folic acid levels in serum and erythrocytes should carefully consider the procedure or commercial kit they will use. It is important that the individual laboratory establish a reference range of normal and deficient serum and erythrocyte folate values based on the procedure selected (54).

Evaluation of folic acid nutriture frequently must also include information on vitamin B_{12} status; hence, simultaneous radioassays for serum folic acid and vitamin B_{12} have been developed (38, 54, 90, 240). Recently, a semiautomated radioassay system for simultaneously measuring the two vitamins in serum was evaluated (38). The analytical results were comparable to those obtained in the individual radioassays (38, 90), but the technical time for analysis was decreased.

Hendel (93) described a radioassay that was specific for the determination of pteroylglutamic acid in plasma, erythrocytes, and urine. The lower limit of detection of pteroylglutamic acid in the assay was 0.1 μg/liter. The radioassay was free of any significant interference from tetrahydrofolic acid, 5-methyltetrahydrofolic acid, or methotrexate. Since little pteroylgutamic acid occurs naturally, this assay has only limited or special research applications.

Radioassays for folates in foods and rat tissues have received limited application in one laboratory (75, 218). However, in view of the large number of folate forms present in foods and tissues and the limited number of folate forms that respond in the radioassay systems, application of this technique to natural materials (197) will require caution and careful evaluations. Converting the folate forms present to their monoglutamate state does not ensure that the various one-carbon-substituted folates will react equally in the radioassays.

Wu et al (252) demonstrated a good correlation between folate levels in serum, erythrocytes, and liver in a study with alcoholic patients. Serum and erythrocyte folate levels may be depressed in thalassemic patients (227) and sickle-cell patients (133). These effects may reflect an increased requirement for folic acid by these patients.

The deoxyuridine suppression test for diagnosing folic acid and vitamin B_{12} deficiencies (96, 241–243) has been the subject of additional evaluation and

modification (33, 44, 47, 49–53, 69, 152, 157, 225, 244, 245, 256). The deoxyuridine suppression test evaluates in vitro the ability of exogenous deoxyuridine to suppress the incorporation of subsequently added [^{3}H]thymidine into the DNA of bone marrow cells. The test correlates well with megaloblastosis (244), and it can determine the presence of either a folate or a vitamin B_{12} deficiency (241). The test is sensitive and may be a better index of folate or vitamin B_{12} status than either erythrocyte folate or serum vitamin B_{12} levels (244). Because bone marrow cells are required to perform the test, its general use is restricted to clinical and research applications.

Subsequently it was reported that the deoxyuridine suppression test could be conducted with PHA-stimulated lymphocytes (50–53). The test was further simplified so that a small amount of whole blood (0.1 ml) could be used instead of isolated lymphocytes (53). It should be noted that an abnormal lymphocyte deoxyuridine suppression test may also indicate a past folate deficiency (50, 51); Das & Herbert (50) reported that the abnormal deoxyuridine suppression in the PHA-stimulated lymphocytes of patients with folic acid or vitamin B_{12} deficiencies persisted for up to 84 days after treatment. These observations must be kept in mind in using and interpreting results obtained from the lymphocyte deoxyuridine suppression test. It should be also noted that the lymphocyte deoxyuridine suppression test is still time-consuming to perform, because the PHA-stimulated lymphocytes require a 72–96-hour incubation period during the test.

The determination of hypersegmentation of neutrophils is easily performed and is a useful measure of folate or vitamin B_{12} deficiency (20, 44, 51, 162). However, neutrophil hypersegmentation is an unreliable indicator of folate deficiency during pregnancy (95). Although iron deficiency was considered to produce hypersegmentation, subsequent investigations revealed that it is probably the result of the existence of a masked folate deficiency (51).

Hypersegmentation of neutrophils occurs during granulopoiesis. Hence, the hypersegmentation persists for approximately two weeks after folic acid treatment of folate-deficient patients (151). Several criteria have been applied to the interpretation of neutrophil hypersegmentation data (44). Commonly, abnormal hypersegmentation has been considered to exist when a lobe average of 3.5 lobes or more per cell is observed or when 5% of the cells have five lobes. More recently, the presence of one six-lobed polymorphonuclear neutrophil per 100 cells was considered a reliable predictor of megaloblastosis in the bone marrow (44, 131, 151).

SUMMARY

This review is limited to progress in the development of new or improved laboratory procedures to assess the nutriture of thiamin, riboflavin, niacin,

vitamin B_6, and folic acid. There has been marked progress in this area for the other vitamins as well. The increased availability of radioassay techniques and HPLC methodologies that have application in nutrition assessment is significant. However, for a number of the vitamins, there is still a need for additional methods that provide functionally interpretable preclinical information and give accurate assessment of body reserves of the nutrient. Often the guides used to interpret the information obtained are tentative and require validation or revision. This situation is further complicated by the frequent lack of suitable reference standards for quality controls and interlaboratory validation.

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Literature Cited

1. Ahluwalia, G. S., Kuczala, Z. J. 1977. Analytical evaluation of a folate radioassay: application to serum and erythrocyte measurements. *Ann. Clin. Lab. Sci.* 7: 113–18
2. Allen, B. A., Newman, R. A. 1980. High-performance liquid chromatographic separation of clinically important folic acid derivatives using ion-pair chromatography. *J. Chromatogr.* 190:241–45
3. Anderson, S. A., Talbot, J. M. 1981. *A Review of Folate Intake, Methodology, and Status.* Life Sci. Res. Off. Fed. Am. Soc. Exp. Biol., Bethesda, Md. 83 pp.
4. Arend, R. A., Brown, R. R. 1981. Comparison of analytical methods for urinary 4-pyridoxic acid. *Am. J. Clin. Nutr.* 34:1984–85
5. Arienti, G., Simonetti, M. S., Fidanza, F. 1982. The effect of niacin deprivation on nicotinic acid mononucleotide pyrophosphorylase of pig erythrocytes. *Int. J. Vitam. Nutr. Res.* 52:143–48
6. Bailey, L. B., Mahan, C. S., Dimperio, D. 1980. Folacin and iron status in low-income pregnant adolescents and mature women. *Am. J. Clin. Nutr.* 33:1997–2001
7. Bailey, L. B., Wagner, P. A., Christakis, G. J., Araujo, P. E., Appledorf, H., Davis, C. G., Masteryanni, J., Dinning, J. S. 1979. Folacin and iron status and hematological findings in predominantly black elderly persons from urban low-income households. *Am. J. Clin. Nutr.* 32:2346–53
8. Bailey, L. B., Wagner, P. A., Christakis, G. J., Davis, C. G., Appledorf, H., Araujo, P. E., Dorsey, E., Dinning, J. S. 1982. Folacin and iron status and hematological findings in black and Spanish-American adolescents from urban low-income households. *Am. J. Clin. Nutr.* 35:1023–32
9. Bamji, M. S., Rameshwar Sarma, K. V., Radhaiah, G. 1979. Relationship between biochemical and clinical indices of B-vitamin deficiency. A study in rural school boys. *Br. J. Nutr.* 41:431–41
10. Baril, L., Carmel, R. 1978. Comparison of radioassay and microbiological assay for serum folate, with clinical assessment of discrepant results. *Clin. Chem.* 24: 2192–96
11. Basu, T. K., Patel, D. R., Williams, D. C. 1974. A simplified microassay of transketolase in human blood. *Int. J. Vitam. Nutr. Res.* 44:319–26
12. Baur, G., Jung, A., Wendel, A. 1982. Activity of the glutathione redox system in human erythrocytes at different ages. *Klin. Wochenschr.* 60:867–69 (In German)
13. Bayoumi, R. A., Rosalki, S. B. 1976. Evaluations of methods of coenzyme activation of erythrocyte enzymes for de-

tection of deficiency of vitamin B_1, B_2, B_6. *Clin. Chem.* 22:327–35
14. Bender, D. A. 1982. The tryptophan load test for vitamin B_6 status is inappropriate for women receiving oestrogens. *Proc. Nutr. Soc.* 41:120A
15. Bender, D. A. 1983. Effects of oestradiol and vitamin B_6 on tryptophan metabolism in the rat: implications for the interpretation of the tryptophan load test for vitamin B_6 nutritional status. *Br. J. Nutr.* 50:33–42
16. Bergmeyer, H. U., Horder, M. 1980. IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase. *J. Clin. Chem. Clin. Biochem.* 18:521–34
17. Bergmeyer, H. U., Horder, M., Moss, D. W. 1978. Provisional recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 3. Revised IFCC method for aspartate aminotransferase. *Clin. Chem.* 24:720–21
18. Bergmeyer, H. U., Scheibe, P., Wahlefeld, A. W. 1978. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clin. Chem.* 24:58–73
19. Bhagavan, H. N., Koogler, J. M. Jr., Coursin, D. B. 1976. Enzymatic microassay of pyridoxal-5'-phosphate using L-tyrosine apodecarboxylase and L-[1-^{14}C] tyrosine. *Int. J. Vitam. Nutr. Res.* 46:160–64
20. Bills, T., Spatz, L. 1977. Neutrophilic hypersegmentation as an indicator of incipient folic acid deficiency. *Am. J. Clin. Pathol.* 68:263–67
21. Böni, L., Kieckens, L., Hendrikx, A. 1980. An evaluation of a modified erythrocyte transketolase assay for assessing thiamine nutritional adequacy. *J. Nutr. Sci. Vitaminol. (Tokyo)* 26:507–14
22. Böni, H., Wassmer, A., Brubacher, G., Ritzel, G. 1977. Assessment of the vitamin B_2 status by means of the glutathione reductase test. *Nutr. Metab.* 21 (Suppl. 1):20–21
23. Bonjour, J. P. 1980. Vitamins and alcoholism. II. Folate and vitamin B_{12}. *Int. J. Vitam. Nutr. Res.* 50:96–121
24. Branfman, A. R., McComish, M. 1978. Rapid separation of folic acid derivatives by paired-ion high-performance liquid chromatography. *J. Chromatogr.* 151: 87–89
25. Brin, M. 1970. Transketolase (sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate dihydroxyacetonetransferase, EC 2.2.1.1) and the TPP effect in assessing thiamine adequacy. *Meth. Enzymol.* 18A:125–33
26. Brown, R. D., Robin, H., Kronenberg, H. 1982. Folate assays—an alternative to microbiological assays and commercial kits. *Pathology* 14:449–53
27. Brown, R. R. 1981. The tryptophan load test as an index of vitamin B-6 nutrition. In *Methods in Vitamin B-6 Nutrition. Analysis and Status Assessment*, ed. J. E. Leklem, R. D. Reynolds, pp. 321–40. New York/London: Plenum. 401 pp.
28. Bruns, D. E., Savory, J., Titheradge, A. C., Cross, R. E., Wills, M. R. 1981. Evaluation of the IFCC-recommended procedure for serum aspartate aminotransferase as modified for use with the centrifugal analyzer. *Clin. Chem.* 27: 156–59
29. Buttery, J. E., Milner, C. R., Chamberlain, B. R. 1982. The NADH-dependent transketolase assay: a note of caution. *Clin. Chem.* 28:2184–85
30. Camp, V. M., Chipponi, J., Faraj, B. A. 1983. Radioenzymatic assay for direct measurement of plasma pyridoxal-5'-phosphate. *Clin. Chem.* 29:642–44
31. Carter, E. G. 1982. Quantitation of urinary niacin metabolites by reversed-phase liquid chromatography. *Am. J. Clin. Nutr.* 36:926–30
32. Cashmore, A. R., Dreyer, R. N., Horváth, C., Knipe, J. O., Coward, J. K., Bertino, J. R. 1980. Separation of pteroyl-oligo-γ-L-glutamates by high-performance liquid chromatography. *Meth. Enzymol.* 66:459–69
33. Chanarin, I. 1977. Isotopes in megaloblastic anaemia. *Clinics in Haematol.* 6:719–30
34. Chanarin, I. 1980. The Folates. In *Vitamins in Medicine*, ed. B. M. Barker, D. A. Bender, 1:247–314. London: Whitefrairs. 459 pp. 4th ed.
35. Chapman, S. K., Greene, B. C., Streiff, R. R. 1978. A study of serum folate by high-performance ion-exchange and ion-pair partition chromatography. *J. Chromatogr.* 145:302–6
36. Chauhan, M. S., Dakshinamurti, K. 1981. Fluorometric assay of B_6 vitamers in biological materials. *Clin. Chim. Acta* 109:159–67
37. Chen, M. F., McIntyre, P. A., Kertcher, J. A. 1978. Measurement of folates in human plasma and erythrocytes by a radiometric microbiologic method. *J. Nucl. Med.* 19:906–12
38. Chen, I. W., Silberstein, E. B., Maxon, H. R., Volle, C. P., Sohnlein, B. W. 1982. Semiautomated system for simultaneous assays of serum vitamin B_{12} and folic acid in serum evaluated. *Clin. Chem.* 28:2161–65
39. Cheng, C. H., Koch, M., Shank, R. E. 1976. Leukocyte transketolase activity as

an indicator of thiamin nutriture in rats. *J. Nutr.* 106:1678–85

40. Clark, B. R., Halpern, R. M., Smith, R. A. 1975. A fluorimetric method for quantitation in the picomole range of N^1-methylnicotinamide and nicotinamide in serum. *Anal. Biochem.* 68:54–61
41. Clark, B. R. 1980. Fluorometric quantitation of picomole amounts of 1-methylnicotinamide and nicotinamide in serum. *Meth. Enzymol.* 66:5–8
42. Clifford, C. K., Clifford, A. J. 1977. High pressure liquid chromatographic analysis of food for folates. *J. Assoc. Off. Anal. Chem.* 60:1248–51
43. Coburn, S. P., Mahuren, J. D. 1983. A versatile cation-exchange procedure of measuring the seven major forms of vitamin B_6 in biological samples. *Anal. Biochem.* 129:310–17
44. Colman, N. 1981. Laboratory assessment of folate status. *Clin. Lab. Med.* 1:755–96
45. Colman, N., Herbert, V. 1979. Kinetic and chromatographic evidence for heterogeneity of the high affinity folate binding in serum. In *Chemistry and Biology of Pteridines*, ed. R. L. Kisliuk, G. M. Brown. New York: Elsevier-Holland. 525 pp.
46. Colman, N., Herbert, V. 1980. Hematologic complications of alcoholism: Overview. *Semin. Hematol.* 17:164–76
47. Colman, N., Herbert, V. 1980. Abnormal lymphocyte deoxyuridine suppression test: a reliable indicator of decreased lymphocyte folate levels. *Am. J. Hematol.* 8:169–74
48. Dakshinamurti, K., Chauhan, M. S. 1981. Chemical analysis of pyridoxine vitamers. See Ref. 27, pp. 99–122
49. Das, K. C., Garewal, G., Mohanty, D. 1980. Derangement of DNA synthesis in erythroleukaemia. Normal deoxyuridine suppression and impaired thymidine incorporation in bone marrow culture. *Acta Haematol. (Basel)* 64:121–30
50. Das, K. C., Herbert, V. 1978. The lymphocyte as a marker of past nutritional status: Persistence of abnormal lymphocyte deoxyuridine (dU) suppression test and chromosomes in patients with past deficiency of folate and vitamin B_{12}. *Br. J. Haematol.* 38:219–33
51. Das, K. C., Herbert, V., Colman, N., Longo, D. C. 1978. Unmasking covert folate deficiency in iron-deficient subjects with neutrophil hypersegmentation: dU suppression tests on lymphocytes and bone marrow. *Br. J. Haematol.* 39:357–75
52. Das, K. C., Manusselis, C., Herbert, V. 1980. In vitro DNA synthesis by bone marrow cells and PHA-stimulated lymphocytes. Suppression by nonradioactive thymidine of the incorporation of ^{3}H-deoxyuridine into DNA: Enhancements of incorporation when inadequate vitamin B_{12} or folate is corrected. *Br. J. Haematol.* 44:51–63
53. Das, K. C., Manusselis, C., Herbert, V. 1980. Simplifying lymphocyte culture and the deoxyuridine suppression test by using whole blood (0.1 ml) instead of separated lymphocytes. *Clin. Chem.* 26: 72–77
54. Dawson, D. W., Delamore, I. W., Fish, D. I., Flaherty, T. A., Gowenlock, A. H., Hunt, L. P., Hyde, K., MacIver, J. E., Thornton, J. A., Waters, H. M. 1980. An evaluation of commercial radioisotope methods for the determination of folate and vitamin B_{12}. *J. Clin. Pathol.* 33:234–42
55. Day, B. P., Gregory, J. F. III. 1981. Determination of folacin derivatives in selected foods by high-performance liquid chromatography. *J. Agric. Food Chem.* 29:374–77
56. Defibaugh, P. W., Smith, J. S., Weeks, C. E. 1977. Assay of thiamine in foods, using manual and semiautomated fluorometric and microbiological methods. *J. Assoc. Off. Anal. Chem.* 60:522–27
57. DeVries, J. X., Günthert, W., Ding, R. 1980. Determination of nicotinamide in human plasma and urine by ion-pair reversed-phase high-performance liquid chromatography. *J. Chromatogr.* 221: 161–65
58. Dong, M. H., Green, M. D., Sauberlich, H. E. 1981. Determination of urinary thiamin by the thiochrome method. *Clin. Biochem.* 14:16–18
59. Duffy, P., Morris, H., Neilson, G. 1981. Thiamin status of a Melanesian population. *Am. J. Clin. Nutr.* 34:1584–92
60. Echols, R. E., Harris, J., Miller, R. H. Jr. 1980. Modified procedure for determining vitamin B_1 by gas chromatography. *J. Chromatogr.* 193:470–75
61. Edwin, E. E. 1979. An improved procedure for the determination of thiamine. *Meth. Enzymol.* 62:51–54
62. Ellefson, W. C., Richter, E., Adams, M., Baillies, N. T. 1981. Evaluation of ion exchange resins and various enzymes in thiamine analysis. *J. Assoc. Off. Anal. Chem.* 64:1336–38
63. Enneking-Ivey, O., Bailey, L. B., Gawley, L., Hananian, J., Hurd, R. 1981. Folic acid and vitamin D status of young children receiving minimal anticonvulsant drug therapy. *Int. J. Vitam. Nutr. Res.* 51:349–52
64. Eto, I., Krumdieck, C. L. 1980. Determination of three different pools of re-

duced one-carbon-substituted folates. I. A study of the fundamental chemical reactions. *Anal. Biochem.* 109:167–84
65. Eto, I., Krumdieck, C. L. 1981. Determination of three different pools of reduced one-carbon-substituted folates. II. Quantitation and chain-length determination of the pteroylpolyglutamates of rat liver. *Anal. Biochem.* 115:138–146
66. Feyns, L. V., Thakker, K. D., Reif, V. D., Grady, L. T. 1982. Purity profiles of pteroylglutamate reference substances by high-performance liquid chromatography. *J. Pharmaceut. Sci.* 71:1242–46
67. Kishi, H., Folkers, K. 1976. Improved and effective assays of the glutamic oxaloacetic transaminase by the coenzyme-apoenzyme system (CAS) principle. *J. Nutr. Sci. Vitaminol. (Tokyo)* 22:225–34
68. Friedman, R. B., Anderson, R. E., Entine, S. M., Hirshberg, S. B. 1980. Effects of disease on clinical laboratory tests. *Clin. Chem.* 26:1D-476D
69. Garand, R., Zittoun, J., Marquet, J., Mouraud, E., Grolleau, J. Y., Le Mevel, A., Zittoun, R. 1980. Deoxyuridine-suppression test and bone marrow culture for the diagnosis of macrocytic refractory anemias. *Scand. J. Haematol.* 35:231–36
70. Garry, P. J., Owen, G. M. 1976. An automated flavin adenine dinucleotide-dependent glutathione reductase assay for assessing riboflavin nutriture. *Am. J. Clin. Nutr.* 28:663–74
71. Gatautis, V. J., Naito, H. K. 1981. Liquid-chromatographic determination of urinary riboflavin. *Clin. Chem.* 27:1672–75
72. Girdwood, R. H. 1977. Drug-induced folate deficiency. In *Folid Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement*, pp. 169–77. Washington D.C.: Natl. Sci. USA. 298 pp.
73. Givas, J. K., Gutcho, S. 1975. pH dependence of the binding of folates to milk binder in radioassay of folates. *Clin. Chem.* 21:427–28
74. Glatzle, D., Vuilleumier, J. P., Weber, F., Decker, K. 1974. Glutathione reductase test with whole blood, a convenient procedure for the assessment of the riboflavin status in humans. *Experientia* 30: 665–67
75. Graham, D. C., Roe, D. A., Ostertag, S. G. 1980. Radiometric determination and chick bioassay of folacin in fortified and unfortified frozen foods. *J. Food Sci.* 45:47–51
76. Gravesen, J. 1977. pH metric method for the determination of nicotinic acid in plasma. *J. Clin. Microbiol.* 5:390–92
77. Gregory, J. F. III. 1980. Determination of pyridoxal-5'-phosphate as the semicarbazone derivative using high-performance liquid chromatography. *Anal. Biochem.* 102:374–79
78. Gregory, J. F. III. 1982. Relative activity of the nonphosphorylated B_6 vitamers for *Saccharomyces uvarum* and *Kloeckera brevis* in vitamin B_6 microbiological assay. *J. Nutr.* 112:1643–47
79. Gregory, J. F. III., Kirk, J. R. 1979. Determination of urinary 4-pyridoxic acid using high performance liquid chromatography. *Am. J. Clin. Nutr.* 32:879–83
80. Gregory, J. F. III, Kirk, J. R. 1981. Determination of vitamin B_6 compounds by semiautomated continuous-flow and chromatographic methods. See Ref. 27, pp. 149–70
81. Grossowicz, N., Waxman, S., Schreiber, C. 1981. Cryoprotected *Lactobacillus casei:* An approach to standardization of microbiological assay of folic acid in serum. *Clin. Chem.* 27:745–47
82. Guilarte, T. R. 1983. Radiometric-microbiological assay of vitamin B_6: Assay simplification and sensitivity study. *J. Assoc. Off. Anal. Chem.* 66:58–61
83. Guilarte, T. R., McIntyre, P. A. 1981. Radiometric-microbiological assay of vitamin B_6: Analysis of plasma samples. *J. Nutr.* 111:1861–68
84. Guilarte, T. R., McIntyre, P. A., Tsan, M. F. 1980. Growth response of the yeast *Saccharomyces uvarum* and *Kloeckera brevis* to the free biologically active forms of vitamin B_6. *J. Nutr.* 110:954–58
85. Guilarte, T. R., Shane, B., McIntyre, P. A. 1981. Radiometric-microbiologic assay of vitamin B_6: Application to food analysis. *J. Nutr.* 111:1869–75
86. Guilarte, T. R., Tsan, M. F. 1983. Microbiological assays of total vitamin B_6 using the yeasts *Saccharomyces uvarum* and *Kloeckera brevis. J. Nutr.* 113:721–24
87. Gubler, C. J., Hemming, B. C. 1979. High-pressure liquid chromatography of thiamine, thiamine analogs, and their phosphate esters. *Meth. Enzymol.* 62:63–68
88. Gubler, C. J., Murdock, D. S. 1979. Differential determination of thiamine and its phosphates and hydroxyethiamine and pyrithiamine. *Meth. Enzymol.* 62:54–57
89. Gutcho, S., Bakke, O. M. 1978. Source of error in determination of erythrocyte folate by competitive binding radioassay—comments. *Clin. Chem.* 24:388–89
90. Gutcho, S., Mansbach, L. 1977. Simul-

taneous radioassay of serum vitamin B_{12} and folic acid. *Clin. Chem.* 23:1609–14
91. Hathcock, J. N. 1978. Thiamin deficiency effects on rat leukocyte pyruvate decarboxylation rates. *Am. J. Clin. Nutr.* 31:250–52
92. Heilmann, E., Bönninghoff, E. 1976. Folic acid determination in the serum and erythrocytes by means of a competitive binding method with 125-iodine labeling. *Schweiz Med. Wochenschr.* 106: 1821–23 (In German)
93. Hendel, J. 1981. Radioimmunoassay for pteroylglutamic acid. *Clin. Chem.* 27: 701–3
94. Hengen, N., Seiberth, V., Hengen, M. 1978. High-performance liquid chromatographic determination of free nicotinic acid and its metabolite, nicotinuric acid, in plasma and urine. *Clin. Chem.* 24: 1740–43
95. Herbert, V., Colman, N., Spivack, M., Ocasio, E., Ghanta, V., Kimmel, K., Brenner, L., Freundlich, J., Scott, J. 1975. Folic acid deficiency in the United States: folate assays in a prenatal clinic. *Am. J. Obstetr. Gynecol.* 123:175–79
96. Herbert, V., Tisman, G., Go, L. T., Brenner, L. 1973. The dU suppression test using ^{125}I-UdR to define biochemical megaloblastosis. *Br. J. Haematol.* 24: 713–23
97. Hilker, D. M., Clifford, A. J. 1982. Thiamin analysis and separation of thiamin phosphate esters by high-performance liquid chromatography. *J. Chromatogr.* 10:433–38
98. Hill, P. W., Dawson, D. W. 1977. Evaluation of a commercial radioisotopic kit for folate assays. *J. Clin. Pathol.* 30:449–53
99. Hubert, C., Poon, M. C. 1975. Glutamic-pyruvic transaminase activity related to red blood cell age. *Can. J. Biochem.* 53:731–34
100. Ishii, K., Sarai, K., Sanemori, H. 1979. Analysis of thiamine and its phosphate esters by high-performance liquid chromatography. *Anal. Biochem.* 97:191–95
101. Ishii, K., Sarai, K., Sanemori, H., Kawasaki, T. 1979. Concentrations of thiamine and its phosphate esters in rat tissues determined by high-performance liquid chromatography. *J. Nutr. Sci. Vitaminol. (Tokyo)* 25:517–23
102. Johnson, I., Guilford, H., Rose, M. 1977. Measurement of serum folate: experience with ^{75}Se-selenofolate radioassay. *J. Clin. Pathol.* 30:645–48
103. Johnson, I., Rose, M. 1978. Measurement of red cell folate with ^{75}Se-selenofolate radioassay. *J. Clin. Pathol.* 31: 47–49
104. Jones, P., Grace, C. S., Rozenberg, M. C. 1979. Interpretation of serum and red cell folate results: A comparison of microbiological and radioisotopic methods. *Pathology* 11:45–52
105. Jung, K., Grützmann, K. D. 1977. Comparative determinations of aminotransferase activities in serum with so-called "optimised" methods. *Clin. Chim. Acta* 81:299–304
106. Kaczmarek, M. D., Nixon, P. F. 1983. Variants of transketolase from human erythrocytes. *Clin. Chim. Acta* 130:349–56
107. Kaufmann, N. A., Guggenheim, K. 1977. The validity of biochemical assessment of thiamine, riboflavin, and folacin nutriture. *Int. J. Vitam. Nutr. Res.* 47:40–45
108. Kawai, C., Wakabayashi, A., Matsumura, T., Yui, Y. 1980. Reappearance of beriberi heart disease in Japan. A study of 23 cases. *Am. J. Med.* 69:383–86
109. Kerkay, J., Coburn, C. M., McEvoy, D. 1977. Effect of sodium ascorbate concentration on the stability of samples for determination of serum folate levels. *Am. J. Clin. Pathol.* 68:481–84
110. Kertcher, J. A., Guilarte, T. R., Chen, M. F., Rider, A. A., McIntyre, P. A. 1979. A radiometric microbiologic assay for the biologically active forms of niacin. *J. Nucl. Med.* 20:419–23
111. Kimura, M., Fujita, T., Itokawa, Y. 1982. Liquid-chromatograhic determination of the total thiamin content of blood. *Clin. Chem.* 28:29–31
112. Kimura, M., Fujita, T., Nishida, S., Itokawa, Y. 1980. Differential fluorimetric determination of picogram levels of thiamine, thiamine monophosphate, diphosphate and triphosphate using high-performance liquid chromatography. *J. Chromatogr.* 188:417–19
113. Kimura, M., Itokawa, Y. 1982. Determination of blood transketolase by high-performance liquid chromatography (a preliminary note). *J. Chromatogr.* 239:707–10
114. Kishi, H., Kishi, T., Williams, R. H., Folkers, K. 1975. Human deficiencies of vitamin B_6. I. Studies on parameters of the assay of the glutamic oxaloacetic transaminase by the CAS principle. *Res. Commun. Chem. Pathol. Pharmacol.* 12:557–69
115. Kjosen, B., Seim, S. H. 1977. The transketolase assay of thiamine in some diseases. *Am. J. Clin. Nutr.* 30:1591–96
116. Knobloch, E., Hodr, R., Janda, J., Herzmann, J., Houdková, V. 1979. Spectrofluorimetric micromethod for determining riboflavin in the blood of new-born

babies and their mothers. *Int. J. Vitam. Nutr. Res.* 49:144–51
117. Kochetov, G. A. 1983. Determination of transketolase activity via ferricyanide reduction. *Meth. Enzymol.* 89D:43–44
118. Krämer, U., Bitsch, R., Hötzel, D. 1977. Applicability of blood and urine analysis for the determination of nutrition status of riboflavin. *Nutr. Metab.* 21 (Suppl 1):22–23
119. Krumdieck, C. L., Eto, I., Krumdieck, R. 1983. New methods for the study of folate coenzymes: endogenous polyglutamate patterns of subcellular hepatocyte fractions and of regenerating rat liver. In *Folyl and Antifolyl Polyglutamates,* ed. I. D. Goldman, B. A. Chabner, J. R. Bertino, pp. 115–37. New York: Plenum. 425 pp.
120. Kubasik, N. P., Volosin, M. T., Sine, H. E. 1975. Comparison of commercial kits for radioimmunoassay: III. Radioassay of serum folate. *Clin. Chem.* 21:1922–26
121. Kum-Tatt, L., Tan, I. K., Seet, A. M. 1975. A new colorimetric method for the determination of NADH/NADPH dependent glutathione reductase in erythrocytes and in plasma. *Clin. Chim. Acta* 58:101–8
122. Kuriyama, M., Yokomine, R., Arima, H., Hamada, R., Igata, A. 1980. Blood vitamin B_1, transketolase and thiamine pyrophosphate (TPP) effect in beriberi patients, with studies employing discriminant analysis. *Clin. Chim. Acta* 108: 159–68
123. Kutnink, M. A., Vannucchi, H., Sauberlich, H. E. 1984. A simple high performance liquid chromatography procedure for the determination of N^1-methylnicotinamide in urine. *J. Liquid Chromatogr.* In press
124. Lankelma, J., Van Der Kleijn, E., Jansen, M. J. 1980. Determination of 5-methyltetrahydrofolic acid in plasma and spinal fluid by high-performance liquid chromatography, using on-column concentration and electrochemical detection. *J. Chromatogr.* 182:35–45
125. Leinert, J., Simon, I., Hötzel, D. 1981. Evaluation of methods to determine the vitamin B_6 status of human. 1. α-EGOT: methods and validation. *Int. J. Vitam. Nutr. Res.* 51:145–54
126. Leinert, J., Simon, I., Hötzel, D. 1982. Methods and their evaluation in the determination of vitamin B_6 status in man. 2. α-EGOT: reliability of the parameter. *Int. J. Vitam. Nutr. Res.* 52:24–35
127. Leklem, J. E., Linkswiler, H. M., Brown, R. R., Rose, D. P., Anand, C. R. 1977. Metabolism of methionine in oral contraceptive users and control women receiving controlled intakes of vitamin B_6. *Am. J. Clin. Nutr.* 30:1122–28
128. Leunis, J. C., Van Riet, C., Brauman, J. 1982. Two-step assay of erythrocyte transketolase activity. *Clin. Chem.* 28: 391–92
129. Lim, K. L., Young, R. W., Driskell, J. A. 1980. Separation of vitamin B_6 components by high-performance liquid-chromatography. *J. Chromatogr.* 188: 285–88
130. Lindemans, J., Van Kapel, J., Abels, J. 1975. Evaluation of a radioassay for serum folate and the effects of ascorbate and methotrexate. *Clin. Chim. Acta* 65: 15–20
131. Lindenbaum, J., Nath, B. J. 1980. Megaloblastic anemia and neutrophil hypersegmentation. *Br. J. Haematol.* 44:511–13
132. Linkswiler, H. M. 1981. Methionine metabolite excretion as affected by a vitamin B-6 deficiency. See Ref. 27, pp. 373–81
133. Liu, Y. K. 1975. Folic acid deficiency in sickle cell anemia. *Scand. J. Haematol.* 14:71–79
134. Longo, D. L., Herbert, V. 1976. Radioassay for serum and red cell folate. *J. Lab. Clin. Med.* 87:138–51
135. Lonsdale, D., Shamberger, R. J. 1978. The potential value of red cell transketolase in the metabolic evaluation of disease. *Cleveland Clin. Q.* 45:267–74
136. Lotter, S. E., Miller, M. S., Bruch, R. C., White, H. B. III. 1982. Competitive binding assays for riboflavin and riboflavin-binding protein. *Anal. Biochem.* 125:110–17
137. Lumeng, L., Lui, A., Ting-Kai, L. 1981. Microassay of pyridoxal phosphate using tryosine apodecarboxylase. See Ref. 27, pp. 57–67
138. Lumeng, L., Ryan, M. P., Li, T. K. 1978. Validation of the diagnostic value of plasma pyridoxal 5'-phosphate measurements in vitamin B_6 nutrition of the rat. *J. Nutr.* 108:545–53
139. Magnus, E. M. 1977. Automated microbiological assay for folic acid. See Ref. 72, pp. 152–155
140. Martinez, O., Roe, D. A. 1977. Effect of oral contraceptives on blood folate levels in pregnancy. *Am. J. Obstetr. Gynecol.* 128:255–61
141. Matsui, M. S., Rozovski, S. J. 1982. Drug-nutrient interaction. *Clin. Ther.* 4: 423–40
142. McGown, E. L., Lewis, C. M., Dong, M. H., Sauberlich, H. E. 1978. Results with commercial radioassay kits compared with microbiological assay of fo-

late in serum and whole-blood. *Clin. Chem.* 24:2186–91
143. McKee, R. W., Kang-Lee, Y. A., Panaqua, M., Swendseid, M. E. 1982. Determination of nicotinamide and metabolic products in urine by high-performance liquid chromatography. *J. Chromatogr.* 230:309–17
144. McMartin, K. E., Virayotha, V., Tephly, T. R. 1981. High-pressure liquid chromatography separation and determination of rat liver folates. *Arch. Biochem. Biophys.* 209:127–36
145. McSharry, W. O., Mahn, F. P. 1979. High-pressure liquid chromatographic assay of folic acid: a collaborative study. *J. Pharm. Sci.* 68:241–43
146. Meadows, G. G., Boze, L., Elmer, G. W. 1977. New rapid determination of pyridoxal phosphate using tryosine phenol-lyase. *J. Pharm. Sci.* 66:1503–5
147. Miller, L. T., Dow, M. J., Kokkeler, S. C. 1978. Methionine metabolism and vitamin B_6 status in women using oral contraceptives. *Am. J. Clin. Nutr.* 31: 619–25
148. Mitchell, G. A., Pochron, S. P., Smutny, P. V., Guity, R. 1976. Decreased radioassay values for folate after serum extraction when pteroylglutamic acid standards are used. *Clin. Chem.* 22:647–49
149. Mortensen, E. 1976. Determination of erythrocyte folate by competitive protein binding assay preceded by extraction. *Clin. Chem.* 22:982–92
150. Mrochek, J. E., Jolley, R. L., Young, D. S., Turner, W. J. 1976. Metabolic response of humans to ingestion of nicotinic acid and nicotinamide. *Clin. Chem.* 22:1821–27
150a. Müller, E. M., Bates, C. J. 1977. The effect of riboflavin deficiency on white cell glutathione reductase in rats. *Int. J. Vitam. Nutr. Res.* 47:46–51
151. Nath, B. J., Lindenbaum, J. 1979. Persistence of neutrophil hypersegmentation during recovery from megaloblastic granulopoiesis. *Ann. Intern. Med.* 90:757–60
152. Okabe, T., Ishizawa, S., Ishii, T., Kataoka, K., Matsuki, S. 1982. Erythrocyte aging changes evaluated by the deoxyuridine suppression tests. *J. Am. Geriatr. Soc.* 30:626–31
153. Paine, C. J., Grafton, W. D., Dickson, V. L., Eichner, E. R. 1975. Oral contraceptives, serum folate, and hematologic status. *J. Am. Med. Assoc.* 231:731–33
154. Park, J. Y. 1975. Comparative study of colorimetric and fluorometric determination of thiamine (vitamin B_1) by automated discrete-sampling technique. *Anal. Chem.* 47:452–58
155. Patrini, C., Rindi, G. 1980. An improved method for the electrophoretic separation and fluorometric determination of thiamine and its phosphates in animal tissues. *Int. J. Vitam. Nutr. Res.* 50:10–18
156. Pelletier, O., Brassard, R. 1977. Automated and manual determination of N^1-methylnicotinamide in urine. *Am. J. Clin. Nutr.* 30:2108–16
157. Pelliniemi, T.-T., Beck, W. S. 1980. Biochemical mechanisms in the Killmann experiment: critique of the deoxyuridine suppression test. *J. Clin. Invest.* 65:449–60
158. Penttinen, H. K. 1978. Determination of thiamine and its phosphate esters by electrophoresis and fluorometry. *Acta Chem. Scand.* B32:609–12
159. Penttinen, H. K. 1979. Fluorometric determination of thiamine and its mono-, di-, and triphosphate esters. *Meth. Enzymol.* 62:58–59
160. Penttinen, H. K. 1979. Electrophoretic separation of thiamine and its mono-, di-, and triphosphate esters. *Meth. Enzymol.* 62:68–69
161. Pietarinen, G. J., Leichter, J., Pratt, R. F. 1977. Dietary folate intake and concentration of folate in serum and erythrocytes in women using oral contraceptives. *Am. J. Clin. Nutr.* 30:375–80
162. Pietrzik, K., Urban, G., Hötzel, D. 1979. Biochemical and hematological measures for determination of folate status in humans. 1. Relation between serum folate and segmentation of neutrophil granulocytes. *Int. J. Vitam. Nutr. Res.* 48:391–96
163. Poloni, M., Patrini, C., Rocchelli, B., Rindi, G. 1982. Thiamin monophosphate in the CSF of patients with amyotrophic lateral sclerosis. *Arch. Neurol.* 39:507–9
164. Powers, H. J., Thurnham, D. I. 1981. Riboflavin deficiency in man: effects on haemoglobin and reduced glutathione in erythrocytes of different ages. *Br. J. Nutr.* 46:257–66
165. Prentice, A. M., Bates, C. J. 1981. A biochemical evaluation of the erythrocyte glutathione reductase (EC 1.6.4.2) test for riboflavin deficiency. 1. Rate and specificity of response in acute deficiency. *Br. J. Nutr.* 45:37–52
166. Prentice, A. M., Bates, C. J. 1981. A biochemical evaluation of the erythrocyte glutathione reductase (EC 1.6.4.2) test for riboflavin status. 2. Dose-response relationships in chronic marginal deficiency. *Br. J. Nutr.* 45:53–65
167. Prentice, A. M., Bates, C. J., Prentice, A., Welch, S. G., Williams, K., McGre-

gor, I. A. 1981. The influence of G-6-PD activity on the response of erythrocyte glutathione reductase to riboflavin deficiency. *Int. J. Vitam. Nutr. Res.* 51: 211–15

168. Purcell, G. V., Behenna, D. B., Walsh, P. R. 1979. Alanine aminotransferase and aspartate aminotransferase measurements with two automated analyzers, SMAC and the ABA-100, compared. *Clin. Chem.* 25:780–82
169. Ramasastri, B. V. 1976. A simple method for estimation of thiamine in foods using base-exchange on sand instead of Decalso. *Int. J. Vitam. Nutr. Res.* 46:403–11
170. Reed, L. S., Archer, M. C. 1976. Separation of folic acid derivatives by high-performance liquid chromatography. *J. Chromatogr.* 121:100–3
171. Reif, V. D., Reamer, J. T., Grady, L. T. 1977. Chromatographic assays for folic acid. *J. Pharmaceut. Sci.* 66:1112–16
172. Reijnierse, G. L., Van Der Horst, A. R., De Kloet, K., Voorhorst, C. D. 1978. A radiochemical method for the determination of transketolase activity in erythrocyte hemolysates. *Clin. Chim. Acta* 90: 259–68
173. Reingold, R. N., Picciano, M. F., Perkins, E. G. 1980. Separation of folate derivatives by in situ paired-ion high-performance liquid chromatography. *J. Chromatogr.* 190:237–40
174. Rettenmaier, R., Vuilleumier, J. P., Müller-Mulot, W. 1979. Assay of vitamin B_1 in food and biological material. *Z. Lebensm. Unters. Forsch.* 26:120–24 (In German)
175. Ribbron, W. M., Stevenson, K. E. 1977. Comparison of semiautomated and manual methods for the determination of thiamine in baby cereals and infant and dietary formulas. *J. Assoc. Off. Anal. Chem.* 60:737–38
176. Robinson, W. T., Cosyns, L., Kraml, M. 1978. An automated method for the analysis of nicotinic acid in serum. *Clin. Biochem.* 11:46–49
177. Rose, C. S., György, P., Butler, M., Andres, R., Norris, A. H., Shock, N. W., Tobin, J., Brin, M., Spiegel, H. 1976. Age differences in vitamin B_6 status of 617 men. *Am. J. Clin. Nutr.* 29:847–53
178. Roser, R. L., Andrist, A. H., Harrington, W. H., Naito, H. K., Lonsdale, D. 1978. Determination of urinary thiamine by high-performance liquid chromatography utilizing the thiochrome fluorescent method. *J. Chromatogr.* 146:43–53
179. Rothenberg, S. P., Da Costa, M., Lawson, J., Rosenberg, Z. 1974. The determination of erythrocyte folate concentration using a two-phase ligand-binding radioassay. *Blood* 43:437–43
180. Rudzki, Z., Nazaruk, M., Kimber, R. J. 1976. The clinical value of the radioassay of serum folate. *J. Lab. Clin. Med.* 87: 859–67
181. Ryan, M. A., Ingle, J. D. Jr. 1980. Fluorometric reaction rate method for the determination of thiamine. *Anal. Chem.* 52:2177–84
182. Sandhu, J. S., Fraser, D. R. 1981. Measurement of niacin metabolites in urine by high pressure liquid chromatography. A simple, sensitive assay of niacin nutritional status. *Int. J. Vitam. Nutr. Res.* 51:139–44
183. Sanemori, H., Ueki, H., Kawasaki, T. 1980. Reversed-phase high-performance liquid chromatographic analysis of thiamine phosphate esters at subpicomole levels. *Anal. Biochem.* 107:451–55
184. Sauberlich, H. E. 1977. Detection of folic acid deficiency in populations. See Ref. 72, pp. 213–31
185. Sauberlich, H. E. 1981. Vitamin B_6 status assessment: Past and present. See Ref. 27, pp. 203–39
186. Sauberlich, H. E. 1981. Recent advances in analytical methods for the water-soluble vitamins. *Prog. Clin. Biol. Res.* 77:151–57
187. Sauberlich, H. E., Dowdy, R. P., Skala, J. H. 1974. *Laboratory Tests for the Assessment of Nutritional Status*, pp. 70–74. Boca Raton, Fla.: CRC
188. Sauberlich, H. E., Herman, Y. F., Stevens, C. O., Herman, R. H. 1979. Thiamin requirement of the adult human. *Am. J. Clin. Nutr.* 32:2237–48
189. Schorah, C. J., Messenger, D. 1975. Flavin nucleotides, glutathione reductase and assessment of riboflavin status. *Int. J. Vitam. Nutr. Res.* 45:39–50
190. Schreiber, C., Waxman, S. 1974. Measurement of red cell folate levels by ^{3}H-pteroylglutamic acid (^{3}H-PteGlu) radioassay. *Br. J. Haematol.* 27:551–58
191. Schrijver, J., Speek, A. J., Klosse, J. A., Van Rijn, H. J., Schreurs, W. H. 1982. A reliable semiautomated method for the determination of total thiamine in whole blood by the thiochrome method with high-performance liquid chromatography. *Ann. Clin. Biochem.* 19:52–56
192. Schrijver, J., Speek, A. J., Schreurs, W. H. P. 1981. Semiautomated fluorometric determination of pyridoxal-5'-phosphate (vitamin B_6) in whole blood by high-performance liquid chromatography (HPLC). *Int. J. Vitam. Nutr. Res.* 51: 216–22

193. Scott, J. M., Ghanta, V., Herbert, V. 1974. Trouble-free microbiological serum and red cell folate assays. *Am. J. Med. Technol.* 40:125–34
194. Shaikh, B., Pontzer, N. J. 1979. Direct urinary assay method of N^1-methylnicotinamide by soap chromatography. *J. Chromatogr.* 162:596–600
195. Shaikh, B., Pontzer, N. J., Huang, S. S., Zielinski, W. L. Jr. 1977. Determination of N^1-methylnicotinamide in urine by high-pressure liquid chromatography. *J. Chromatogr. Sci.* 15:215–17
196. Shane, B. 1982. High performance liquid chromatography of folates: identification of poly-gamma-glutamate chain lengths of labeled and unlabeled folates. *Am. J. Clin. Nutr.* 35:599–608
197. Shane, B., Tamura, T., Stokstad, E. L. R. 1980. Folate assay: A comparison of radioassay and microbiological methods. *Clin. Chim. Acta* 100:13–19
198. Sharda, D., Bamji, M. S. 1972. Erythrocyte glutathione reductase activity and riboflavin concentration in experimental deficiency of some water soluble vitamins. *Int. J. Vit. Nutr. Res.* 42:43–49
199. Shin, Y. S., Rasshofer, R., Friedrich, B., Endres, W. 1983. Pyridoxal-5'-phosphate determination by a sensitive micromethod in human blood, urine and tissues; its relation to cystathioninura in neuroblastoma and biliary atresia. *Clin. Chim. Acta* 127:77–85
200. Shin-Buehring, Y., Rasshofer, R., Endres, W. 1981. A new enzymatic method for pyridoxal-5-phosphate determination. *J. Inher. Metab. Dis.* 4:123–24
201. Shojania, A. M. 1980. Problems in the diagnosis and investigation of megaloblastic anemia. *Can. Med. Assoc. J.* 122:999–1004
202. Shojania, A. M. 1982. Oral contraceptives: effect of folate and vitamin B_{12} metabolism. *Can. Med. Assoc. J.* 126:244–47
203. Shultz, T. D., Leklem, J. E. 1981. Urinary 4-pyridoxic acid, urinary vitamin B-6 and plasma pyridoxal phosphate as measures of vitamin B-6 status and dietary intake in adults. See Ref. 27, pp. 297–320
204. Simon, I., Leinert, J., Hötzel, D. 1982. Methods and their evaluation in estimating the vitamin B_6 status in humans. 3. Determination of 4-pyridoxic acid in urine. *Int. J. Vitam. Nutr. Res.* 52:280–86
205. Skala, J. H., Taylor, P. C., Johnson, H. L. 1982. Determination and use of erythrocyte aminotransferase activities for the evaluation of vitamin B_6 status in humans. *Fed. Proc.* 41:469
206. Skala, J. H., Waring, P. P., Lyons, M. F., Rusnak, M. G., Alletto, J. S. 1981. Methodology for determination of blood aminotransferases. See Ref. 27, pp. 171–202
207. Smeets, E. H. J., Muller, H., DeWael, J. 1971. A NADH-dependent transketolase assay in erythrocyte hemolysates. *Clin. Chim. Acta* 33:379–86
208. Smith, G. P., Samson, D., Peters, T. J. 1983. A fluorimetric method for the measurement of pyridoxal and pyridoxal phosphate in human plasma and leucocytes, and its application to patients with sideroblastic marrows. *J. Clin. Pathol.* 36:701–6
209. Smith, M. D. 1980. Rapid method for determination of riboflavin in urine by high-performance liquid chromatography. *J. Chromatogr.* 182:285–91
210. Stout, R. W., Cashmore, A. R., Coward, J. K., Horváth, C. G., Bertino, J. R. 1976. Separation of substituted pteroyl monoglutamates and pteroyl oligo-γ-L-glutamates by high pressure liquid chromatography. *Anal. Biochem.* 71:119–24
211. Tafolla, W. H., Sarapu, A. C., Dukes, G. R. 1981. Rapid and specific high-pressure liquid chromatographic assay for folic aid in multivitamin-mineral pharmaceutical preparations. *J. Pharmaceut. Sci.* 70:1273–76
212. Takikawa, K., Miyazaki, K., Arita, T. 1982. High-performance liquid chromatographic determination of nicotinic acid and its metabolites, nicotinuric acid and nicotinamide, in plasma. *J. Chromatogr.* 233:343–48
213. Tennant, G. B. 1977. Continuous-flow automation of the *Lactobacillus casei* serum folate assay. *J. Clin. Pathol.* 30: 1168–74
214. Terry, R. C., Simon, M. 1982. Determination of niacin metabolites 1-methyl-5-carboxylamide-2-pyridone and N-1-methylnicotinamide in urine by high-performance liquid chromatography. *J. Chromatogr.* 232:261–74
215. Thanassi, J. W., Cidlowski, J. A. 1980. A radioimmunoassay for phosphorylated forms of vitamin B_6. *J. Immunol. Meth.* 33:261–66
216. Theobald, R. A., Batchelder, M., Sturgeon, M. F. 1981. Evaluation of pteroylglutamic acid and N^5-methyltetrahydrofolic acid reference standards in a new "No-Boil" radioassay for serum folate. *Clin. Chem.* 27:553–55
217. Thurnham, D. I., Rathakette, P. 1982. Incubation of $NAD(P)H_2$: glutathione oxidoreductase (EC 1.6.4.2) with flavin adenine dinucleotide for maximal stimulation in the measurement of riboflavin status. *Br. J. Nutr.* 48:459–66

218. Tigner, J., Roe, D. A. 1979. Tissue folacin stores in rats measured by radioassay. *Proc. Soc. Exp. Biol. Med.* 160:445–48
219. Tillotson, J. A., Bashor, M. M. 1980. Fluorometric apoprotein titration of urinary riboflavin. *Anal. Biochem.* 107:214–19
220. Tryfiates, G. P., Sattsangi, S. 1982. Separation of vitamin B_6 compounds by paired-ion high-performance liquid chromatography. *J. Chromatogr.* 227:181–86
221. Vanderslice, J. T., Brownlee, S. G., Maire, C. E., Reynolds, R. D., Polansky, M. 1983. Forms of vitamin B_6 in human milk. *Am. J. Clin. Nutr.* 37:867–71
222. Vanderslice, J. T., Maire, C. E. 1980. Liquid chromatographic separation and quantification of B_6 vitamers at plasma concentration levels. *J. Chromatogr.* 196:176–79
223. Vanderslice, J. T., Maire, C. E., Beecher, G. R. 1981. B_6 vitamer analysis in human plasma by high performance liquid chromatography: A preliminary report. *Am. J. Clin. Nutr.* 34:947–50
224. Vanderslice, J. T., Maire, C. E., Beecher, G. R. 1981. Extraction and quantitation of B-6 vitamers from animal tissues and human plasma: A preliminary study. See Ref. 27, pp. 123–47
225. Van Der Weyden, M. B. 1979. Deoxyuridine metabolism in human megaloblastic marrow cells. *Scand. J. Haematol.* 23:37–42
226. Vannucchi, H., Kutnink, M. A., Kretsch, M. J., Sauberlich, H. E. 1984. Measurement of urinary 4-pyridoxic acid excretion by vitamin B_6 depleted-repleted young women. *Proc. West. Hemisph. Nutr. Cong. VII.* In press
227. Vatanavicharn, S., Anuvatanakulchai, M., Na-Nakorn, S., Wasi, P. 1979. Serum erythrocyte folate levels in thalassaemic patients in Thailand. *Scand. J. Haematol.* 22:241–45
228. Viceps-Madore, D., Cidlowski, J. A., Kittler, J. M., Thanassi, J. W. 1983. Preparation, characterization, and use of monoclonal antibodies to vitamin B_6. *J. Biol. Chem.* 258:2689–96
229. Vo-Khactu, K. P., Clayburgh, R. H., Sandstead, H. H. 1974. An improved NADH-dependent transketolase assay for assessing thiamine nutriture. *J. Lab. Clin. Med.* 83:983–89
230. Vo-Khactu, K. P., Sims, R. L., Clayburgh, R. H., Sandstead, H. H. 1976. Effect of simultaneous thiamin and riboflavin deficiencies on the determination of transketolase and glutathione reductase. *J. Lab. Clin. Med.* 87:741–48
231. Waddell, C. C., Domstad, P. A., Pircher, F. J., Lerner, S. R., Brown, J. A., Lawhorn, B. K. 1976. Serum folate levels. Comparison of microbiologic assay and radioisotope kit methods. *Am. J. Clin. Pathol.* 66:746–52
232. Waldenlind, L. 1979. An improved method for the determination of endogenous thiamine and its phosphate esters in biological material. *Nutr. Metab.* 23:38–41
233. Waring, P. P., Fisher, D., McDonnell, J., McGown, E. L., Sauberlich, H. E. 1982. A continuous-flow (Auto Analyzer II) procedure for measuring erythrocyte transketolase activity. *Clin. Chem.* 28:2206–13
234. Warnock, L. G. 1975. Transketolase activity of blood hemolysate, a useful index for diagnosing thiamin deficiency. *Clin. Chem.* 21:432–36
235. Warnock, L. G., Prudhomme, C. R., Wagner, C. 1979. The determination of thiamin pyrophosphate in blood and other tissues, and its correlation with erythrocyte transketolase activity. *J. Nutr.* 108:421–27
236. Waxman, S., Schreiber, C. 1977. Measurement of serum folate levels: Current status of the radioassay methodology. See Ref. 72, pp. 98–109
237. Waxman, S., Schreiber, C. 1980. Determination of folate by use of radioactive folate and binding proteins. *Meth. Enzymol.* 66:468–83
238. Waxman, S., Schreiber, C., Herbert, V. 1971. Radioisotopic assay for measurement of serum folate levels. *Blood* 38: 219–28
239. Waxman, S., Schreiber, C., Rose, M., Johnson, I., Sheppard, R., Sumbler, K., Keen, A., Guilford, H. 1978. Measurement of serum folate by ^{75}Se-selenofolate radioassay. Results of a multi-institutional clinical trial. *Am. J. Clin. Pathol.* 70:356–63
240. Whigham, W. R., Sudarsanan, L. 1977. Simultaneous assay for vitamin B_{12} and folate compared with separate assay. *Clin. Chem.* 23:1778–79
241. Wickramasinghe, S. N. 1981. The deoxyuridine suppression test: a review of its clinical and research applications. *Clin. Lab. Haematol.* 3:1–18
242. Wickramasinghe, S. N., Longland, J. E. 1974. Assessment of deoxyuridine suppression test in diagnosis of vitamin B_{12} or folate deficiency. *Br. Med. J.* 3:148–50
243. Wickramasinghe, S. N., Saunders, J. E. 1975. Deoxyuridine suppression test. *Br. Med. J.* 2:87
244. Wickramasinghe, S. N., Saunders, J.

1976. Correlation between the deoxyuridine-suppressed value and some conventional haematological parameters in patients with folate or vitamin B_{12} deficiency. *Scand. J. Haematol.* 16:121–27

245. Wickramasinghe, S. N., Saunders, J. E. 1977. Results of three year's experience with the deoxyuridine suppression test. *Acta Haematol.* 58:193–206
246. Williams, A. K. 1979. High-performance chromatography of vitamin B_6. *Meth. Enzymol.* 62:415–22
247. Williams, D. G. 1976. Methods for the estimation of three vitamin dependent red cell enzymes. *Clin. Biochem.* 9:252–55
248. Williams, W. J., Beutler, E., Erslev, A. J., Rundles, R. W. 1977. *Hematology*. New York: McGraw-Hill. 1504 pp. 2nd ed.
249. Wilson, S. D., Horne, D. W. 1982. Use of glycerol-cryoprotected *Lactobacillus casei* for microbiological assay of folic acid. *Clin. Chem.* 28:1198–1200
250. Wolfe, H., Brown, R. R., Arend, R. A. 1980. The kynurenine load test, an adjunct to the tryptophan load test. *Scand. J. Clin. Lab. Invest.* 40:9–14
251. Wood, B., Gijsbers, A., Goode, A., Davis, S., Mulholland, J., Breen, K. 1980. A study of partial thiamin restriction in human volunteers. *Am. J. Clin. Nutr.* 33:848–61
252. Wu, A., Chanarin, I., Slavin, G., Levi, A. J. 1975. Folate deficiency in the alcoholic—its relationship to clinical and haematological abnormalities, liver disease and folate stores. *Br. J. Haematol.* 29:469–78
253. Yang, B. I., Sawhney, A. K., Pitchlyn, R. C., Peer, P. M. 1981. Simple assay for femtomoles of pyridoxal and pyridoxamine phosphate. See Ref. 27, pp. 79–98
254. Yasumoto, K., Tadera, K., Tsuji, H., Mitsuda, H. 1975. Semiautomated system for analysis of vitamin B_6 complex by ion-exchange column chromatography. *J. Nutr. Sci. Vitaminol. (Tokyo)* 21: 117–27
255. Young, D. S., Pestaner, L. C., Gibberman, V. 1975. Effects of drugs on clinical laboratory tests. *Clin. Chem.* 21:1D-432D
256. Zittoun, J., Zittoun, R., Marquet, J., Bilski-Pasquier, G., Sulton, C. 1978. Anaemia induced by vitamin B_{12} or folate deficiency: diagnostic value of the "dU suppression" test. *Nouv. Presse Med.* 7: 1617–20 (In French)