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Title: Stability of Folic Acid in 25 Percent Dextrose, 3.5 Percent Amino Acids and Multivitamin Solution

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The chemical stability of folic acid in 25 percent dextrose, 3.5 percent amino acids and multivitamin solution was investigated.

Solutions of 2.5, 5.0, 7.5 and 1.0 mg/liter of folic acid admixed in the study solution were prepared. Solutions were stored in light-room temperature, light-refrigeration, dark-room temperature, or dark-refrigeration. Samples were drawn to determine initial folic acid concentration, and again at 4, 8, 12, 18, 24, 36, and 48 hours after admixture. Folic acid concentration was determined by competitive binding radioassay and results are expressed as percent of initial folic acid concentration.

Folic acid was stable for 48 hours with each tested concentration and stability was found to be independent of temperature or light storage effects.

Folic acid admixed in the study solution is chemically stable for up to 48 hours after initial admixture under normal TPN storage conditions.

STABILITY OF FOLIC ACID IN 25 PERCENT DEXTROSE, 3.5 PERCENT AMINO ACIDS AND MULTIVITAMIN SOLUTION

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I. INTRODUCTION

Quality assurance of drugs and drug products is essential in providing quality health care. The pharmacist is responsible for providing a sterile, physically and chemically compatible drug product in the preparation of intravenous admixture products. The labeled quantity of the right drug should be available to the patient upon administration, and also should be bioavailable once administered. The pharmacist relies upon current textbooks and scientific journals for updated research in order to make rational decisions regarding stability and compatibility.

Total parenteral nutrition (TPN) solutions composed of dextrose, amino acids, electrolytes and vitamins are admixed daily by pharmacy IV services. Stability and compatibility data are currently available concerning the admixture of some TPN additives.[1,2] However, data currently available do not define the chemical stability of the vitamin, folic acid, in TPN solutions although it is routinely prepared this way.

Folic acid is an essential vitamin important to man as a hematopoetic agent necessary for the regeneration and function of blood forming elements. It also acts as a coenzyme in several metabolic reactions such as the interconversion of amino acids where transfer

of one carbon units is involved.[3] Therapeutically, it is used in the treatment of megaloblastic anemia and in the replacement of daily folate losses.

Despite the importance and established essentiality of folic acid, patients may still suffer deficiencies.[4] In hospitalized patients, the incidence of water soluble vitamin deficiencies is high; folic acid deficiencies ranked the highest with 45 percent of 105 hypovitaminemic patients deficient in folic acid.[4] In total parenteral nutrition therapy where the TPN solution is often the sole source of nutritional support, the importance of quality assurance must be emphasized. Chemical and physical stability must be determined for TPN components.

Recent investigations report folic acid is physically compatible in TPN solutions.[5,6] However, the data do not address chemical stability. Current texts state that folic acid is chemically unstable when combined with other vitamins.[2,7,8] The original references cited in the texts date back to the late 1940's and early 1950's and define folic acid stability not in TPN fluids, but in oral multivitamin solutions.[9-11] Biamonte and Schneller[11] measured folic acid loss over 16 months and Scheindlin[9] studied degradation of this drug for 22 days.

The effects of the B-complex vitamins on decomposition of folic acid were first quantified in 1947 by Foss et al.[10] Riboflavin was found to have the greatest and most injurious effect on folic acid decomposition followed in descending order by thiamine, ascorbic acid,

nicotinamide, pantothenic acid and pyridoxine. A year later, Scheindlin studied the stability of oral liquid preparations and found that polyvitamin mixtures exerted a destructive effect on folic acid at room temperature.[9] It was suspected at this time that the destructive effects were due to the B-complex vitamins and perhaps also to vitamin C. Room temperature storage and the presence of other vitamins appear to be favorable conditions for folic acid degradation. These conditions apply to TPN solutions. Folic acid is often admixed with vitamins in TPN solutions and hangs at room temperature for up to 24 hours during infusion.

Biamonte and Schneller continued investigation in 1951 and found that the rate of folic acid decomposition was increased proportionately with an increase in the amount of folic acid actually dissolved. Folic acid admixed in TPN solutions is (by definition) totally dissolved, but with the variety of dosages commonly administered, the concentrations of folic acid admixed in TPN solutions will differ. Higher concentrations may be less stable than lower concentrations.

Biamonte and Schneller found that riboflavin had the greatest effect on accelerated decomposition of folic acid. Folic acid decomposition was accompanied by the liberation of equivalent amounts of an aromatic amine. This was presumed to be para-amino-benzoyl-glutamic acid (PABG).[11] The degradation of folic acid by ascorbic acid was measured by Scheindlin and Griffith.[9] Alone, ascorbic acid decomposition of folic acid occurred rather slowly. In aqueous medium, only about 20 percent of the folic acid cleaved in four weeks.[9]

In 1952, Scheindlin, Lee and Griffith were able to describe the reactions which take place in the chemical decomposition of folic acid by riboflavin.[12] Even at low concentrations of riboflavin, folic acid cleavage continued to proceed to completion. It was concluded that the role of riboflavin was that of a hydrogen acceptor as it is in many biochemical reactions, in the form of the coenzymes flavin mononucleotide and flavin adenine dinucleotide. The reaction was accelerated by light and also affected by air. It was suggested that air is not the source of oxygen for the primary oxidation, but rather supplies oxygen for the regeneration of riboflavin from leucoriboflavin.[12] Riboflavin is a component of most multivitamin products used in TPN solutions. Although present in small quantities, the early data suggest that, in the presence of air and light, it may be sufficient to cleave folic acid to completion. TPN solutions are admixed into air-evacuated containers, but are exposed to light as they often hang in room light during infusion. Scheindlin, Lee and Griffith described this chemical degradation. Under the combined effect of light and riboflavin, a dehydrogenation occurs with the loss of hydrogens and reduction of riboflavin to leucoriboflavin (Figure 1.)[12] The dehydrogentated folic acid then undergoes rapid oxidative cleavage with the formation of PABG and a carbonyl compound, presumably 2-amino, 4-hydroxy, 6-pteridinecarboxaldehyde. The leucoriboflavin is readily reoxidized by air back to riboflavin.[12] More recent investigations have confirmed PABG and the carbonyl compound as the products of the oxidative cleavage of folic acid by riboflavin.[13,14]

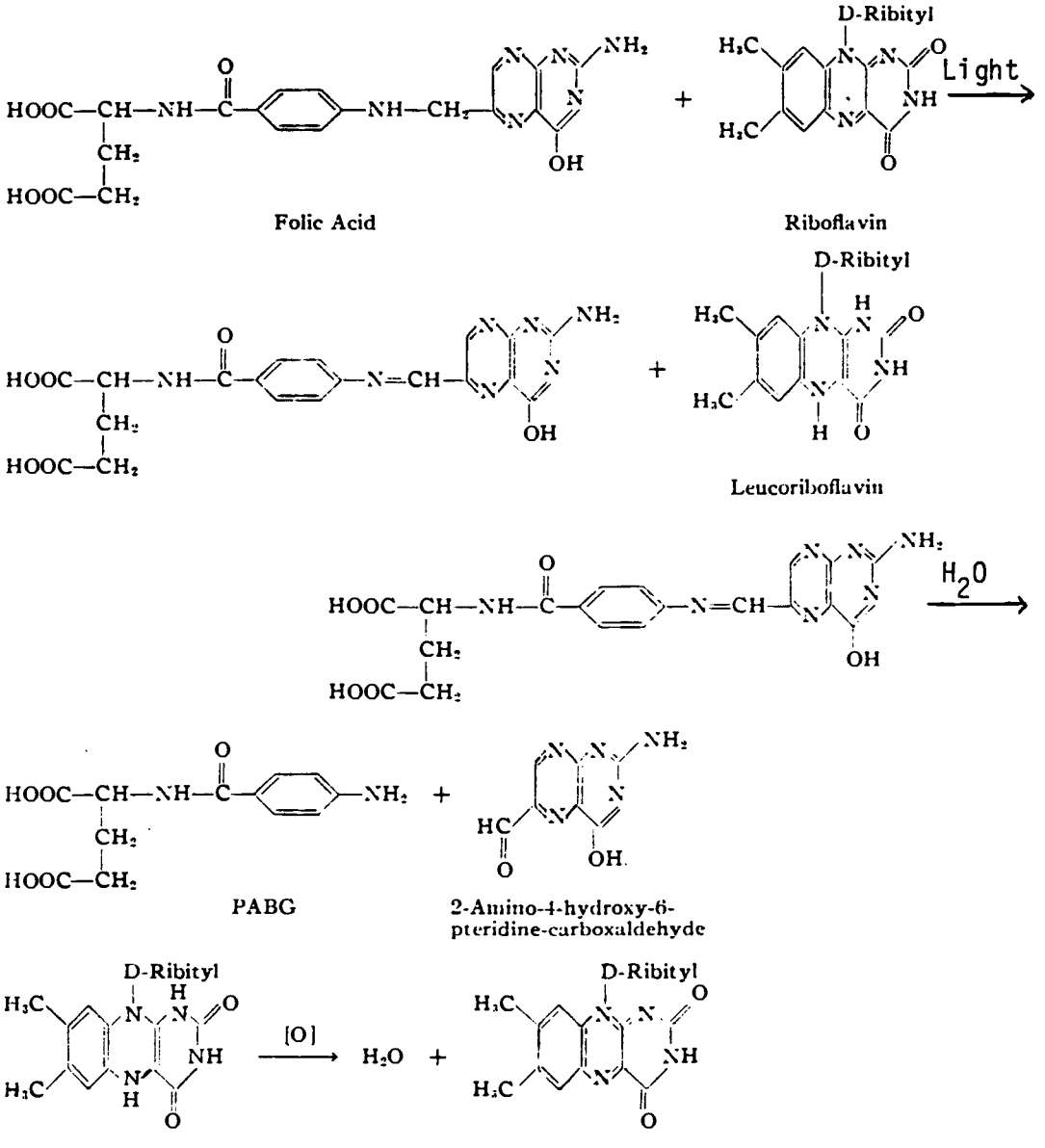

Figure 1. Chemical degradation of folic acid under the combined effects of light and riboflavin

The early folic acid stability studies have identified components which may affect folic acid degradation, and a chemical equation for the reaction has been proposed.[12] Much of this information may also apply to folic acid stability in TPN solutions. However, the purpose of the early studies was investigation of long term shelf-life stability of folic acid rather than short term infusion-time stability as is important in the administration of TPN solutions. Early investigations were conducted in oral multivitamin solutions. There is some similarity in the composition of oral multivitamin solutions and TPN solutions. However, many components and qualities of the solutions differ, and application of the early data to folic acid stability in TPN solutions may lead to erroneous conclusions.

Recent literature examines folic acid stability in parenteral solutions. In 1977, Kobayashi and King studied compatibility of common additives in protein hydrolysate and dextrose solutions.[5] However, their study was limited to assessment of physical stability of folic acid and consisted of noting the formation of precipitate while in solution. Chemical degradation of folic acid does not result in the formation of a precipitate,[13,14] and thus, these data are limited to conclusions concerning physical stability of folic acid. Compatibility and stability of electrolytes, vitamins and antibiotics in combination with 8 percent amino acid solution were studied by Schuetz and King in 1978.[6] They confirmed only that therapeutic admixtures of folic acid were not of sufficient concentration for scanning by ultraviolet spectrophotometry.[6]

Without adequate data, current and frequently used reference texts continue to cite the early research findings of folic acid instability in oral vitamin solutions even when referring to intravenous folic acid stability. Based upon the early work, the texts refer to folic acid instability with riboflavin, thiamine, light,[2,7,8] reducing agents, oxidizing agents and heavy metal ions.[2] These conclusions have not been widely accepted by practicing hospital pharmacists. The lack of current scientific data and guidelines for folic acid admixture in TPN solutions has led to inconsistencies in the admixture of TPN solutions. IV pharmacy services currently either overlook the potential for folic acid instability problems, or precautionary measures are taken to separate folic acid from its degradative components in the same TPN solution. Although separation of folic acid from other vitamins may be more work and can limit batch preparation of TPN solutions, it is the desired method of practice from a therapeutic and quality assurance standpoint.

One milligram of folic acid is commonly prescribed to TPN patients in clinical practice. But, recent American Medical Association Nutrition Advisory Group (AMA/NAG) recommendations suggest that 0.4 mg of folic acid per day are required in parenterally fed patients.[15] Before accepting this recommendation as the therapeutic standard, the AMA/NAG urge that adequate tests for safety, stability and compatibility be performed. Clinical testing should then be carried out in order to ensure that appropriate concentrations of vitamins are offered with a variety of diseases.[15] Clinical

investigations differ from the AMA/NAG recommendation and report that 0.6-0.9 mg of folic acid per day are required by the TPN patient.[16,17]

Actual folic acid requirements may vary depending upon the patient's disease state. These various requirements are yet to be defined, and currently a variety of folic acid dosages may be prescribed. Although this argument is not resolved, admixture of folic acid to TPN solutions without regard to stability problems may result in the administration of less than prescribed dosages. Repletion of folic acid stores requires several days. The dose and length of therapy required for repletion is increased in patients with histories of chronic deficiency.[4] Many TPN patients may suffer from chronic nutritionally depleting diseases. Thus, the effects of administering an unstable folic acid dose are significant. Degraded doses may result in increased length of therapy, increased number of hospitalized days and a resultant increase in hospital costs.

Stability data necessary to make decisions concerning the admixture and storage of folic acid in TPN solutions currently are not available. Older literature has reported effects of other vitamins, temperature, folic acid concentration, light and time on the degradation of folic acid in oral multivitamin solutions as discussed above. These effects may also apply to folic acid admixed in TPN solutions. This study was conducted to define the chemical stability of folic acid as a component of TPN solutions. Effects of temperature, light, initial folic acid concentration, and time on the chemical degradation of folic acid by multivitamins were investigated.

Folic acid stability when admixed in 25 percent dextrose, 3.5 percent amino acids and multivitamin solutions was studied for 48 hours. Temperature, light and initial folic acid concentration were analyzed for significant effects on folic acid concentration over the 48 hour study period. If temperature, light and initial folic acid concentration and the parent solutions do not enhance the chemical degradation of folic acid within 48 hours, then folic acid solutions when stored under these conditions can be used clinically. Statistical null hypotheses are: (1) the effect of storage at room temperature on folic acid concentration is the same as the effect of storage at refrigerated temperatures; (2) the effect of storage under room light is the same as the effect of storage in the dark; (3) differing initial folic acid concentrations have a similar and consistent effect on folic acid decomposition. Alternative hypotheses are: (1) the effects of storage at room temperature are different from the effects of storage at refrigerated temperatures; (2) the effects of storage under room light are different from the effects of storage in the dark; (3) differing initial folic acid concentrations have a different and consistent effect on folic acid decomposition.

II. METHODS

Folic Acid Assay

Several assay methods are available for analysis of folic acid from folic acid tablets or capsules.[18] These include spectrophotometric USP assay, ultraviolet spectrophotometry, fluorometry and chromatography. The USP assay is designed for estimation of folic acid in folic acid or multivitamin tablets and capsules.[19] Long term instability of folic acid has discouraged manufacturing of oral or injectable solutions of multivitamins with folic acid. A USP test to quantitatively assay folic acid in these solutions has not been developed.

Folic acid injection in this study is admixed in a complex solution. The four listed folic acid assays offer many disadvantages to analyze the study solution. These include poor sensitivity to the low concentrations of folic acid used clinically in TPN therapy,[6] amino acid interference with oxidation and reduction reagents,[20] poor separation of folic acid from other vitamins and amino acids,[20,21] and similar absorbance of other study solution components.[22]

Microbiological and radioassays are available for assay of folic acid from serum samples. They are highly specific and are designed for separation analysis of folic acid in complex solutions containing proteins and other vitamins.[23,24] Competitive binding radioassays have improved greatly in accuracy and precision in recent years.

Values obtained are comparable to microbiological assays, but are determined with greater speed, simplicity, and specificity.[24,25] Radioassays are preferred in many clinical laboratories for analysis of folic acid, as microbiological assays are becoming outdated. Competitive binding radioassay was used in this investigation.

The assay kit used includes a boiling step designed to destroy endogenous binding proteins present in human serum.[26] This step is not necessary because proteins were not used in this study. The boiling step was eliminated for samples after preliminary runs showed a small loss of folic acid after boiling. Incubation pH is important in the assay to allow equivalent competition of the labeled tracer, ^{125}I folic acid in the pteroylglutamic acid (PGA) form, with serum folic acid in the methyl-tetrahydrofolic acid (MH_4) form.[27] PGA and MH_4 have similar affinities for the binder in the competitive radioassay at a pH of approximately 9.3.[13] Folic acid admixed in this study solution is in the PGA form. Binder affinity of PGA is independent of pH in the pH range from 6.9 to 9.3.[13] Since both of the competing compounds, tracer and sample, are in like chemical forms in this study, the high alkaline pH was not essential for equivalent competitive binding.

The assay binder binds on the pteridine structure of the folic acid molecule.[28] However, the pteridine compound formed in folic acid degradation does not bind to the assay binder. When tracer folic acid (PGA) was allowed to degrade for 10 weeks, the formation of pteridine resulted in a proportionate gradual increase in non-specific binding

in the assay blank. The proportionate increase in non-specific binding was found to be due to the formation of the pteridine compound and PABG.[13] These degradation products are not adsorbed by charcoal and thus an increase in non-specific binding during assay may serve as a confirmation of folic acid degradation.

When PGA is added to serum for analysis, recovered folic acid concentrations are generally larger than theoretical.[29] Preliminary assays in this study showed recovered concentrations to be an average of 0.9 ng/ml larger than theoretical folic acid concentrations in sample solutions (according to label strength). Stability determinations throughout this study are expressed as percent of initial concentration.

Sample Preparation

Twenty-four liter bottles composed of approximately 500ml of 50 percent dextrose in water[a] and 500 ml of 7 percent crystalline amino acids[b] were admixed with aseptic technique. Final concentration of this solution was 25 percent dextrose and 3.5 percent amino acids. Five milliliters of multivitamin concentrate emulsion[c] (Table 1) was added to each bottle. Storage conditions and folic acid content studied are listed in Table 2. One of four different quantities of folic acid[d] was added to each bottle. Bottles contained 0.25, 0.5, 0.75,

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[a]Fifty percent dextrose in water, Abbott, North Chicago, IL, Lot 21-297-DM-04.
[b]Seven percent crystalline amino acids, Abbott, North Chicago, IL, Lot 21-249-DM-01.
[c]Multivitamin concentrate, USV, Tuchahoe, NY, Lot 57679.
[d]Folic acid injection, Lederle, Pearl River, NY, Lot 593-311.

TABLE 1.

Multivitamin Formulation[a]

Ascorbic Acid	500 mg
Riboflavin (as 5-phosphate)	10 mg
Thiamine HCl	50 mg
Niacinamide	100 mg
Pyridoxine HCl	15 mg
Dexpanthenol	25 mg
Vitamin A	10,000 IU
Vitamin A	1,000 IU
Vitamin E	5 IU

[a]Multivitamin Concentrate, USV, Tuckahoe, NY, Lot 57679

TABLE 2.

Solution Storage and Folic Acid Content

Storage Condition		Folic Acid Content
Lighting	Temperature	mg/liter
Light[a]	Rm. Temp.[b]	0.25
Light[c]	Ref.[d]	0.25
Dark	Rm. Temp.	0.25
Dark	Ref.	0.25
Light	Rm. Temp.	0.5
Light	Ref.	0.5
Dark	Rm. Temp.	0.5
Dark	Ref.	0.5
Light	Rm. Temp.	0.75
Light	Ref.	0.75
Dark	Rm. Temp.	0.75
Dark	Ref.	0.75
Light	Rm. Temp.	1.0
Light	Ref.	1.0
Dark	Rm. Temp.	1.0
Dark	Ref.	1.0

[a] 148 foot candles

[b] 21°C

[c] 250 foot candles

[d] 5-8°C

or 1.0 mg of folic acid (based on label strength). Final concentrations of folic acid were calculated to be 250, 500, 750, or 1000 ng/ml, respectively. Room temperature storage was $21^{o}C \pm 2^{o}C$ and refrigeration storage was $5\text{-}8^{o}C \pm 2^{o}C$. Room temperature fluorescent light was 148 foot candles and refrigeration fluorescent light was 250 foot candles. Dark storage conditions were provided by aluminum foil wrapping the bottle. Each bottle of solution was thoroughly mixed according to USP guidelines before storage and again before each sampling time.

One half milliliter from each of the 24 admixed TPN solutions was immediately removed and diluted aseptically prior to analysis. The standard curve (described later) assay range of folic acid was most accurate from 0.5-24 ng/ml. Dilution of samples prior to each assay run provided folic acid concentrations in the most accurate range of the assay. The 0.5 ml sample was diluted volumetrically with 25 percent dextrose and 3.5 percent amino acid solution to a final volume of 50 ml. Samples were drawn from each TPN solution at 0, 4, 8, 12, 18, 24, 36 and 48 hours after initial admixture. Samples were diluted and assayed immediately for folic acid concentration with one exception. Samples drawn at time zero were actually assayed two hours after initial admixture because volumetric dilution and fresh sample and solution preparation required the extra working time. These first samples were analyzed and values used as the initial folic acid concentrations.

Folic Acid Analysis

Folic acid concentration was determined by ^{125}I competitive binding radioassay. Folic acid assay kits[e] from a single lot number were used throughout the entire experiment. The kits contain ^{125}I labeled folic acid, borate-potassium cyanide buffer, dithiothreitol, charcoal-dextran, milk protein binder, and seven folic acid standards of 0.0, 0.5, 1.0, 3.0, 6.0, 12.0, and 24.0 ng/ml. ^{125}I labeled folic acid, binder, standards and charcoal-dextran were reconstituted or prepared according to kit manufacturer instruction.

The experiment required conducting eight complete assay runs within the 48 hour study period. Folic acid is unstable in freeze-thaw processes[30] and thus samples were assayed immediately after dilution. A single "master" volume of KCN buffer, ^{125}I folic acid and dithiothreitol (working solution) was prepared according to manufacturer's instructions. This single preparation was prepared initially and used throughout the study period to decrease required time to conduct each assay run and to insure uniformity of the working solution. The working solution was refrigerated ($5^{o}C \pm 2^{o}C$) between assay runs and brought back to room temperature before each run. Working solutions are stable for seven days after mixing when stored at $4^{o}C$.[30] The total volume of working solution prepared was calculated to provide a volume of 950 microliters or borate-potassium cyanide buffer, 50 microliters

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[e]Folic acid assay kits, Diagnostic Products, Los Angeles, CA, Lot 5FOD2077.

of ^{125}I folic acid and 20 microliters of dithiothreitol per sample or standard tube.

Each run contained duplicate tubes of the 24 samples, 7 standards, 2 controls[f], 1 total count tube, 1 standard non-specific binding and 1 sample non-specific binding tube. The standards were used to evaluate intrassay run variability. At least two controls of known concentration were determined with each assay run to ensure working solution stability and to assess interassay human variation. The total tube was held for counting only, after addition of working solution and provided the total count available from the labeled ^{125}I folic acid in the working solution used in the assay run. The non-specific binding tubes account for interferring binding which may occur in the standard or sample solutions which do not contain folic acid. Standard non-specific binding is subtracted before determing folic acid concentrations of each standard. Preliminary runs determined that a small amount of binding occurred in solutions containing no folic acid. A sample solution without folic acid was used to determine non-specific binding of components in the TPN solution.

For each assay run, test tubes were labeled in duplicate for each standard, sample, total, control and non-specific binder. One milliliter of working solution was pipetted into each test tube. The total tube was removed and held for counting only. Two hundred microliters of each standard, sample, control, or solution for non-specific bind-

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[f]Folic acid assay controls, Ortho Diagnostic System, Raritan, NJ, Lot 10T102 B&C.

ing was added to appropriately labeled tubes containing the working solution. Each tube was lightly capped and all tubes except samples were placed in a boiling water bath for 15 minutes. Preliminary test runs revealed a small loss of folic acid from the sample solution after boiling. Although the manufacturer reports no significant loss occurs after boiling, any loss may be significant to the determination of folic acid stability.[30] The boiling step is conducted to destroy endogenous proteins present in human serum.[30] Since the sample solution does not contain protein, boiling the sample to destroy endogenous proteins is not necessary. All tubes were placed in a room temperature bath for five minutes.

One hundred microliters of binder was added to all tubes except tubes used to determine non-specific binding. All tubes were vortexed and incubated at room temperature for 45 minutes. Incubation pH of standards and controls was measured at 9.0 and pH of samples at 7.4. During incubation, unlabeled folic acid competes with labeled folic acid for the limited number of available specific binding sites on the binder. Complexes are formed and the amount of labeled folic acid that is bound is reduced proportionately. The greater the concentration of folic acid in the sample, the greater the reduction in bound labeled folic acid. Four hundred microliters of charcoal-dextran suspension was slowly pipetted into all tubes to separate bound and free fractions of folic acid. Each tube was vortexed for 10 seconds and incubated at room temperature for 15 minutes. All tubes were centrifuged for 20 minutes at 3000 rpm. The clear supernatant

was decanted into correspondingly marked tubes and the charcoal precipitate of free folic acid fraction was discarded. The supernatant contained bound folic acid, both labeled and unlabeled. The precipitate contained unbound folic acid both labeled and unlabeled. Performance and reproducibility of counting precipitate or supernatant is good; the choice of which to count is one of convenience and preference.[30] Each tube of supernatant was counted for 10 minutes in a gamma scintillation counter[g]. The level of radioactivity measured in the supernatant represents bound labeled folic acid and is inversely related to the folic acid concentration in the sample or standard. Standard curves were run simultaneously with each assay run (0, 4, 8, 12, 18, 24, 36 and 48 hours) to calculate concentrations for unknowns. Seven separate standard curves were generated in the 48 hour time period.

Calculation of Concentration

Counts from supernatant were corrected and standard curves plotted by programmed computer[h]. Folic acid concentration of the sample was expressed in ng/ml. This determination involved several calculations. Duplicated counts were averaged for all tubes. The average count due to non-specific binding of standard was subtracted from all tubes except samples to obtain corrected counts. The

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[g]Gamma scintillation counter, Nuclear Chicago Co.; Des Plaines, IL.
[h]Programmable Computer, Medical Data System, Ann Arbor, MI.

average count of non-specific binding of sample solutions was subtracted from all samples to obtain corrected counts of samples. Only corrected counts were used in calculations. Percent of zero standard bound ($\%B^{o}$) was calculated for standards and samples by dividing each corrected count by the corrected count of the zero standard. Percent of zero standard bound was plotted against ng/ml on logit-log paper and the best fitting line was drawn (Figure 2). Folic acid concentration of samples expressed in ng/ml was determined from the corresponding $\%B^{o}$ value as read from the curve.

Statistical Methods

Analysis of variance among bottles was computed for main [concentration (C), light (L) and temperature (T)] effects and interaction (CxL, CxT, LxT, CxLxT) effects. Each temperature, light and initial folic acid concentration was assessed and the mean square for error among bottles was computed. Multiple regression analysis[31] of the effects of temperature, light and initial folic acid concentration on folic acid stability over the 48 hour study period was analyzed with repeated measures. The mean square for error within bottles as sampled over time was computed. This error term is an assessment of bottle variation due to random error after main and interaction effects have been taken into consideration. Mean squares for each main effect and interaction effect was compared to the mean square for error to calculate an F-statistic. Values of p less than 0.05 were considered significant.

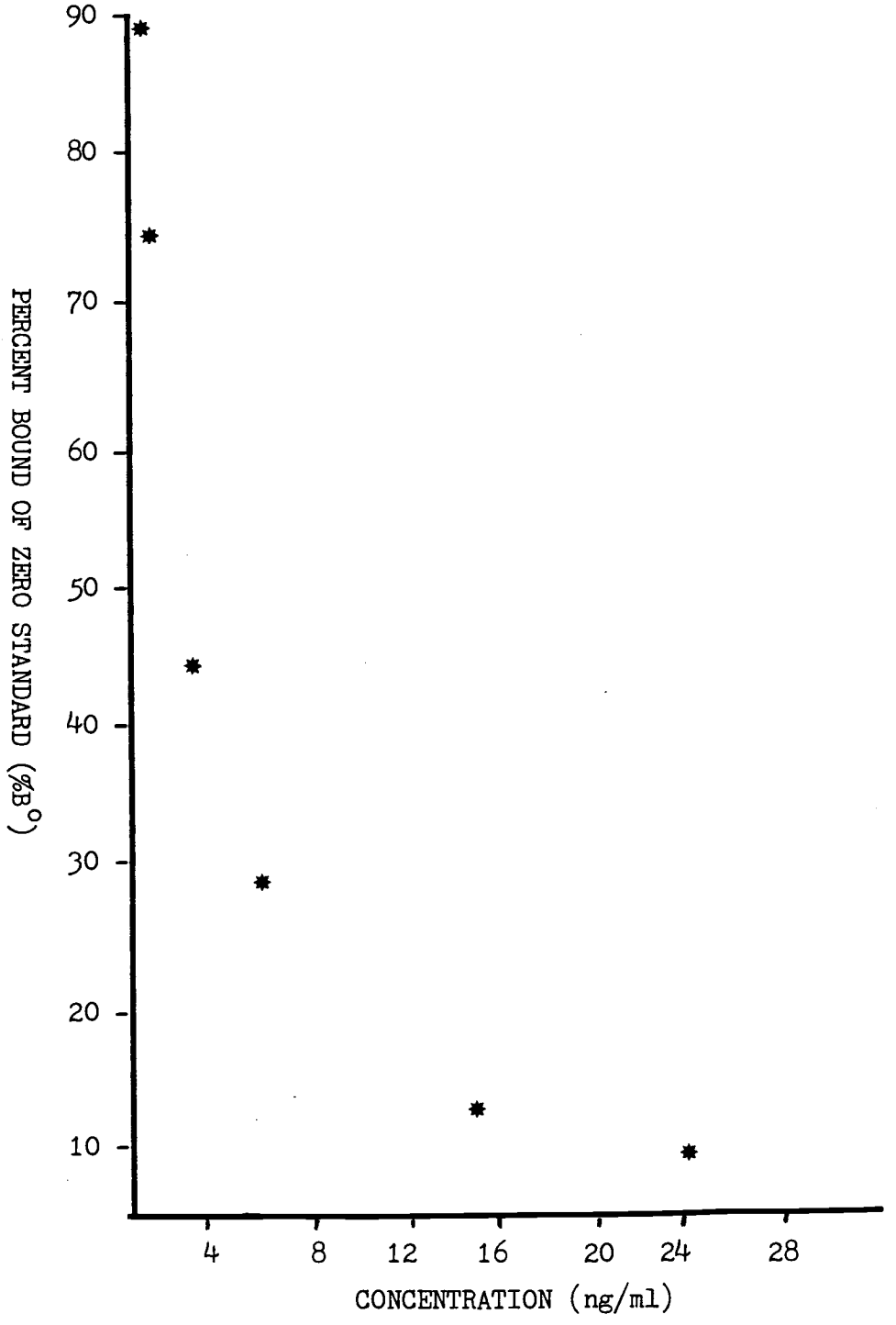

Figure 2. Assay Standard Curve.(Concentration x %B°)

III. RESULTS

Each bottle of solution was kept at a single storage condition throughout the 48 hour study period. The standard curves plotted for each run had correlation coefficients of 0.9993 to 0.9998 (Figure 2). The average inversely estimated percent of theory was 100.8 percent, with an average coefficient of variation of 3.61 percent. The mean square for random error among bottles was computed with 8 degrees of freedom (Figure 3). The mean square for random error of concentrations determined within the 48 hour time period was computed with 48 degress of freedom (Figure 4). Results due to temperature and light effects are presented in Table 3 and Table 4, respectively. Mean concentration of folic acid determined throughout the 48 hour study period illustrates stability at both the room temperature and refrigeration and at both light and dark storage conditions. Results from the effects of varying the initial concentration of folic acid are presented in Table 5. Mean concentrations of folic acid determined throughout the 48 hour study period illustrate stability in all four initial concentrations. Non-specific binding, which may serve as confirmation of folic acid stability,remained constant throughout the 48 hour study.

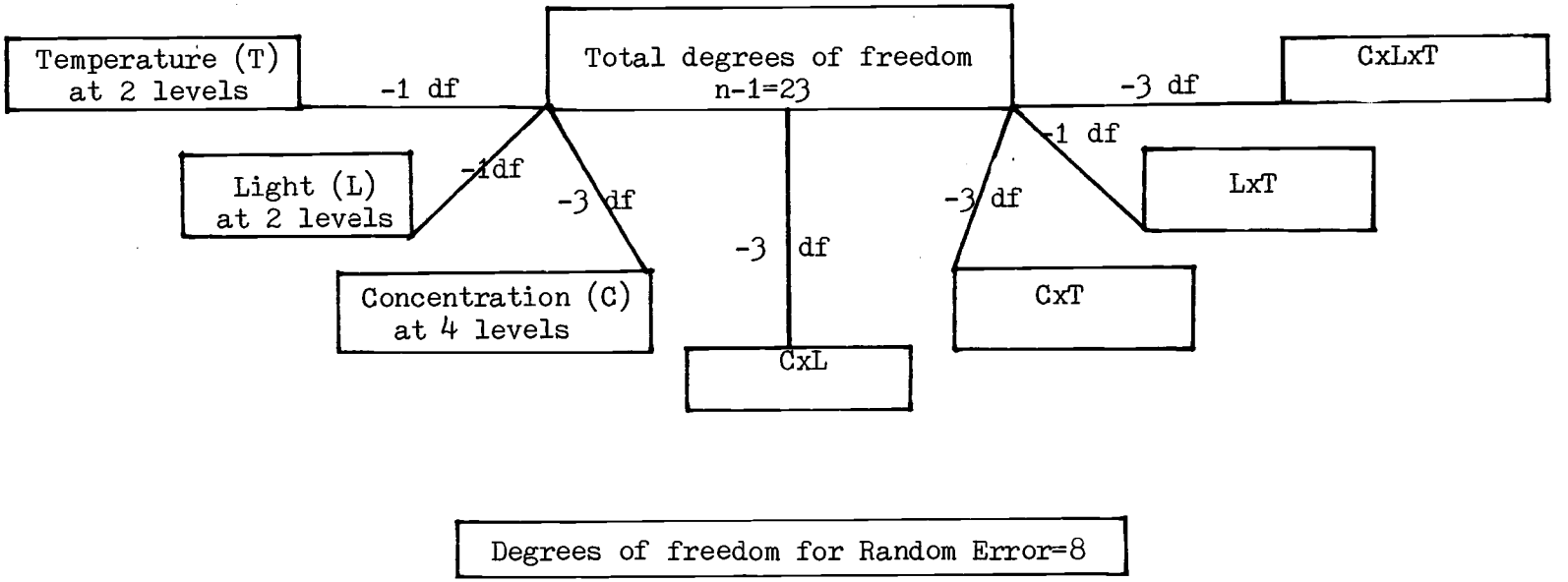

Figure 3. Determination of degrees of freedom for random error among bottles. (df = degrees of freedom)

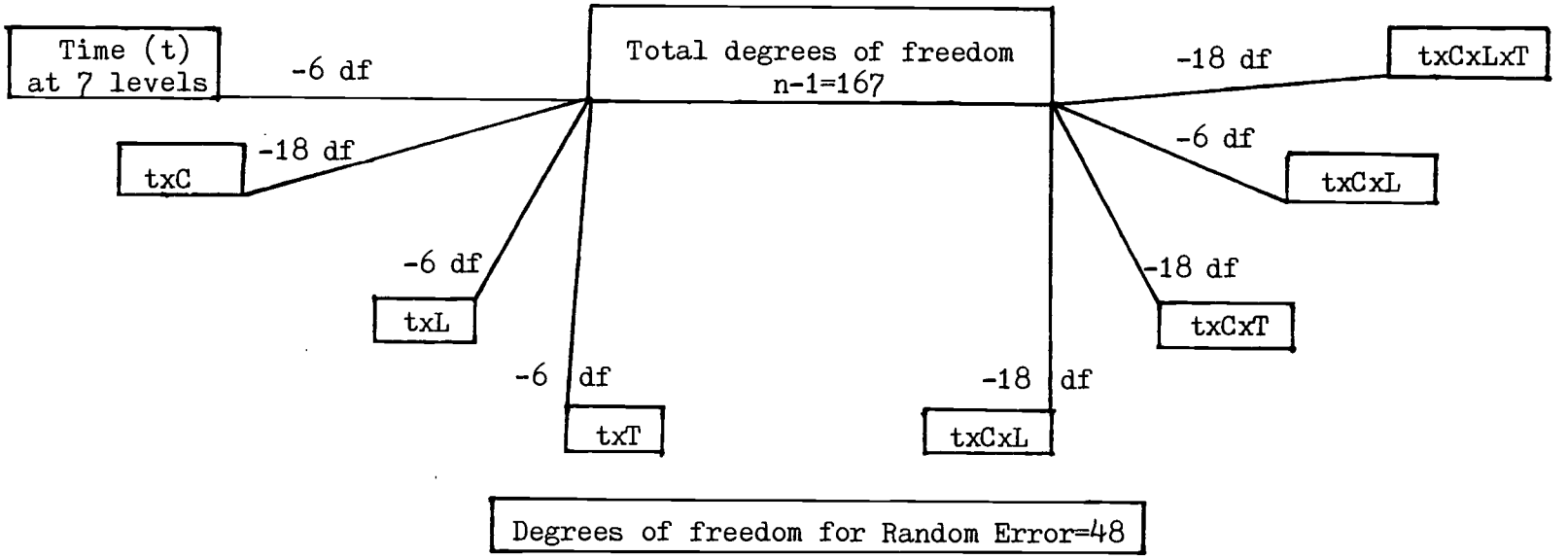

Figure 4. Calculation of the degrees of freedom for random error of concentrations within bottles over 48 hours. (df = degrees of freedom)

TABLE 3.

Temperature Effects: Mean[a] Percent of Added Folic Acid Remaining over 48 hours (± Standard Deviation)

	Time After Initial Admixture						
	4 hours	8 hours	12 hours	18 hours	24 hours	36 hours	48 hours
Rm. Temp.[b]	100.6 (7.9)	89.9 (8.4)	93.2 (8.8)	94.7 (8.5)	98.8 (7.9)	96.2 (7.6)	102.4 (8.4)
Ref.[c]	95.7 (6.4)	97.5 (6.8)	92.3 (7.0)	93.0 (7.3)	101.4 (7.1)	97.6 (6.9)	104.4 (7.1)

[a]Means for duplicate determinations from 12 bottles stored at each temperature. Each mean was calculated by collapsing across non-significant effects of light storage condition and initial folic acid concentration.

[b]21°C ± 2°C.

[c]5-8°C ± 2°C.

TABLE 4.

Light Effects: Mean[a] Percent of Added Folic Acid Remaining over 48 Hours (± Standard Deviation)

	Time After Initial Admixture						
	4 hours	8 hours	12 hours	18 hours	24 hours	36 hours	48 hours
Light[b]	96.2 (10.4)	89.3 (9.4)	94.0 (8.8)	92.6 (9.6)	98.3 (8.7)	95.8 (7.8)	103.0 (8.1)
Light[c]	93.9 (7.4)	87.8 (8.7)	90.6 (8.0)	89.8 (9.8)	99.8 (7.9)	93.8 (7.6)	101.2 (7.7)
Dark[d]	101.3 (3.7)	91.3 (6.7)	93.2 (6.5)	96.5 (9.3)	101.1 (5.9)	97.8 (6.4)	104.7 (5.7)

[a]All means were calculated by collapsing across non-significant effects of initial folic acid concentration.

[b]Means for duplicate determinations of six bottles stored in room temperature (21°C) and Light (148 foot candles).

[c]Means for duplicate determinations of six bottles stored in refrigeration (5-8°C) and Light (250 foot candles).

[d]Means for duplicate determinations of twelve bottles stored in room temperature (21°C) or refrigeration (5-8°C) and Dark.

TABLE 5.

Concentration Effects: Mean[a] Percent of Added Folic Acid Remaining over 48 Hours (± Standard Deviation)

Folic Acid Added per Bottle (mg)	Time After Initial Admixture						
	4 hours	8 hours	12 hours	18 hours	24 hours	36 hours	48 hours
0.25	102.1 (6.4)	92.9 (5.4)	92.3 (4.8)	92.2 (4.9)	95.6 (4.7)	95.6 (5.1)	99.4 (6.0)
0.5	93.3 (6.0)	89.3 (5.8)	91.0 (6.1)	90.9 (6.3)	95.2 (5.9)	93.1 (6.4)	100.1 (5.4)
0.75	102.0 (3.8)	90.8 (5.8)	94.5 (6.5)	95.5 (5.5)	105.9 (6.9)	99.6 (7.1)	110.5 (8.0)
1.0	96.4 (6.4)	86.6 (6.8)	93.3 (6.3)	98.2 (10.2)	102.8 (8.8)	96.8 (9.1)	99.9 (9.0)

[a]Means for duplicate determinations of 4 bottles containing 0.25 mg, 4 bottles of 1.0 mg, 8 bottles of 0.5 mg and 8 bottles of 0.75 mg. Means were calculated by collapsing across non-significant effects of light and temperature storage condition.

IV. DISCUSSION

Stability in pharmaceutics is frequently defined as less than 10 percent loss of the labeled drug quantity from time of manufacture to time of complete administration.[35] Mean concentrations of folic acid are generally greater than 90 percent of original concentration throughout the 48 hour study period. Statistical analysis reveals significant interaction of only one of the conditions which were tested on stability. However, therapeutically, this finding is not significant. Mean concentrations were constant at levels greater than 90 percent of initial concentration.

Temperature Effects

Statistical interaction effects of storage at room temperature or at refrigerated temperature on folic acid concentration were not significant ($p > 0.07$) throughout the 48 hour study period. The null hypothesis that room temperature and refrigeration have similar effects on folic acid stability over a 48 hour period cannot be rejected, and the alternative hypothesis that room temperature and refrigeration have different effects on folic acid stability for 48 hours cannot be accepted. Statistically, it cannot be concluded that storage at room temperature affects folic acid degradation for up to 48 hours differently than does storage at refrigerated temperature. However, room temperature or refrigerated storage effects on folic acid stability are not of therapeutic importance. Mean concentrations of

folic acid were not significantly affected when solutions were stored at either room temperature or refrigeration for up to 48 hours (Table 3).

Light Effects

Statistical interaction effects of storage in light or dark on folic acid concentration throughout the 48 hour study period were not significant ($p > 0.26$). The null hypothesis that light and dark have similar storage effects on folic acid stability over a 48 hour period cannot be rejected, and therefore, the alternative hypothesis that room temperature and refrigeration have different effects on folic acid stability for up to 48 hours cannot be accepted. Statistically, it cannot be concluded that storage in light affects folic acid degradation for up to 48 hours differently than does storage in dark. However, therapeutically, the light or dark storage effects on folic acid stability are not important in this study. The data illustrate (Table 4) that mean concentrations of folic acid were not significantly affected when solutions were stored in light or dark throughout the 48 hours study period.

Initial Folic Acid Concentration Effects

Statistically, varying the initial folic acid concentration significantly affects folic acid stability throughout the 48 hour study period ($p < 0.0005$). The null hypothesis that differing initial folic acid concentrations have similar and consistent effects on folic acid decomposition over a 48 hour period can be rejected. It can be concluded that differing initial folic acid concentrations do not have

similar and consistent effects on folic acid stability over 48 hours. This statistically significant effect may be examined graphically. Figure 5 illustrates that the effects of each different concentration on folic acid stability are not consistent over the 48 hour study period; graphically this appears as line cross-over. If these effects were consistent, then graphically, these would appear as four separate and distinct lines. Although the interaction appears statistically significant, when examined graphically, the lines are not separate and distinct. A consistent initial folic acid concentration effect does not occur throughout the 48 hour study period. The alternative hypothesis that differing initial folic acid concentrations have different and consistent effects on folic acid stability over a 48 hour period cannot be accepted. It cannot be concluded that initial folic acid concentrations have a different and consistent effect on folic acid stability over a 48 hour period. Percent concentrations means of folic acid (Table 5) were not significantly affected over 48 hours when solutions were admixed with any of the four initial folic acid concentrations.

Combined Effects

Three way [(time (t) xLxC, txLxT, txCxT)] and four way (txLxTxC) interaction effects of temperature, light and initial folic acid concentration on folic acid stability determined over 48 hours were analyzed and found to be statistically non-significant ($p = 0.08$ to 0.3). Folic acid concentration remained constant for 48 hours.

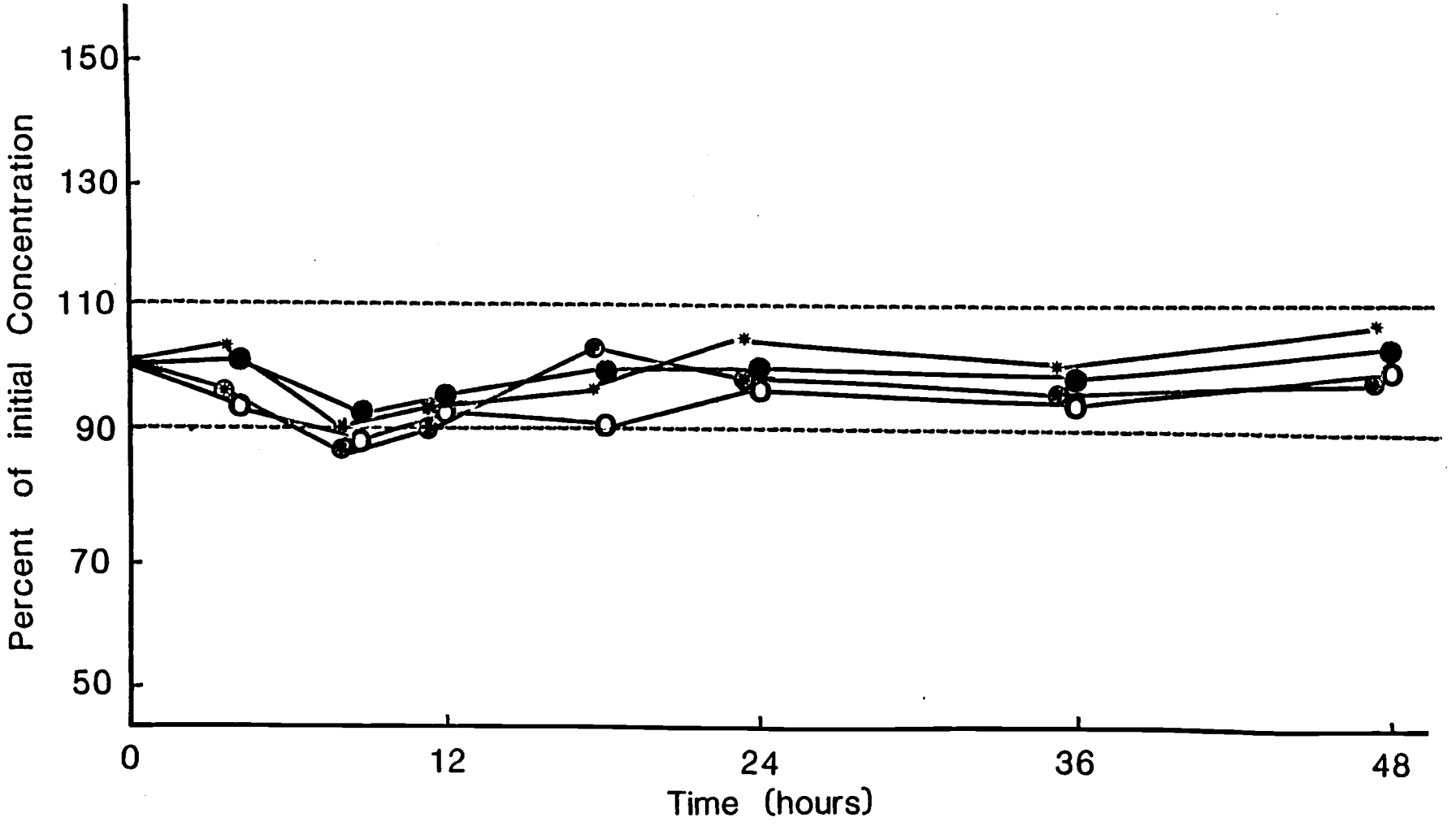

Figure 5. Statistical interaction of Concentration x Time. (—●—)=0.25 ng/liter, (—O—)=0.5 ng/liter, (—*—)=0.75 ng/liter, (—⊛—)=1.0 ng/liter.

V. CONCLUSIONS

Storage at room temperature, exposure to room light and higher initial folic acid concentrations were determined to be factors which enhanced folic acid degradation in early investigations. These investigations studied the stability of oral multivitamin preparations with shelf lives of up to 16 months.[11] This study illustrates that early conclusions on folic acid stability in oral multivitamin solutions do not apply to folic acid admixed with multivitamins in a commonly administered TPN solution. Several differences which apply to TPN solutions and not to oral multivitamin solutions may contribute to these findings.

The American Society of Hospital Pharmacists National Coordinating Committee on Large Volume Parenterals recommend that TPN solutions be administered immediately after admixture; if not administered immediately then refrigerated, and if refrigerated, the solution should be hung within 24 hours.[33] The policy of most IV services is to discard TPN solutions 24 hours after admixture as a safeguard to microbial growth. Therefore, TPN solutions are stored for much shorter times than were early multivitamin solutions.

The absence of air in the sterile air-evacuated container used for TPN solution preparation is another factor which differs from conditions studied in early research. Oral multivitamin solutions are prepared in glass bottles which are not air-evacuated. Additionally,

riboflavin, which is a component of the multivitamin product added to many TPN solutions (Table 1), may act as a hydrogen acceptor while folic acid oxidative cleavage proceeds to completion. The absence of air slows the rate of oxidative cleavage of folic acid in the presence of riboflavin. Preparing TPN solutions in air-evacuated containers maintains sterility and may also act to protect folic acid.

Folic acid is reported to be physically unstable with heavy metal ions.[4,7,34] Folic acid complexing may occur with formation of a precipitate.[35] Heavy metal ions as electrolytes or trace elements are commonly components of TPN solutions. Neither electrolytes nor trace elements were added to the TPN solution in this study. The purpose of this study was determination of the chemical stability of folic acid admixed with multivitamins in TPN solution. The formation of a precipitate would interfere with this determination and thus components which may precipitate were not added to the solution. Physical stability of folic acid with heavy metal electrolytes is well documented.[2,5,6] However, physical compatibility of folic acid with heavy metal trace elements is an area which requires further study.

Folic acid admixed in 25 percent dextrose, 3.5 percent amino acids and multivitamin solution is chemically stable for 48 hours after initial admixture. This stability is maintained independently of storage effects at room temperature or refrigeration, or storage in light or dark conditions. Different initial folic acid concentrations admixed in the solution also had no clinically significant effect on folic acid stability throughout the 48 hour study period.

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