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LITERATURE SURVEY

Vitamins in Pharmaceutical Formulations

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CONTENTS

The formulation of pharmaceutical vitamin products having adequate physical and chemical stability as well as suitable taste, odor, color, and freedom from bacterial contamination can entail numerous problems arising from the differing physical form, stability, and solubility characteristics of the individual vitamins. For liquid products, choice of the optimal pH is a crucial factor. Interactions between some of the vitamins and between vitamins and other product constituents must also be considered. Successful development of vitamin products requires knowledge of the fundamental aspects of the physical and chemical properties of the various forms of the vitamins available, the use of adequate techniques of manufacture, and the addition of suitable manufacturing overages based on critical stability studies.

SOLUBILITY OF THE VITAMINS

Vitamins are categorized into two general groups: fat-soluble and water-soluble. The fat-soluble vitamins include vitamins A, E, and K and cholecalciferol (vitamin D). Vitamin A is available as the free alcohol, retinol, and its acetate, palmitate, and propionate esters. Vitamin E is listed by USP as both *d*- and *dl*-forms of tocopherol and its acetate and acid succinate esters. Cholecalciferol is normally utilized in pharmaceuticals as ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3), both of which are equally active in humans. Oil-soluble forms of vitamin K include phytonadione (vitamin K_1), menadione, menadiol diacetate, and menadiol dibutyrate. Incorporation of fat-soluble vitamins into an aqueous preparation requires the use of an efficient emulsifying agent (*e.g.*, polysorbate 80) to produce homogeneous and physically stable formulations. In the case of vitamin K, there are a number of water-soluble forms such as menadiol sodium diphosphate, the dipotassium salt of menadiol disulfate, and menadione sodium bisulfite.

Solubilities of the water-soluble vitamins at 25° in water are listed in Table I. For several of the relatively insoluble vitamins such as riboflavin and biotin, the solubility is dependent on the nature of the crystal structure. For acids such as biotin and folic acid, addition of alkali significantly increases solubility. In the case of biotin it is possible to prepare a 20% aqueous solution of the sodium salt by this method. Figure 1 illustrates the increasing solubility of

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Table I—Solubility of Vitamins in Water at 25°

	mg/ml
Panthenol	Freely soluble
Thiamine hydrochloride	1000
Thiamine mononitrate	27
Niacinamide	1000
Sodium ascorbate	620
Ascorbic acid	333
Calcium pantothenate	356
Pyridoxine hydrochloride	220
Riboflavin-5′-phosphate sodium	43–112
Riboflavin	0.066–0.33
Cyanocobalamin	12.5
Biotin	0.3–0.4
Folic acid	0.0016

folic acid with increasing pH and shows the solubilizing effect on folic acid of gentisic acid ethanolamide as related to pH (1). An amorphous form of *d*-calcium pantothenate was reported (2) to dissolve ~10% faster than the crystalline form in aqueous media (pH 7.0 buffer).

STABILITY CHARACTERISTICS OF THE VITAMINS

Factors influencing the stability of the individual vitamins and their relative effects on different forms of a particular vitamin are listed in Table II. Although temperature is not mentioned in the tabulation, it is normal that an increase in temperature will accelerate any of the decomposition reactions. Macek (5) has reviewed the stability problems encountered with some vitamins.

A number of the vitamins may be classified as stable since they usually present no major problems regarding stability in pharmaceutical dosage forms. These include cholecalciferol, vitamin E acetate or acid succinate, biotin, niacin or niacinamide, pyridoxine, and riboflavin. The labile vitamins which are likely to present problems of instability in dosage forms are vitamin A (retinol and retinyl esters), vitamin K, ascorbic acid, cyanocobalamin, folic acid, pantothenic acid, panthenol, and thiamine.

The presence of one or more double bonds in the structures of vitamin A, cholecalciferol, and phytonadione makes them subject to isomerization under conditions frequently encountered in pharmaceutical products. For example, vitamin A undergoes isomerization in aqueous preparations at an acid pH. Vitamin A added to such products as essentially the all-*trans* form will isomerize on storage to an equilibrium mixture of approximately two-thirds all-*trans* and one-third *cis* isomers, the predominant *cis* isomer being 2-*cis* or neovitamin A, which has biological activity of 75% of that of the all-*trans* isomer. Lesser amounts of the 6-*cis* and 2,6-di-*cis* isomers may also form. The latter have only ~23% of the biological potency of all-*trans* vitamin A. Lehman *et al.* (6) have reported on the extent of isomerization that occurs in stored multivitamin drops. Equations have been proposed (7, 8) for calculating the biological potency of the isomerized vitamin A from the maleic values determined by Carr-Price colorimetry before and after reaction of vitamin A with maleic anhydride, in which isomers having a *cis* configuration at the terminal double bond (the 2-position) do not react to form an adduct.

Vitamin A suppliers have made available mixtures of vitamin A palmitate isomers at the equilibrium ratio of

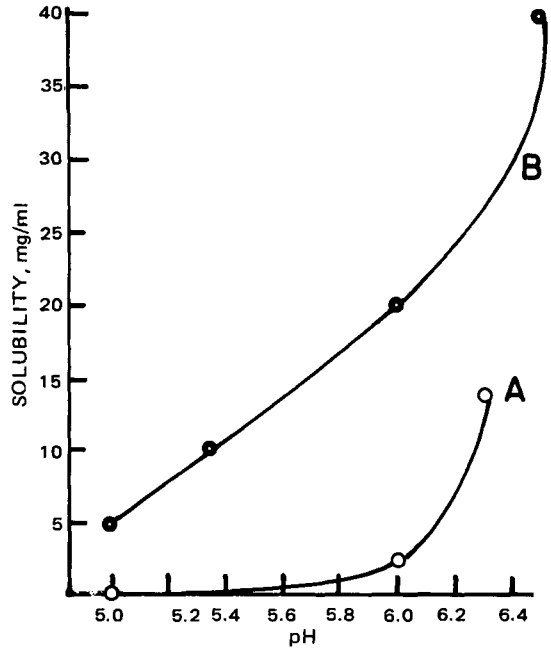

Figure 1—*Solubility of folic acid* versus *pH. Key: (A) with no solubilizer, (B) with 3% gentisic acid ethanolamide.*

Table II—Stability Characteristics of Vitamins

Vitamin A	sensitive to atmospheric oxygen (retinol less stable than its esters); trace metals catalyze decomposition; inactivated by UV light; isomerizes at acid pH; stable in alkali; in aqueous dispersions palmitate ester more stable to heat than retinol <pH 5.5; palmitate most stable ester under moisture stress; stabilized by antioxidants and protective coatings.
Cholecalciferol (vitamin D)	sensitive to atmospheric oxygen; trace metals and carriers with acid surface activity catalyze isomerization or decomposition; generally more stable than vitamin A; stabilized by antioxidants and protective coatings.
Vitamin E	tocopherol sensitive to atmospheric oxygen, especially in alkali and sensitive to UV light; esters very stable.
Vitamin K	fairly stable to heat; decomposed by sunlight and alkali.
Ascorbic acid (vitamin C)	stable when dry; readily oxidized in solution; decomposition catalyzed by metal ions (copper and iron); greatest instability at ~pH 4.
Biotin	stable to air and acid and at neutral pH; slightly unstable in alkali.
Cyanocobalamin (vitamin B_{12})	decomposed by oxidizing and reducing agents; slightly unstable in acid or alkaline solution; ascorbic acid and thiamine–niacinamide accelerate the decomposition; sensitive to light in very dilute solutions for assay.
Folic acid	unstable <pH 5; decomposed by sunlight and riboflavin; decomposed by reducing agents.
Niacin and niacinamide	normally very stable.
Calcium pantothenate	unstable in acid (< pH 5) and alkali; maximum stability at pH 6–7.
Panthenol	more stable than calcium pantothenate at ≤pH 5.
Pyridoxine (vitamin B_6)	normally very stable; metal ions can catalyze decomposition; dilute solutions for assay are sensitive to light.
Riboflavin (vitamin B_2)	stable in acid solution; unstable in alkaline solution; sensitive to light, especially in alkaline solution or in very dilute acid solutions for assay; decomposed by reducing agents.
Thiamine (vitamin B_1)	increasingly unstable in solution as pH rises; decomposed by oxidizing or reducing agents; cleaved by sulfite very rapidly at high pH (Ref. 3); hydrochloride more hygroscopic than mononitrate (Ref. 4).

two-thirds all-*trans* and one-third *cis* for use in aqueous products where isomerization is known to occur (9). With such use, the drop in potency by USP assay due to isomerization does not occur in the multivitamin product and, consequently, the stability of vitamin A in the product is somewhat better with the preisomerized mixtures than with all-*trans* vitamin A. Figure 2 illustrates this advantage of the preisomerized equilibrium mixture over all-*trans* vitamin A palmitate in a number of multivitamin drop preparations at pH 5.0 stored at room temperature for 12 months. On the average, after 6–12 months, vitamin A retention is 7% greater with the preisomerized vitamin A. A similar difference was found in storage tests at 45°.

The reversible isomerization reaction in solutions of cholecalciferol has been discussed by Keverling Buisman *et al.* (10). In this reaction an equilibrium is formed over time between ergocalciferol and precalciferol, the equilibrium ratio of the isomers being dependent on temperature. The isomerization rates of ergocalciferol and cholecalciferol are virtually equal (11) and are not influenced by the solvent, acidity, UV light, catalysts, and free-radical reaction inhibitors (12, 13).

Inactive byproducts that may be present from the irradiation process used for manufacture of cholecalciferol, such as tachysterol, isotachysterol, and *trans*-cholecalciferol, give the same pink color with Nield's reagent used for assay of ergocalciferol plus precalciferol. The interference by tachysterol and *trans*-cholecalciferol can be eliminated by forming an adduct of those compounds with maleic anhydride, which can be done in 30 min at 20°. Isotachysterol can be estimated after formation of adducts of ergocalciferol and precalciferol with maleic anhydride by heating for 3 hr at 100°. Isotachysterol does not react significantly with maleic anhydride (14) under these conditions.

In a series of papers, Takahashi and Yamamoto (15) studied the isomerization of ergocalciferol that occurs in powders prepared with various excipients such as $CaSO_4$, $CaHPO_4$, talc, aluminum silicate, and magnesium trisilicate. This isomerization is catalyzed by the surface acid of the excipients. Compounds such as monoethanolamine and polyoxyethylene glycol 4000, which are able to reduce the surface acidity of the excipients, act as stabilizers of ergocalciferol. Storage of ergocalciferol powders at high humidity increases their stability because the surface acidity is reduced through absorption of moisture. However, coating agents such as shellac, ethylcellulose, or cellulose acetate phthalate were unable to prevent the isomerization of the vitamin. The isomers identified were 5,6-*trans*-ergocalciferol, precalciferol, isocalciferol, a *cis* isomer of isocalciferol, isotachysterol, and tachysterol.

Phytonadione, having a double bond in the side chain, exists in the form of both *trans* and *cis* isomers. The *trans* isomer is the naturally occurring form, which is biologically active. The *cis* form has no significant biological activity.

DEGRADATION STUDIES

Vitamin A—The degradation of retinol in solution in an oil with polysorbate 80 was shown by Carstensen (16) to be a pseudo first-order reaction. Carstensen (16) and Tardif (17) reported first-order decomposition of vitamin A in tablets with adherence to an Arrhenius equation, and Shah *et al.* (18) found that vitamin A in two multivitamin preparations decomposed in accordance with a first-order reaction.

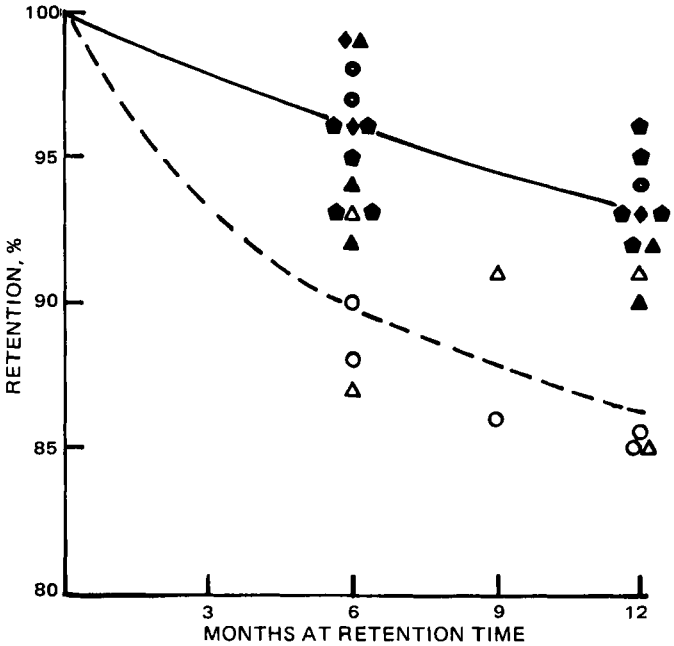

Figure 2—*Stability of vitamin A palmitate in aqueous multivitamin drops at pH 5; symbols represent different formulations with all-*trans *(●, △) or preisomerized (○, ▲, ■, ◆) vitamin A.*

Cholecalciferol (Vitamin D)—The decomposition of ergocalciferol and cholecalciferol at 25 and 40° under dry, humid conditions was studied by Grady and Thakker (19). Ergocalciferol decomposed rapidly at both temperatures in dry air, leading to formation of products of higher polarity. It was more stable at higher humidity than in dry air. Cholecalciferol was not as labile in dry air but decomposed rapidly at 40° and high humidity levels.

Vitamin E (α-Tocopheryl Acetate)—Vitamin E is relatively stable, but the unesterified α-tocopherol is less stable due to the free phenolic hydroxy group. Oxidation of α-tocopherol with agents such as nitric acid, ferric chloride, or ceric sulfate leads successively to the formation of α-tocopherolquinone, α-tocored, the *para*-quinone, and α-tocopurple (20, 21). In the absence of air, α-tocopherol is stable to heat but will be degraded if exposed to air. Thus, during saponification for analysis, protection by an antioxidant such as ascorbic acid or pyrogallol is practiced. Exposure to light will cause gradual darkening.

Vitamin K—Vire *et al.* (22) studied the degradation of menadione and menadione bisulfite in neutral and alkaline solution using polarographic methods. In alkaline solution the predominant degradation of the bisulfite addition product is to menadione, but in neutral solution isomerization to a naphthoquinone sulfinate becomes significant. In the absence of oxygen, menadione is degraded primarily *via* rearrangement to epoxynaphthohydroquinone.

Thiamine (Vitamin B_1)—The chemistry of the degradation of thiamine under the influence of heat and pH has been reported by Dwivedi *et al.* (23–25). The kinetics of the degradation of thiamine have been studied by various workers (17, 18, 25–29) who have reported first-order reactions with a rate increasing with increasing pH. Mulley *et al.* (28) also studied the degradation of cocarboxylase,

which was less stable than thiamine hydrochloride between pH 4.5 and 6.5 in phosphate buffer. Kobayashi (30) found that decomposition of thiamine in the neutral range is accelerated by the presence of Cu^{+2} at high temperature. The decomposition was affected by initial concentrations of thiamine and Cu^{+2}, time and temperature of heating, pH, and kind of buffer. In the presence of sodium nitrite, Kaya (31) found that heating of thiamine solutions for 60 min at 75° caused formation of elemental sulfur and thiochrome as well as 4-methyl-5-(β-hydroxyethyl)thiazole. The cleavage of thiamine by sulfite or bisulfite as noted in Table II is very rapid at high pH. Almost complete destruction of thiamine within 24 hr has been observed when thiamine was added to parenteral infusion fluids containing bisulfite as a preservative and having a pH ≥6.

Stepuro and Ostrovskii (32) studied the effect of pH on the photochemical reaction rate of thiamine, thiamine derivatives, and decomposition products using UV irradiation at 253.7 nm. The reactions take place at pH 3.0–9.0 with a rapid speeding up at pH 4–5 for thiamines. At pH < 3, the photolyzed thiazoles produce thiamide precipitates. Moorthy *et al.* (33) reported the reversible 1-electron redox potential of thiamine to be ~−0.5 V.

Riboflavin—Irradiation of riboflavin in neutral or acid solution yields lumichrome and in alkaline solution lumiflavine (34). The rate of decomposition is dependent on temperature, pH, and light intensity and wavelength. The decay of riboflavin-5′-phosphate exposed to light is dependent on the same factors (35).

Pantothenic Acid—Degradation has been shown to be a first-order reaction in solution at pH 3.8 by Frost and McIntire (36), in tablets by Campbell *et al.* (37–39), and in multivitamin liquids by Shah *et al.* (18).

Cyanocobalamin (Vitamin B_{12})—The decomposition of cyanocobalamin in tablets follows a first-order reaction (37–39). Marcus and Stanley (40) stored solutions of hydroxocobalamin in either acetate or citrate–phosphate buffer at 70, 80, and 90° and found the vitamin to degrade by a first-order reaction. Macek and Feller (41) show the decomposition of cyanocobalamin at higher temperatures to be catalyzed by thiamine decomposition and, hence, to be complicated. Shah *et al.* (18) found that cyanocobalamin in two multivitamin liquids stored at higher temperatures did not decompose in accordance with first-order kinetics. The oxidation–reduction thermodynamics of cyanocobalamin, cobalamins, and cobinamides have been reported by Ely (42).

The rates of destruction of cyanocobalamin in neutral aqueous solutions when exposed to various light sources have been reported by DeMerre and Wilson (43). Below 300 foot candles no destruction is noticeable. Sunlight at a brightness of 8000 foot candles caused 10% loss for each 30 min of exposure.

Losses of cyanocobalamin in solutions sterilized by gamma irradiation were lower at −78 and −196° than at −22 or −50° and were greatest at 0 and 18° (44).

Folic Acid—Dick *et al.* (45) studied the thermal stability of folic acid in buffered solutions at 100 and 120 ± 1°. Considerable destruction was found <pH 4, but >pH 5 there was no destruction in 1 hr at 100° or 15 min at 121°. Stokstad *et al.* (46) found rapid destruction of folic acid when a solution was exposed to direct sunlight. Degradation was most rapid at pH 7.0 and most slow in alkaline solution. Exposure to fluorescent light for 6 hr produced only slight inactivation. By extrapolation of results obtained at 55, 70, and 85° and 30, 50, and 70% relative humidity, Tripet and Kesselring (47) calculated 1%/year decomposition rate for folic acid either in solid form or in solutions with 5, 10, and 50% avicel. In two multivitamin liquids at higher temperatures, Shah *et al.* (18) found that folic acid did not follow first-order kinetics.

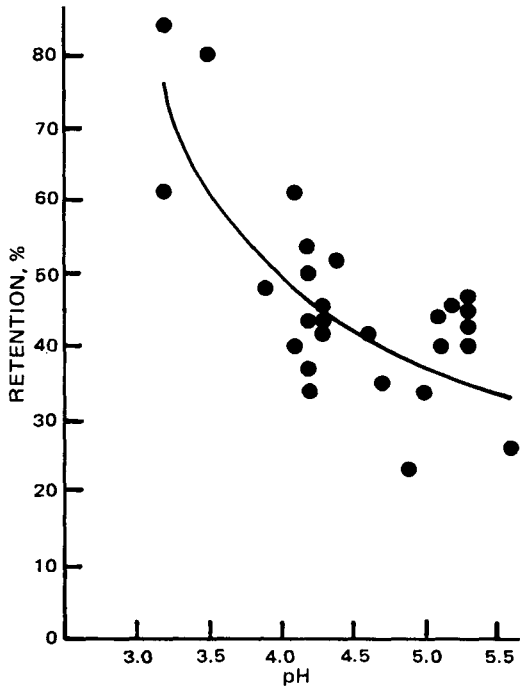

Figure 3—*Stability of thiamine* versus *pH in multivitamin drops; 6 weeks/45°.*

Ascorbic Acid (Vitamin C)—Reversible oxidation of ascorbic acid yields dehydroascorbic acid which has full ascorbic acid activity. Levandoski *et al.* (48) demonstrated that this reaction proceeds through an intermediate compound, identified as monodehydroascorbic acid, which may be complexed with ascorbic acid. Ascorbic acid stability in certain aqueous and fruit juice vehicles was reported by Uprety *et al.* (49). Ogata *et al.* (50) studied the kinetics of cupric salt-catalyzed autoxidation of ascorbic acid in aqueous solutions. Kassem *et al.* (51) reported that metal-catalyzed degradation of ascorbic acid in 10% injectable solutions took place by first-order kinetics. The order of effectiveness of metal ions as catalysts was $Cu^{+2} > Fe^{+2} > Zn^{+2}$; Fe^{+3}, Mn^{+2}, and Mg^{+2} had negligible effect. Dissolved oxygen had a deleterious effect increasing with concentration. Hayakawa and Hayashi (52) identified a Cu^{+2}–ascorbate complex as intermediate in the oxidation of ascorbic acid in the presence of Cu^{+2} ions.

The rate of anaerobic degradation of ascorbic acid in aqueous solution and the corresponding rate of formation of carbon dioxide as a breakdown product were reported by Finholt *et al.* (53, 54). Rogers and Yacomeni (55) and Blaug and Hajratwala (56) studied the effect of pH on the aerobic degradation of ascorbic acid in aqueous solution, which proceeded by a first-order reaction. Maximum rate of degradation occurred at pH 4 near the pK_{a_1} of ascorbic acid, and the minimum rate was at pH 5.6. Tingstad *et al.* (57) reported first-order degradation of ascorbic acid in a

multivitamin emulsion containing sodium fluoride stored at 70° and pH 3.15, 3.40, and 3.80.

Sattar *et al.* (58) found fluorescent light to have practically no effect on the destruction of ascorbic acid in pure solution, but the addition of riboflavin accelerated the decomposition. Losses of ascorbic acid were markedly increased by Cu^{+2} and Fe^{+3} under light exposed and unexposed conditions. The pro-oxidant effect of Cu^{+2} increased with increasing temperature and was relatively low under limited oxygen supply as compared to that in air, whereas oxygen supply had no influence on the effect of Fe^{+3}. Addition of Zn^{+2}, Ni^{+2}, and Mn^{+2} exhibited almost no effect under limited or excess oxygen availability. Uno *et al.* (59) and Switek and Modrzejewski (60) described the decomposition of ascorbic acid due to high frequency or gamma radiation used to sterilize aqueous solutions.

Vitamins, General—Huettenrauch (61) recommended the following as useful experimental parameters for kinetic studies of interactions and degradation of water-soluble vitamin preparations: temperature, pressure, solvent, degree of ionization, pH, concentration, and catalytic activity.

MUTUAL INTERACTIONS OF THE VITAMINS

Thiamine–Riboflavin—An incompatibility in aqueous vitamin B complex solutions has been described by Gambier and Rahn (62). The oxidative action of riboflavin on thiamine leads to the formation and precipitation of thiochrome. Subsequently, chloroflavin, the reduction product of riboflavin, may also precipitate. In vitamin B complex solutions containing ascorbic acid, thiochrome formation is not observed.

Thiamine–Folic Acid—Biamonte and Schneller (63) found thiamine to cause considerable decomposition of folic acid at pH 5.9 and 6.9 in aqueous, buffered solutions. Darnule and Colah (64) found the breakdown of folic acid to be accelerated by the presence of decomposition products of thiamine. The key element was the hydrogen sulfide produced during the breakdown of thiamine.

Thiamine–Cyanocobalamin—Blitz *et al.* (65) reported that the combination of thiamine and niacinamide in a vitamin B complex solution caused considerable decomposition of cyanocobalamin. It was shown by Macek and Feller (41, 66) that the breakdown of cyanocobalamin could be attributed largely to the 4-methyl-5-(β-hydroxyethyl)thiazole formed by cleavage of thiamine. At low levels of thiamine (1–10 mg/ml) losses of cyanocobalamin after a year at room temperature were small, but the losses were much higher at higher levels of thiamine or at elevated temperatures.

Riboflavin–Niacinamide—The presence of niacinamide in aqueous solution increases the solubility of riboflavin, due apparently to a complex formation between the two vitamins. This effect of niacinamide was useful in the preparation of vitamin solutions before the more soluble riboflavin-5′-phosphate became available. El-Khawas and El-Gindy (67) studied this interaction and reported that below 1% niacinamide, the effect on the solubility of riboflavin is small, but as the concentration of niacinamide is increased above 1%, the solubilizing effect on riboflavin becomes more pronounced and greater than that of urea.

Riboflavin–Folic Acid—Biamonte and Schneller (63) reported the deleterious effect of riboflavin on folic acid in aqueous, buffered solutions, particularly at pH ≥ 5.0. The combined action of light and riboflavin causes rapid oxidative cleavage of folic acid. Scheindlin *et al.* (68) studied this reaction and found it to occur more rapidly at pH 6.5 than at pH 4.0. The reaction is retarded, but not halted, by the exclusion of air and will proceed even when the solution is kept in amber glass.

Riboflavin–Ascorbic Acid—Hand *et al.* (69) and Sattar *et al.* (58) have reported that riboflavin catalyzes the photochemical decomposition of ascorbic acid during exposure of solutions to light and air.

Niacinamide–Ascorbic Acid—Wenner (70) described the preparation of a niacinamide–ascorbic acid complex in solid form. This yellow compound (71), containing one molecule each of niacinamide and ascorbic acid, forms readily in solution by what appears to be a charge–transfer reaction. It has been claimed (72) that preforming of this complex prevents difficulties with thickening and hardening of the mixtures employed in soft gelatin capsules. Guttman and Brooke (73) studied the formation of this yellow complex in the acid pH range at room temperature by measuring the absorbance at 365 nm. Maximum color was formed at pH 3.8 under the conditions employed. Osberger (74) described the commercial preparation of this complex and its applications in direct-compression tablets.

Niacinamide–Folic Acid—Taub and Lieberman (75) found that niacinamide acts as a solubilizer of folic acid. A 10% solution of niacinamide maintained a concentration of 5 mg/ml of folic acid at pH as low as 5.6, whereas the normal solubility of folic acid at pH 6.0 is 2 mg/ml.

Ascorbic Acid–Folic Acid—The cleavage of folic acid due to the reducing action of ascorbic acid was studied by Scheindlin and Griffith (76). In an acid medium the reaction products are *p*-aminobenzoylglutamic acid and 2-amino-4-hydroxy-6-methylpteridine. The free amino group of *p*-aminobenzoylglutamic acid is then destroyed by ascorbic acid or its oxidation products. The decomposition of folic acid was rapid at pH 3.0–3.3 and slow at pH 6.5–6.7.

Ascorbic Acid–Cyanocobalamin—Gakenheimer and Feller (77) observed an incompatibility between ascorbic acid and cyanocobalamin with losses of cyanocobalamin being least at pH 0–1 and increasing to a maximum at pH 7. Trenner *et al.* (78) found that hydroxycobalamin was much less stable in the presence of ascorbic acid then cyanocobalamin. Studies by Frost *et al.* (79) indicated that cyanocobalamin analogs in which the cobalt atom is strongly coordinated are the most stable toward ascorbate. Bartilucci and Foss (80) reported that decomposition products of ascorbic acid may play an important role in the effect on cyanocobalamin stability. Since copper ions catalyze the decomposition of ascorbic acid, it is not surprising that Stapert *et al.* (81) found that copper ions greatly enhance the destructive action of ascorbic acid on cyanocobalamin. Studies by Rosenberg (82) showed that Cu^{+2} itself has no effect on cyanocobalamin, and that ascorbic acid in the complete absence of Cu^{+2} causes relatively little decomposition of cyanocobalamin, but the combinations of ascorbic acid–Cu^{+2} or dehydroascorbic acid–Cu^{+2} were destructive of cyanocobalamin.

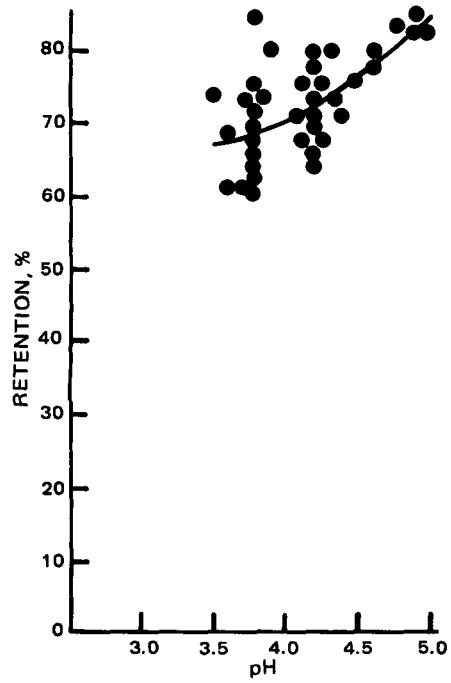

Figure 4—*Stability of ascorbic acid* versus *pH in vitamin B complex–ascorbic acid injectables; 6 weeks/45°.*

Utsumi *et al.* (83) studied the effect of various cobalamin analogs in catalyzing the oxidation of ascorbic acid in solution in acetate buffer at pH 5. The catalytic activity decreased in the order of hydroxycobalamin > hydroxocobinamide > cyanocobalamin coenzyme > cyanocobalamin > cyanocobinamide.

Other Interactions—Papp *et al.* (84) reported that several binary mixtures of thiamine, riboflavin, pyridoxine, niacin, and ascorbic acid showed nonlinear interferometric and conductometric curves, suggesting interactions. Hsu (85) found that niacinamide added to a solution of riboflavin-5′-phosphate sodium and ascorbic acid significantly increased the loss of riboflavin-5′-phosphate during photolysis, whereas added tryptophan stabilized both vitamins. Valls *et al.* (86) determined the stability of pyridoxal-5-phosphate and found it to be poor at pH 6 in aqueous solution. Increase in the degradation rate was caused by either thiamine, thiamine diphosphate, riboflavin-5′-phosphate, cobamamide, pyridoxal, or pyridoxine but not by riboflavin or cyanocobalamin. Takahashi and Yamamoto (87) found that ergocalciferol in powder preparations was readily isomerized by ascorbic acid, folic acid, thiamine hydrochloride, or pyridoxine hydrochloride but not by niacinamide or calcium pantothenate.

pH-STABILITY RELATIONSHIPS

In Aqueous Model Systems—*Thiamine versus Pantothenic Acid*—As noted in Table II, the stability of thiamine is best at low pH and decreases rapidly as the pH increases toward neutrality. In contrast, the stability of calcium pantothenate is optimal at pH 6.5–7 and decreases as the pH decreases. Frost and McIntire (36) studied the retention of thiamine hydrochloride and calcium pantothenate in 0.1% solutions at various pH levels after autoclaving for 15 min at 6.81 kg. Equal retentions of thiamine and calcium pantothenate were found at ~pH 4.5, the point at which the two curves of stability *versus* pH

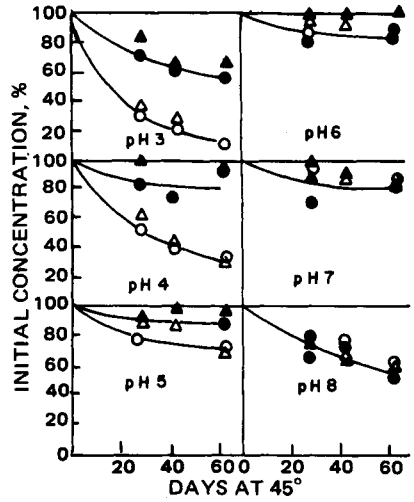

Figure 5—*Comparative stability of pantothenic acid (○, △) and panthenol (●, ▲) at pH 3–8.*

intersected. It may be noted that Figs. 3 and 4, which show 6 week/45° stability curves for thiamine and calcium pantothenate, respectively, in multivitamin drops, indicate 40% retention of both vitamins at pH 4.7.

Panthenol versus Calcium Pantothenate—Rubin (88) studied the comparative stability of these two forms of the vitamin in pure solutions with added buffers and a preservative. Both microbiological and rat bioassays were employed to assess stability. Figure 5 shows the comparative stability data obtained in tests at 45° for as much as 63 days. In the pH range of 3–5, commonly encountered in multivitamin liquids, panthenol shows markedly better stability than pantothenic acid.

Ascorbic Acid—The degradation of ascorbic acid in

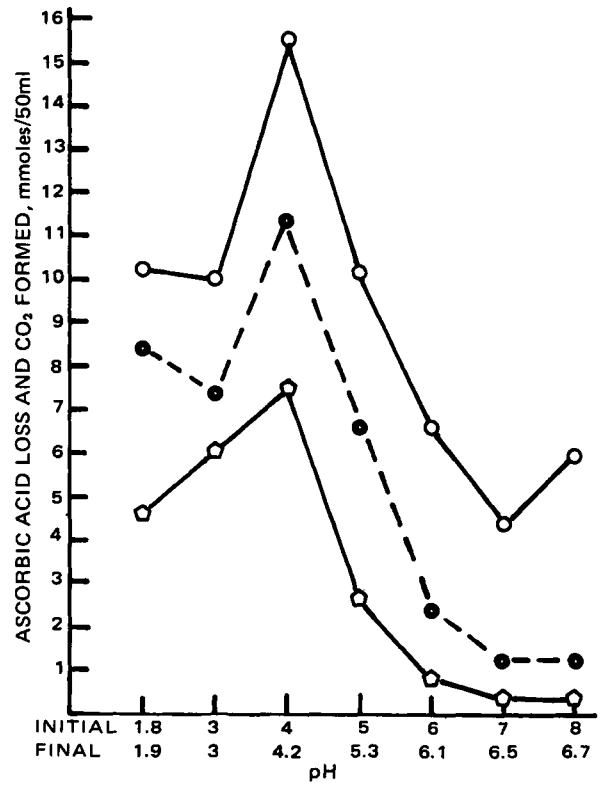

Figure 6—*Effect of pH on decomposition of ascorbic acid in 25% aqueous solution sealed in ampuls and stored 93 hr at 55°. Key: (○) ascorbic acid; (●) total ascorbic acid; (□) carbon dioxide formed.*

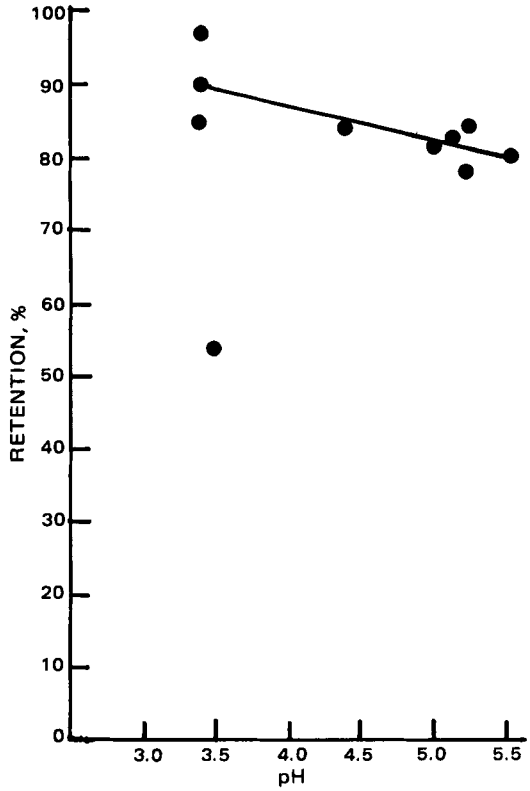

Figure 7—*Stability of thiamine* versus *pH in multivitamin products for teaspoon dosage; 6 weeks/45°.*

solution is pH-dependent. Finholt *et al.* (53) studied the pH–rate profile of the anaerobic degradation of ascorbic acid in solution at 96°. A maximum rate was observed at pH 4.1 which corresponds to the pK_1 of ascorbic acid. A study conducted in the author's laboratory also showed maximum degradation at ~pH 4.1 in solutions of 25% ascorbic acid in 50-ml sealed ampuls stored for 93 hr at 55°. Assays were made for ascorbic acid and dehydroascorbic acid contents and carbon dioxide formed. The results are shown in Fig. 6. The three graphs showing losses of ascorbic acid and total ascorbic acid (reduced and dehydro) and carbon dioxide formed all show a peak at ~pH 4.1. Pressure build-up in containers of products containing ascorbic acid can be especially troublesome at that pH as compared to either lower or higher pH.

In Aqueous Multivitamin Products—The influence of pH on the stability of some of the labile vitamins has been studied in a number of commercial as well as experimental multivitamin formulations. The pH–stability relationships are illustrated in Figs. 3, 4, 7–12 which show the results of storage for 6 weeks at 45°.

Thiamine—Figures 3, 7, and 8 show the retention of thiamine over the usual pH range for multivitamin drops, teaspoon dosage forms, and vitamin B complex and ascorbic acid injectables, respectively. All three types of products show progressively decreasing stability with increasing pH. Thiamine stability is particularly poor in the drop preparations, intermediate in the injectables, and best in the syrups and elixirs for teaspoon dosage. In the latter case, the better stability may be due to the influence of a high sugar content.

Ascorbic Acid—Figures 4, 9, and 10 show the pH–stability relations for ascorbic acid in multivitamin drops, teaspoon dosage forms, and vitamin B complex–ascorbic

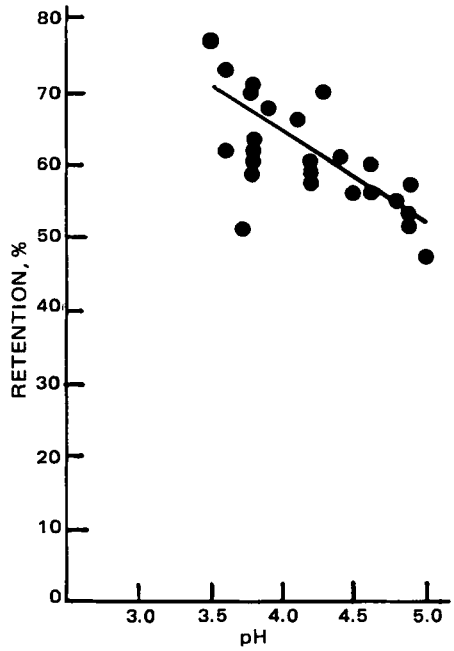

Figure 8—*Stability of thiamine* versus *pH in vitamin B complex–ascorbic acid injectables; 6 weeks/45°.*

acid injectables, respectively. In the drop preparations (Fig. 9), the curve shows maximum loss to occur at ~pH 4.2, which is similar to the pattern found in model systems (Fig. 6). In the other two types of preparation (Figs. 4 and 10), the losses at a given pH are similar and both show progressively decreasing loss as the pH increases from 3.5 to 5 or 5.5.

Panthenol and Calcium Pantothenate—The relative stability of panthenol and calcium pantothenate *versus* pH is shown in Fig. 11 for multivitamin drops and teaspoon dosage forms, which show similar stability patterns for panthenol. Figure 12 gives a similar comparison for vitamin B complex–ascorbic acid injectables. Panthenol shows the expected trend toward increasing losses with decreasing pH similar to the losses observed in the model systems shown in Fig. 5. Below pH 5 the losses of calcium pantothenate are much higher than those of panthenol.

Vitamin A—The relation of vitamin A palmitate stability to pH in multivitamin preparations for teaspoon dosage is shown in Fig. 13. Increasing the pH improves vitamin A stability. In the case of multivitamin drops, no clear-cut pattern of pH stability could be established due to wide variations found at any given pH level due to non-pH-related factors in the formulations tested.

FACTORS THAT ENHANCE VITAMIN STABILITY

Reduction of Water Content—Anmo *et al.* (89) determined the stability of retinol in 60–100% aqueous ethanol and found increasing losses as the water content increased. In a study of an oral liquid vitamin preparation made with various proportions of water, glycerin, and/or propylene glycol, Delgado *et al.* (90) reached the general conclusion that formulations containing lesser amounts of water possessed relatively higher stability values than those containing larger amounts of water. Parikh and Lofgren (91) demonstrated increased stability of ascorbic acid and thiamine when glycerin or propylene glycol was substituted for part of the water in an oral multivitamin

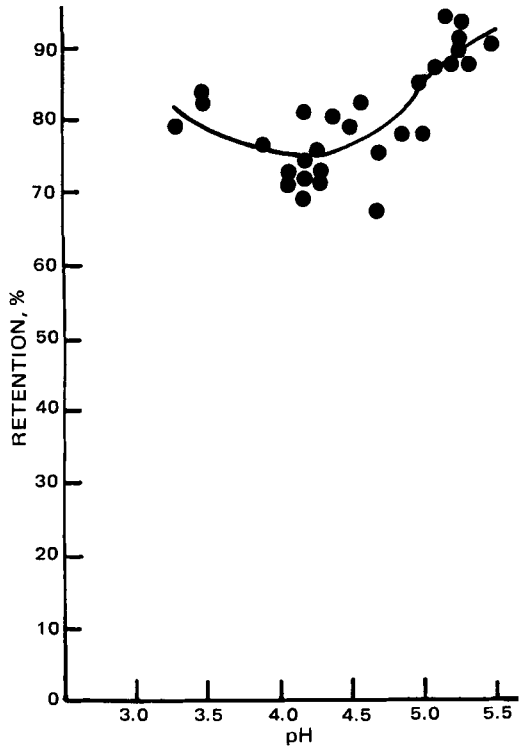

Figure 9—*Stability of ascorbic acid* versus *pH in multivitamin drops; 6 weeks/45°.*

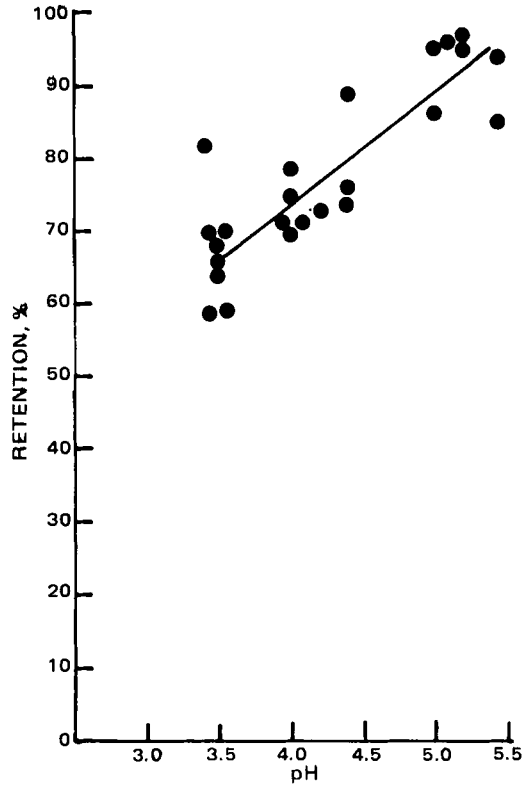

Figure 10—*Stability of ascorbic acid* versus *pH in multivitamin products for teaspoon dosage; 6 weeks/45°.*

liquid. Bandelin and Tuschhoff (92) reported similar findings on ascorbic acid and, in addition, found that ethanol or sugars such as sucrose, corn syrup, and dextrose also provide a stabilizing effect on ascorbic acid. Poust and Colaizzi (93) found that the first-order rate constants for oxidative decomposition of ascorbic acid at 30° decrease as a function of polysorbate 80 concentration up to 30%. In a study of the stability of ascorbic acid *per se* for 3 weeks at 45°, DeRitter *et al.* (94) found increasing stability as the moisture content decreased. Gerber *et al.* (95) reported increased stability of cyanocobalamin with high levels of sorbitol or sorbitol and glycerin. These authors found reasonable stability of both cyanocobalamin and ascorbic acid in a mixture with ferrous gluconate in 70% sorbitol. Gulesich (96) described an aqueous preparation containing ascorbic acid and iron, preferably ferrous sulfate, with 60–75% sorbitol.

Antioxidants—The stability of vitamins sensitive to oxidative decomposition can be increased in many cases by addition of antioxidants. Vitamin A and cholecalciferol are decomposed by exposure to air and are generally stabilized, in concentrates as well as pharmaceutical products, by addition of small amounts of antioxidants such as tocopherol, butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, ascorbyl palmitate, or a combination of several antioxidants (97–100). Koslov *et al.* (101) described model systems for studying the effectiveness of antioxidants in stabilizing vitamin A acetate and palmitate in thin films. At 10–40° butylated hydroxytoluene was superior to butylated hydroxyanisole, but ≥50° both were equally effective.

Ascorbic acid may also be more stable in the presence of antioxidants. Gladkikh (102) has reviewed methods for increasing the stability of ascorbic acid in medicinal forms. According to Tansey and Schneller (103), phenolic antioxidants such as butylated hydroxyanisole, nordihydroguaiaretic acid, and ethyl hydrocaffeate at levels of 0.02–0.05% retard the decomposition of folic acid by light in the presence of riboflavin and also protect folic acid somewhat in the dark.

Chelating Agents—The presence of trace metal ions, especially Cu^{+2} and to a lesser extent Fe^{+2}, accelerate the breakdown of ascorbic acid. The addition of a chelating agent such as edetic acid and its salts has been shown to enhance the stability of ascorbic acid *via* formation of metal chelates (90, 104). Kirkova *et al.* (105) tested nine stabilizers for ascorbic acid solutions and found a mixture of 0.1% disodium edetic acid and 0.25% $Na_2S_2O_4$ most effective. Reyes (106) found chelates of ascorbic acid with stearates of magnesium, calcium, or aluminum to provide improved stability of ascorbic acid in aqueous solutions.

Pyridoxine in solution is also sensitive to trace metal ions (107). Huang (108) found that Fe^{+2} in multivitamin products led to low recoveries of pyridoxine in microbiological assays. Recoveries of pyridoxine were 75 or 77% for 20:1 or 12:1 ratios of Fe^{+2}–pyridoxine, respectively. Addition of edetic acid before acid hydrolysis of such samples brought recovery of pyridoxine to 100%.

Cort *et al.* (109) found that the degradation of *dl*-α-tocopherol and *dl*-γ-tocopherol by Cu^{+2} could be completely inhibited by the combination of ascorbic acid and edetic acid, whereas ascorbic acid alone could prevent the oxidation of tocopherol in the presence of Fe^{+3}. Pure tocopherols when undiluted were stable to air and light over a period of years.

Other Compounds—Knobloch *et al.* (110) reported that the decomposition of vitamin A and cholecalciferol catalyzed by fine grain silicic acid can be inhibited by trolamine. An adduct of cholecalciferol with cholesterol

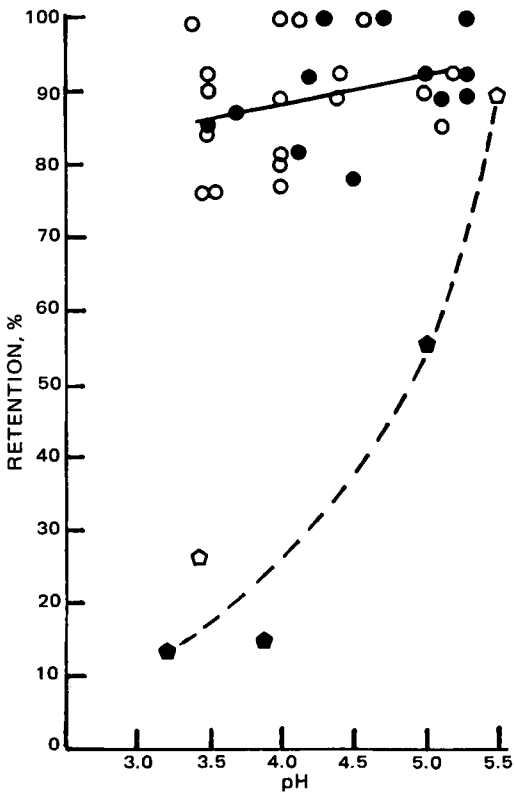

Figure 11—*Stability of panthenol (—●, ○) and pantothenate (- - -■, □) versus pH in multivitamin solutions-drops (●, ■) and teaspoon dosage forms (○, □); 6 weeks/45°.*

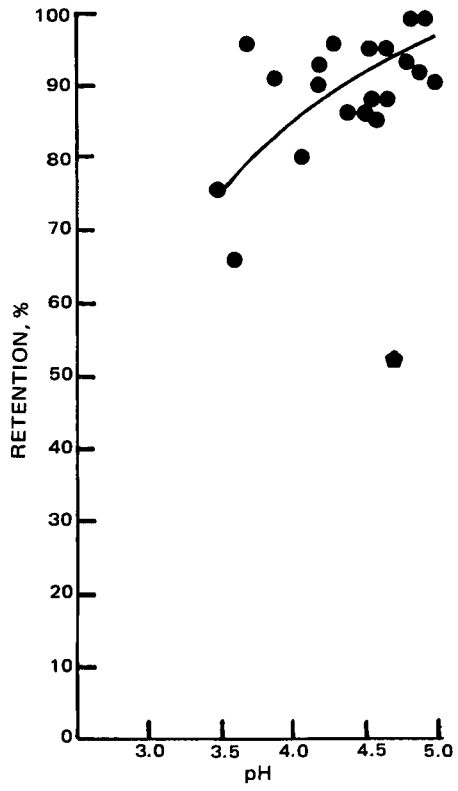

Figure 12—*Stability of panthenol (●) and pantothenate (■) versus pH in vitamin B complex injectables; 6 weeks/45°.*

was also found to be more stable than ergocalciferol or cholecalciferol.

Stabilization of riboflavin was achieved by Nobukuni *et al.* (111) by the addition of 1–20 moles of 2′-, 3′- and/or 5′-guanylic acid/mole of riboflavin. Protection of riboflavin, flavin mononucleotide, and flavin-adenine dinucleotide from photodecomposition was studied by Hata *et al.* (112–114). *para*-Substituted phenol derivatives, salicylic acid, tryptophan, and caffeine increased the stability of flavin mononucleotide through interactions in aqueous solution. Antipyrine and sulpyrine added to solutions of the flavins caused changes in their absorption spectra in the visible region suggesting interactions.

O'Broin *et al.* (115) compared the stability of folic acid to that of 5-formyl-, 10-formyl-, 5-methyl-, and unsubstituted tetrahydropteroylglutamate. Ascorbate was a superior stabilizing agent to 2-mercaptoethanol at comparable concentrations.

The effect of various compounds as stabilizers of B complex vitamins during gamma irradiation for the purpose of sterilizing aqueous solutions has been determined by Kishore *et al.* (116, 117). The combinations of nitrous oxide and glucose or oxygen and glucose as well as thiourea, tryptophan, and tyrosine provided good protection to thiamine, riboflavin, pyridoxine, niacinamide, and folic acid. Irradiation of aqueous solutions of these vitamins in the frozen state and with addition of glucose reduces radiolytic degradation. Irradiation in the dry, solid state causes no detectable damage, indicating that the best method for radiation sterilization of vitamin preparations may be to irradiate the components in the dry, solid state and then compound them together.

Rosenblum and Woodbury (118) reported the stabilizing effect of ferrous and ferric ions on cyanocobalamin in a multiple vitamin capsule. Newmark (119) described the effective stabilization of cyanocobalamin in solution in the presence of thiamine, niacinamide, and ascorbic acid by addition of various iron compounds and salts, including iron peptonate, ferric ammonium citrate, ferric chloride, ferrous gluconate, ferric glycerophosphate, ferric or ferrous sulfate, ferric or ferrous oxide, and ferric or ferrous complexes with such substances as edetic acid and its salts. Zuck and Conine (120) compared the relative effectiveness of complex cyanides and iron salts and found the cyanides to provide more effective stabilization of cyanocobalamin under conditions designed to exclude air as well as in partially filled containers having a large volume of air over the product. The complex cyanides were also effective in reducing the destruction of cyanocobalamin induced by UV irradiation.

Stabilization of thiamine solutions by addition of 0.05–5% monothioglycerol, thiosorbitol, or thioglucose has been the subject of a patent by Bray (121). Sodium formaldehyde sulfoxylate is used also for stabilizing injectable solutions of thiamine.

A considerable number of compounds have been reported to be stabilizers of ascorbic acid in solutions. In extracting solutions used in analyses of ascorbic acid, the stabilizers that have been used include metaphosphoric acid (122), oxalic acid (123), acetic acid (124, 125), and trichloroacetic acid. A combination of metaphosphoric acid and edetic acid has been recommended for use in the presence of Cu^{+2} and Fe^{+2} (104). Flavonoids inhibit the Cu^{+2} catalyzed oxidation of ascorbic acid (126). The order of activity reported by Takamura and Ito (127) is 3-hydroxyflavone < rutin < quercetin, and no inhibition was observed for flavone. Other compounds reported to have

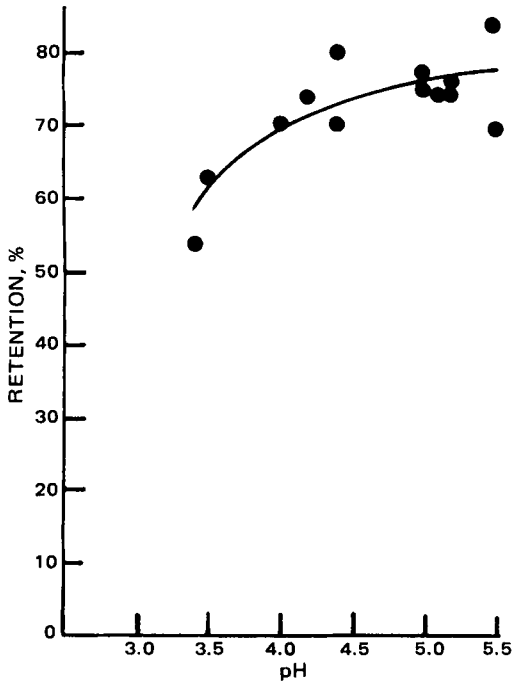

Figure 13—*Stability of vitamin A palmitate* versus *pH in multivitamin products for teaspoon dosage; 6 weeks/45°.*

similar activity are *O*-diphenols (128), aryl thioureas (129), rubeanic acid (130), acidic polysaccharides (131), sodium alginate (132), high concentrations of sucrose, glucose, or fructose (133), lysine and glutamic acid (117), a combination of *dl*-*N*-acetylhomocystine-thiolactone and sodium sulfite (134), gelatin (135), urocanic acid and/or its alkyl esters (136), and RNA, DNA, or salts (137). A review on the stabilization of ascorbic acid in liquid and solid preparations has been published by Gladkikh (102).

Coating and Encapsulation—A list of the various coating materials and processes used on single vitamins or combinations of several vitamins is given in Table III. Improving stability of labile vitamins under stress conditions is an important function of coating agents, but they are also useful for converting liquid vitamins into free-flowing, dry powders, masking taste in chewable tablets, improving handling and tableting characteristics, or stabilizing the color of ascorbic acid tablets, which otherwise may develop a tan color on aging.

Preparation of Adsorbates—Adsorption of fat-soluble vitamins on suitable adsorbents has been utilized as a means of conversion of the vitamins to dry, free-flowing powders as well as to enhance their stability. Espoy (182) described the preparation of an adsorbate of vitamin A on calcium silicate[1]. Neutral or weakly alkaline carriers such as magnesium oxide tend to stabilize vitamin A and cholecalciferol, whereas carriers with acid surface activity catalyze their decomposition (110, 183). This acid catalysis on fine grain silicic acid[2] can be inhibited by trolamine. Takahashi and Yamamoto (15) reported that ethanolamines and polyoxyethylene compounds were effective in preventing the isomerization of ergocalciferol caused by surface acidity of excipients.

Cannalonga and Czarecki (184) described a process for preparing agglomerated, free-flowing powders containing

[1] Microcel-E.
[2] Aerosil.

Table III—Coating or Encapsulation Processes for Vitamins

Vitamin	Stabilization Process	Reference
	Fat-Soluble Vitamins[a]	
A	Emulsify oil solution in gelatin, agar, or pectin or combinations thereof, plus sugar solution, cast into sheets, dry, freeze, and grind.	138, 139
A	Emulsify in gelatin–sugar solution and spray into starch ester containing hydrophobic groups[b].	140
A	Emulsify solution in hot well oil in wax and spray chill.	141
A	Granulate with partially hydrolyzed casein–formaldehyde and dry.	142
A	Emulsify in water-soluble gelable colloid, freeze, grind, and dry.	143
A	Emulsify in alkaline gelatin solution–pectin and water-soluble calcium salt and spray dry.	144
A	Emulsify in solution of low molecular weight amylose–glucose, dry on glass plate, and powder.	145
A	Emulsify vitamin A palmitate in hydrolyzed gelatin solution, spray dry, and agglomerate.	146
A and/or cholecalciferol	Emulsify in solution of gelatin–dextrin and maltose and spray dry.	147
A and/or cholecalciferol	Prepare clathrate in desoxycholic acid and subdivide.	148
A and/or cholecalciferol	Emulsify in wax or hydrogenated oil–vegetable flour and lecithin or polyoxyethylene sorbitan monopalmitate and spray.	149
A and/or cholecalciferol	Emulsify in film former (selected from dextrin, gelatin, casein or sodium carboxymethyl cellulose, maltose, sucrose, gum arabic)–emulsifier and spray into a current of atomized dehydrating agent (ethylene glycol, propylene glycol, glycerin, sorbitol, benzyl alcohol, ethanol, propanol, or butanol).	150
E	Emulsify in gum acacia–sugar solution and drum dry.	151
E	Emulsify in gum acacia solution–surface-active agent and convert into dry product.	152
E (acetate)	Emulsify in hydrolyzed gelatin solution with preservatives and spray dry.	153, 154
E (acetate)	Emulsify in hydrolyzed gelatin solution with preservatives and spray dry with agglomeration.	155
A, E, K, and/or cholecalciferol	Emulsify in solution of gum acacia or polyoxyethylene derivative of a partial ester of sorbitol and a fatty acid–glucose, sucrose, or corn syrup, cool and drop from a candy drop machine.	156
A, E, K, and/or cholecalciferol	Emulsify in gelatin and/or gum–a sugar alcohol–emulsifier such as lecithin and spray dry.	98
A, E, K, and/or cholecalciferol	Emulsify in solution of gum arabic or gum ghatti–lactose and spray dry; add 2% calcium silicate[c] as flour agent.	157
A, E, K, and/or cholecalciferol	Emulsify in wax, lecithin–synthetic polysaccharide[d], spray, and dust with one part soya flour to two parts of vitamin beadlets.	158
A, E, K, and/or cholecalciferol	Emulsify in solvent solution of a prolamine[e]–polymerized corn syrup and spray onto warm surface to remove solvent.	159
A, E, K, and/or cholecalciferol	Mix with gum acacia solution and dry gum acacia to form a plastic dough, dry, and comminute.	160
A, E, K, and/or cholecalciferol	Emulsify into gelatin solution previously heated with ascorbic acid–citric acid–plasticizer and spray into alcohol.	161

Continued on next page

Table III—*Continued*

Vitamin	Stabilization Process	Reference
A, E, K, and/or cholecalciferol	Emulsify into gelatin solution–powdered milk–a solid fat (mp 35–70°).	162
A, E, K, and/or cholecalciferol	Emulsify in a starch ester containing hydrophobic groups[b]–gelatin and sucrose or glucose and spray dry or spray into mineral oil or water.	163
A, E, K, and/or cholecalciferol	Solution in sunflower oil microencapsulated in gelatin.	164
A, E, or cholecalciferol	Emulsify into gelatin solution–chelating agent, protective polymer, antifoaming agent, and powdered whey.	165
	Water-Soluble Vitamins	
Thiamine	Microencapsulation in cellulose acetate phthalate with an acetone–benzene mixture as solvent.	166
Cyanocobalamin	Spray dry in starch succinate base–buffers and preservatives and add silicic acid.	167
Cyanocobalamin	Spray or vacuum dry in a galactomannan base from guar seed.	168
Ascorbic acid	Spray dry in methylcellulose, gum acacia, or gelatin–sucrose or lactose and magnesium stearate.	169
Ascorbic acid–salts and esters	Coating with small percentage of methyl polysiloxane.	170
Ascorbic acid	Spray with silicone lacquer and ethanol in fluidized bed dryer.	171
Ascorbic acid	Application of silicone oil coating.	172
Ascorbic acid	Microencapsulation in ethyl polymers using methyl ethyl ketone or with acetylcellulose in acetone.	166
Ascorbic acid	Disperse solid ascorbic acid in polyethylene glycol 6000 or 4000.	173
Ascorbic acid (calcium salt)	Microencapsulate in hydrogenated castor oil in ethanol.	174
Ascorbic acid	Coating with fats (mp 50–80°), lecithin, and glycerin esters.	175
Thiamine, riboflavin, or pyridoxine	Suspend in molten mixture of mono- and diglycerides of fatty acids and spray chill.	176
Niacinamide	Suspend in molten stearic acid, spray chill, heat for 14 days at 45°, and dust with silicic acid.	177
Niacinamide	Suspend in molten mixture of mono- and diglycerides of fatty acids, heat 14 days at 45°, and dust with silicic acid.	178
Thiamine, riboflavin, or niacinamide	Suspend in molten stearic, palmitic, or myristic acid or combinations thereof, and spray chill.	179
Thiamine, riboflavin, or niacinamide	Encapsulate in ethylcellulose–polyethylene in cyclohexane.	180
Vitamins	Coat with resins and polyvinyl compounds.	181

[a] Antioxidants and preservatives added are not listed. [b] Dry-Flo. [c] Micro-Cel. [d] Polyose D. [e] Zein.

vitamin A palmitate, *dl*-α-tocopheryl acetate, riboflavin, or perfumes which could be easily tableted. The adsorbents used included SiO_2, kaolin, $CaHPO_4$, and $MgCO_3$.

Protection from Light—Vitamins such as riboflavin and phytonadione, which are sensitive to light, particularly in solution, should be protected from light by suitable packaging of the vitamin solution. Surowiecki and Krowcznyski (185) reported that decay of riboflavin-5′-phosphate depended on temperature and intensity and wavelength of light. To prevent such decomposition, storage in amber glass capable of absorbing light up to a wavelength of 650 nm was recommended.

Lyophilization—For single or multivitamin solutions where instability of one or more vitamins in the formulation is a serious problem, lyophilization is an effective means of achieving improved vitamin stability. The lyophilization process has been applied in the preparation of multidose vials of vitamin B complex vitamins for parenteral use.

FORMULATION OF VITAMIN PRODUCTS

Aqueous Emulsions of Fat-Soluble Vitamins—Various surface-active agents (Table IV) have been used in the preparation of aqueous dispersions of fat-soluble vitamins. Optically clear dispersions can be made with efficient surfactants. Dispersions of single vitamins and multivitamin products containing vitamins A and E and cholecalciferol are prepared in aqueous media for both oral and intramuscular uses. A water-dispersible vitamin E compound was prepared by Cawley and Stern (193), namely, the polyethylene glycol ester of α-tocopheryl acid succinate.

Injectables—*Vitamins A and E and Cholecalciferol (vitamin D)*—Sobel (194) prepared veterinary injectables containing 0.0001–0.1% of vitamin A ester plus 0.005–0.06 g/ml of sorethytan monooleate in an aqueous, isotonic vehicle. Robeson (195) described a clear parenteral preparation in which vitamin A and cholecalciferol plus α-tocopherol or α-tocopheryl acetate were dispersed in an aqueous solution of α-tocopheryl polyoxyethylene glycol 1000 succinate. Aiello and Bauernfeind (196) prepared veterinary injectables of vitamins A, A–cholecalciferol, and A–E–cholecalciferol using a glyceryl triester of low molecular weight fatty acids (6–12 carbons and not more than 10% C-14 or greater) plus a polyoxyethylene-sorbitan ester of palmitic, stearic, oleic, or ricinoleic acid. A semisolid preparation for intramuscular injection of vitamins A and E and cholecalciferol was described by Feigh (197) as a substantially water-free mixture of the vitamins with polyethylene glycol 4000 and 200 plus antioxidant and preservative. Gherghinof *et al.* (198) prepared an aqueous injectable solution containing 22,000 IU/ml of vitamin A palmitate with 10 parts of polysorbate 80[3] to 1.1 parts of vitamin A. Butylated hydroxytoluene was added as antioxidant, the pH adjusted to 3–4 with ascorbic acid, and the solution sealed in vials under nitrogen and sterilized three times at 80° for 60 min with intervals of 24 hr. A solution was described which contained 300,000 IU of cholecalciferol/ml made with 15% (w/v) polysorbate 80[3] and 2.25% (w/v) isoamyl alcohol with ascorbic acid added to bring pH to 3–4. The vials were sealed under nitrogen without heat sterilization.

Vitamin K—Water-soluble derivatives of menadione such as menadiol tetrasodium diphosphate salt are used in injectable formulations. The fat-soluble phytonadione has been prepared in colloidal emulsion form with an efficient surfactant such as polyoxyethylene vegetable oil[4]. Fujita *et al.* (199, 200) prepared an injectable in which vitamin K was dissolved in a mixture of a hardened castor oil polyoxyethylene derivative and propylene glycol, after which sorbitol, trolamine, and water were added prior to sealing in ampuls and autoclaving for 30 min at 4.54 kg. Koshiro *et al.* (201) studied the compatibility of three

[3] Tween 80.
[4] Emulphor-620.

Table IV—Surfactants Used in Preparation of Aqueous Dispersions of Fat-Soluble Vitamins

Surfactants or Dispersing Agents	Reference
Polyalkalene oxide derivatives of partially esterified polyhydric alcohols or their anhydrides.	186
Bone gelatin from the lime process, ascorbyl palmitate, and a monoester of glycerin or of propylene glycol.	187
Polyoxyethylene glycol monoricinoleate containing 30–50 oxyethylene units.	188
Monoester (laurate, oleate, *etc.*) of polyethylene glycol 400 and a predominantly nonionic surfactant.	189
Polyoxyethylene vegetable oil[a] or other emulsifier[b].	190
Polysorbate[c] 40, 60, or 80–polyethylene glycol 200–600.	191
Polysorbate[d] 20 and algin[e].	192

[a] Emulphor EL-620. [b] Prosol E-4329. [c] Tween 40, 60, or 80. [d] Tween 20. [e] Tagat R, S, or O.

parenteral solutions of vitamin K with 98 commercial injectables.

Thiamine—For a single vitamin injectable with thiamine, the hydrochloride salt is generally preferred, but purity of the vitamin is an important factor. A special ampul grade of thiamine hydrochloride has been made for this purpose. Monciu and Boteanu (202) prepared 2.5 and 5% solutions of thiamine hydrochloride and found retention of at least 90% of initial concentration in the 2.5% solution after 4 years at room temperature and in the 5% solution after 5 years at room temperature. Various additives have been tested for their effect on the stability of thiamine hydrochloride solutions for injection. Ammar (203) compared the effects of edetic acid, *N*-hydroxyethyledetic acid, and pentetic acid and found the latter two to be superior to edetic acid in stabilizing thiamine. The optimum concentration of edetic acid was 3 mmoles/liter and 0.5 mmole/liter of *N*-hydroxyethyledetic acid. Ammar (204) also studied the effect of DL-α-lipoic acid, which at low concentrations (0.5–1.0 mmole/liter) increased the stability of solutions of thiamine hydrochloride for injection, but at higher concentrations (3–10 mmoles/liter) decreased the stability of thiamine hydrochloride. In the presence of 20 ppm of copper, the concentration of lipoic acid for maximum stability of thiamine was shifted from 0.5–1.0 mmole/liter, indicating that the concentration of additive for maximum stability of thiamine is dependent on the level of trace metals in the solution. Kirkova and Nedelova (205) studied the effect of different buffers, propyl gallate, benzyl alcohol, sodium edetate, sorbitol, glucose, and heat sterilization on the stability of thiamine solutions for injection. The most important factor was found to be the quality of the thiamine hydrochloride.

Taub *et al.* (206) reported that thiamine in parenteral solutions in admixture with riboflavin and niacinamide exhibited maximum stability with respect to potency and clarity of solution at pH 4 under a nitrogen atmosphere. In admixture with iron compounds, thiamine was more stable in the presence of ferrous gluconate than of iron peptonate or ferric ammonium citrate, and again, the optimum pH was 4. In these studies it was found that thiamine mononitrate is somewhat more stable than the hydrochloride.

Ascorbic Acid—Stable, buffered solutions containing 50–200 mg of ascorbic acid/ml were prepared by Cimenera and Wilcox (207) by buffering to pH 6.0–6.5 with trisodium phosphate, adding 0.5% phenol, heating 2-ml sealed ampuls at 100° for 10 min and cooling rapidly. Stability was achieved by adhering to rigid anaerobic conditions by the use of nitrogen throughout manufacture and avoiding contact with metals. Popovic *et al.* (208) obtained best stability in a parenteral solution containing 10 g of ascorbic acid, 4.75 g of $NaHCO_3$, 1 g of $Na_2S_2O_5$ and 0.1 g of edetic acid/liter and through which carbon dioxide was passed. Kassem *et al.* (209) studied the stabilizing effect of various metal complexing agents on ascorbic acid solutions. *N*-Hydroxyethylethylenediaminetriacetic acid was most effective, but the optimum concentration depended on the amount of heavy metal ions in the solution and had to be determined experimentally for each formulation.

Pyridoxine (Vitamin B_6)—Stabilized 2.5 and 5.0% solutions of pyridoxine were prepared by Nikolov and Nedelova (210) at pH 2.8–3.0 with 0.1% Na_2SO_3 added. The filling was done under a stream of nitrogen and aseptic conditions were used. Inoue *et al.* (211) determined the stability of an injectable solution of pyridoxamine phosphate in the presence of 70 other injectables.

Multivitamins—Ban (212) reviewed the preparation, storage, and usage of injectable solutions of the water-soluble vitamins. Boyazhieva *et al.* (213) described a veterinary preparation for intramuscular use containing ferro-dextran, gamma globulin, trace elements, and vitamins, which was claimed to be stable for 1 year. Blitz *et al.* (214) studied the stability of cyanocobalamin in vitamin B complex injectables and found cyanocobalamin to be unstable in the presence of thiamine and niacinamide at concentrations from 25–100 mg/cm^3 of each at pH 4.25 but relatively stable at lower concentrations of each component. Rigoli (215) reported good stability of cyanocobalamin and ascorbic acid after lyophilization of a solution containing both vitamins. Haeger and Nash (216) described a two-compartment syringe for injectable water-soluble vitamins with sodium ascorbate, folic acid, and niacinamide in the upper compartment and thiamine hydrochloride, riboflavin-5′-phosphate sodium, niacinamide, panthenol, pyridoxine, cyanocobalamin, and ferrous citrate in the lower compartment. A complete multivitamin injectable preparation was also described (217) which is similar to that described previously but with vitamin A palmitate, *d*-α-tocopheryl acetate, and ergocalciferol emulsified in the solution in the upper compartment by means of polysorbate 80 and propylene glycol.

The stability of vitamins after addition of injectable preparations to intravenous fluids is also a matter of concern. Although storage of such mixtures is normally for short periods, losses of labile vitamins such as vitamin A, thiamine, cyanocobalamin, and ascorbic acid can be significant and are higher during light exposure than in the dark. In addition, sorption of vitamins on the surface of plastic infusion fluid bags can contribute to unavailability of a portion of the vitamin content to the patient. Moorhatch and Chiou (218) studied the sorption of 10 vitamins on such bags. Howard *et al.* (219) studied the effect on vitamin A activity of storage of a parenteral nutrient solution in polyvinyl bags in the dark at 4°. After 8 and 72 hr and 2 weeks, the vitamin A content was 86, 73, and 23% of the initial concentration. Approximately 30% of the vitamin A losses were due to sorption on the plastic bags. The

use of bisulfite as a preservative in parenteral infusion fluids can cause high losses of added thiamine, particularly at ≥pH 5.5. In the laboratory only 3–8% retention of thiamine was found after 24 hr at room temperature in an amino acid infusion fluid at pH 6.5 and containing, according to label, 1 mg of sodium bisulfite/ml. Kobayashi and King (220) reported that the addition of vitamins to a protein hydrolysate–dextrose parenteral solution caused no physical incompatibilities.

Tablets—The fat-soluble vitamins A and E and cholecalciferol are normally incorporated into tablets in the form of dry, stabilized coated products or adsorbates as described above. The B complex vitamins, thiamine, riboflavin, niacinamide, and pyridoxine, which can contribute off-flavor to chewable tablets, are available in coated forms, usually containing 25–33% of the vitamin. Fatty acids or mono- and diglycerides of fatty acids are utilized as coating agents for effective masking of taste and also contribute to the stability of thiamine. Ascorbic acid is stabilized with a small percentage of ethylcellulose and is also available as granulations containing 90–95% ascorbic acid, which are particularly suited for preparation of high-potency ascorbic acid tablets by direct compression. Biotin and cyanocobalamin are used in tablets in the form of triturates, adsorbates, or spray-dried powders containing 0.1–1.0% of the vitamin to facilitate the distribution of the microgram quantities normally used. Nessel *et al.* (221) have studied the uniformity of distribution of cyanocobalamin in tablet formulations using 0.1 and 1% gelatin products and a 1% resinate in both wet-granulated and dry-blended formulations.

Single or multiple vitamin tablets have been made by both wet and dry granulation processes and by direct compression and either left uncoated or coated by compression, film, or sugar-coating processes. A number of reports dealing with vitamin tablet technology are listed in Table V. Seugling (239) has reviewed the use of various tableting aids in the development of pharmaceutical products, including conventional and chewable vitamin tablets. Lieberman and Lachman (240) have presented a comprehensive review of tablet technology which also deals with vitamin products. Wai *et al.* (241) studied stability of vitamin A, thiamine, and ascorbic acid compressed in eight commonly used solid vehicle matrixes. Mannitol and lactose were found to yield superior stability. All three vitamins were quite stable when the moisture content of the tablets was ≤1%. In multivitamin chewable tablets, these authors found that the use of coated ascorbic acid, thiamine, riboflavin, and niacinamide enhanced vitamin stability. The best stability results were obtained when vitamin A, ergocalciferol, and thiamine were granulated with one-fourth of the mannitol-magnesium stearate, ascorbic acid with one-half of the matrix, and the remaining B vitamins with one-fourth of the matrix. The use of water or dilute alcohol for granulation caused more rapid discoloration on storage than did alcohol granulation or dry slugging procedures.

Campbell and McLeod (39) studied the stability of vitamins in several commercial tablet formulations and found a rather wide range of stability for some of the labile vitamins. Maekawa *et al.* (242) studied the stability of vitamins in sugar-coated decavitamin tablets and the mutual interactions of the various vitamins. The best stability was obtained when vitamin A, ergocalciferol, calcium pantothenate, cyanocobalamin, and folic acid were in the coatings and the other vitamins in the tablet core. Bojarski *et al.* (243) compared vitamin stability in decavitamin tablets and dragees and found better stability in the tablets. Only thiamine and calcium pantothenate were unstable, and water had a major influence, especially on the stability of thiamine. Thiamine nitrate was more stable in these tests than thiamine hydrochloride. Ragazzi and Veronese (244) reported much better stability of vitamin A, ergocalciferol, and cyanocobalamin when these vitamins were protected by gelatin-sugar coating prior to incorporation into multivitamin tablets. Coated ascorbic acid, however, was not different from uncoated. Maekawa *et al.* (245) found much better stability of ascorbic acid in sugar-coated tablets than in uncoated tablets upon storage for 3 weeks at 37° and 74% relative humidity. Bolatre *et al.* (246) determined that ascorbic acid in tablets can discolor badly without much change in ascorbic acid content. Carstensen *et al.* (247) studied the degradation of thiamine tableted with magnesium stearate and microcrystalline cellulose. As the moisture level was increased to 5.5%, thiamine loss increased, but at higher moisture levels stability was enhanced with increased moisture content. The model proposed to explain this phenomenon was that thiamine dissolved in the water present adsorbs on the microcrystalline cellulose, and the thiamine present in the monolayer degrades totally, whereas the thiamine in layers beyond the monolayer does not degrade.

The preparation of sustained-release vitamin tablets has been a matter of interest, particularly in the case of high-potency ascorbic acid, but very little has been published on the technology of formulation of such tablets. Nuernberg *et al.* (248) described the preparation and *in vitro* release testing of 250-mg ascorbic acid tablets in which the release of the vitamin was sustained by the addition of more than 20% galactomannans. Cazals (249) prepared tablets of cyanocobalamin containing a mixture of mannitol and aminoacetic acid, which were claimed to yield prolonged and enhanced effectiveness. Kassem *et al.* (250) described a tablet of riboflavin-5′-phosphate with a maintenance dose in the core and an initial dose in the coating. The vitamin was embedded in a matrix of tragacanth, acacia, ethylcellulose, and stearic acid and granulated through a sieve to an optimal size of 1.6 μm. Subsequent coating with a thin layer of ethylcellulose suppressed the initial release. Hardening the granules at 30° for 2 hr improved the release. Lubrication with magnesium stearate and talc was recommended.

The major problem in formulating a sustained-release multivitamin product is to achieve full bioavailability of the vitamins in addition to sustained-release for a significant number of hours. Riboflavin is particularly troublesome in this regard due to its very low solubility in water. Morrison *et al.* (251) studied *in vitro* release rates and the corresponding physiological availability of riboflavin in sustained-release vitamin preparations and pointed out the frequent association of incomplete bioavailability with prolonged *in vitro* release. Other work by this group (252–255) pointed out similar difficulties with availability of riboflavin in normal, enteric coated, and chewable vitamin tablets. It was concluded that *in vitro* disintegration times longer than 1 hr or coating processes

Table V—Vitamin Tablet Technology

Vitamins	Formulation Technology	Reference
A, E, and cholecalciferol	Absorbed from oil solution on magnesium oxide with ascorbic acid and *dl*-α-tocopherol added; granulate with calcium pantothenate, starch, and calcium stearate before tableting.	184
Thiamine	Granulation with 1.5% ethylcellulose or 15% polyethylene glycol in alcohol plus dry binder.	222
Ascorbic acid	Addition of ~1% of sorbose, lactose, or a mixture of the two in the granulation tends to stabilize tablet color.	223
Ascorbic acid	Granulate 50–80 parts of ascorbic acid with 5–16 parts starch and 2.5–5 parts hydroxypropyl methylcellulose (viscosity 20–4000); add 0.25–0.35 liter of alcohol/kg and 5.5–16 parts starch; dry and pass through 10–16 mesh screen. Add lubricants (0.25–2 parts colloidal silicon, 0.5–2 parts hydrogenated vegetable oil, 0.1–0.5 parts zinc stearate, and 2.8–8 parts starch) and compress.	224
Ascorbic acid	Add physically modified starch, magnesium silicate as hardener, and hydrogenated vegetable oil as lubricant and compress directly.	225
Ascorbic acid	Vinyl acetate–crotonic acid copolymer yields harder and less friable tablets by direct compression than polyethylene glycol 6000.	226
Ascorbic acid	Microcrystalline cellulose is superior to microfine cellulose (less compression required and less capping).	227
Thiamine, ascorbic acid, or folic acid	Tablets formulated[a] and subjected to accelerated aging; ascorbic acid was unstable but thiamine and folic acid were stable.	228
Thiamine and ascorbic acid	Tablets coated with 2% aqueous solution of hydroxypropyl cellulose (viscosity 6.1 cps at 20°) were more stable than tablets coated with a sugar solution.	229
Thiamine and ascorbic acid	Tablets coated with syrup containing sugar, gelatin, and gum arabic over a subcoating of carboxymethyl–starch–talc disintegrated in 14 min while controls without the carboxymethyl–starch in subcoating took 42 min.	230
Thiamine and ascorbic acid	pH of tablets also containing aspirin adjusted to 2.5–4 with tartaric acid; tablets coated with 10% povidone and 4% shellac.	231
Cyanocobalamin and ascorbic acid	Granulating cyanocobalamin and ascorbic acid with trace element caseinates improved vitamin stability; without ascorbic acid caseinates did not improve cyanocobalamin stability.	232
Cyanocobalamin, thiamine, and pyridoxine	Best stability of cyanocobalamin achieved with cyanocobalamin in a separate layer in a sugar-coated tablet.	233
Multivitamin	Granulation with alcohol and addition of 5% povidone.	234
Multivitamin	Vitamin E adsorbed on silica gel and 0.5–5% finely divided silica gel added to smoothing syrup.	235
Multivitamin	Granulation of vitamin E and ascorbic acid.	236
Multivitamin	Effervescent tablets stabilized with 0.1 g of cyclamic acid and 0.2 g of α-aminoacetic acid/tablet.	237
Multivitamin	Lyophilization of vitamin substance in a solvent containing a binder, granulating and compressing; binders used were gum arabic, alginates, pectins, dextrins, glucose, povidone, polyvinyl alcohol, carboxymethyl cellulose, polyethylene glycol, *etc.*	238

[a] Formulated with Encompress Std.

can reduce the bioavailability of vitamins to humans and that any product with such characteristics should be tested in humans to determine that the vitamins are available.

Oral Single Vitamin Liquids—Vitamin A, in the form of an aqueous dispersion with an effective surface-active agent, yields much more rapid absorption *in vivo* than oil solutions. Adamski and Sawick (256) found the stability of vitamin A palmitate in aqueous dispersions to depend on the concentration of the vitamin A, the nature of the surfactant, and the buffer applied. A derivative of castor oil and ethylene oxide[5] yielded the best stability.

Stabilized aqueous solutions of thiamine were prepared by Bray (121) using thioglycerol, thiosorbitol, and thioglucose as stabilizing agents. Italfarmaco S.p.A. (257) described a 1% solution of thiamine hydrochloride with 5% povidone, 0.05% propyl gallate, and 0.005% sodium edetic acid, which was chemically and organoleptically stable for more than 2 years. Riboflavin and pyridoxine were also incorporated into similar compositions. Genova and Papazova (258) found cyanocobalamin stable for 5 months at 40° in a solution containing 100 μg/ml of cyanocobalamin in 1% solutions of orotic acid and monoethanolamine. Exposure to UV light caused destruction of cyanocobalamin, which was more rapid at pH 4.9–5.2 than at pH 3.9–4.2. Bajeva and Jonega (259) studied the stability of ascorbic acid–citrate formulations and found maximum stability with addition of 30% (v/v) propylene glycol and 0.1% cysteine hydrochloride.

Oral Multivitamin Liquids—General directions for preparation of stabilized multivitamin liquids have been given by Djourno and Thoumyre (260) and Belova and Litvinenko (261). Delgado *et al.* (90) prepared 10 different multivitamin drop formulations with various combinations of water, glycerin, and/or propylene glycol as vehicle. The most stable preparation contained 80% propylene glycol + 20% water with ethyl hydrocaffeate and Na_2Ca edetic acid. Parikh and Lofgren (91) studied the stability of seven different oral multivitamin drop preparations with sorbitol alone or in combinations with propylene glycel and glycerin in the aqueous vehicle. The studies showed: (*a*) butylated hydroxyanisole retards oxidative decomposition of vitamin A; (*b*) glycerin is a better substitute for a portion of the water than propylene glycol as far as stability of thiamine is concerned; (*c*) reducing the percentage of water by substituting propylene glycol or glycerin yields better stability of ascorbic acid; (*d*) stability of cyanocobalamin increased as pH was increased from 3.5 to 4.5; (*e*) folic acid, when in solution in preparations containing propylene glycol, was very unstable at pH 3.4. Biamonte and Schneller (63) also found folic acid in solution very un-

[5] Cremophor EL.

stable at acid pH, but when in suspension at low pH due to insolubility, folic acid is quite stable.

Stone (262) determined the stability of drop formulations containing thiamine, riboflavin, and niacinamide with and without ascorbic acid, using propylene glycol as a vehicle either alone or with 25 and 50% glycerin, simple syrup, or water. Greater retention of thiamine was found in solutions containing the greater percentage of water, whereas higher stability values were obtained for ascorbic acid in formulations containing lesser amounts of water. Similar relationships are seen in Figs. 3, 7, 9, and 10 where drop preparations show higher losses of thiamine than the more dilute elixirs or syrups, whereas ascorbic acid is more stable at pH 4 in the drop formulations than in the teaspoon dosage forms. Discoloration by yellowing and darkening was found by Stone (262) to be proportional to the percentage of water present.

Zoni and Lazzeretti (263) obtained satisfactory stability in a drop preparation containing B vitamins and L-lysine by adding povidone to the aqueous base. Synergistic enhancement of the stabilizing effect of povidone was found with the addition of polymerized povidone[6], 0.05% propyl gallate, and 0.005% (w/v) edetate disodium at pH 4. Maekawa and Egawa (264) achieved stability of an aqueous multivitamin preparation by the use of a partitioned container with vitamin A palmitate, niacinamide, and ascorbic acid in one chamber and thiamine in the other. The liquids contained stabilizing and flavoring agents and were admixed just prior to use. Youssef *et al.* (265) reported that the decomposition of vitamins and sodium metabisulfite is accelerated by storage of aqueous solutions in polyethylene containers.

Ointments, Creams, and Lotions—The utility of topically applied vitamins has been discussed by Siemers and Sleezer (266), DeRitter *et al.* (267), and Clement and Jones (268). Rubin (269) reviewed the percutaneous absorption of vitamins. Formulations of topical preparations containing panthenol were described by Rubin *et al.* (270), and the incorporation of various topically active vitamins into application forms was reviewed by DeRitter *et al.* (267). Anmo and Fueller (271) described a stable vitamin A ointment, and Haronikova and Mandak (272) found that the stability of vitamin A and cholecalciferol in an ointment was increased by adding a mixture of two antioxidants, nordihydroguaiaretic acid, butylated hydroxyanisole, and/or quercitin, or one antioxidant plus edetic acid or potassium pectinate. The composition of the ointment base influences the stability of vitamin A in the ointment (273, 274); the presence of polyethylene glycol and sodium lauryl sulfate in the base enhances the degradation of vitamin A. The decomposition of vitamin A palmitate in emulsion ointments is accelerated by lime water and lavindin (275), and the stability of vitamin A in cosmetic creams has been reported to be highly variable (276).

Syrups—Yashiki *et al.* (277) described a stable vitamin A palmitate syrup that prevented the sorption of vitamin A to plastic containers on storage. Bandelin and Tuschhoff (278) studied the stability of ascorbic acid in syrups at pH 3.0 and at concentrations of 25 and 100 mg/teaspoonful with two sucrose and one glycerin–sorbitol bases. Smaller losses of ascorbic acid were found with the glycerin–sorbitol combination, and the losses were relatively less at the higher concentration of ascorbic acid. The rate of loss of ascorbic acid in solution was determined (92) with ethanol, glycerin, propylene glycol, sorbitol, sucrose, corn sugar, or dextrose added and all yielded a stabilizing effect on ascorbic acid. Sucrose, sorbitol, glycerin, and propylene glycol were superior to the others in this respect. Vegetable gums added to such solutions to increase viscosity accelerated destruction of the vitamin. Vitamin B complex factors added to ascorbic acid syrups in USP syrup and in sorbitol appeared to increase the stability of ascorbic acid. The stability of ascorbic acid in glycerin and sorbitol bases containing salts and stabilizers was investigated by Agarwal and Agarwal (279). In solutions at pH 4 stored for 90 days at 47°, glycerin afforded better protection to ascorbic acid than sorbitol; the effect of stabilizers was less pronounced in the case of the glycerin base. Yao and Hsu (280) prepared multivitamin solutions in syrup, glucose, sorbitol, or sucrose solutions at pH 3.2, 4.5, and 7.0. In stability tests at 41.5 and 60° thiamine was more stable at pH 3.2 and ascorbic acid at pH 7.0. The use of antioxidants, including ascorbyl palmitate and butylated hydroxyanisole, and lecithin and an oxidized starch derivative, W14/S, was found by Fabrizzi *et al.* (281) to stabilize vitamin A, thiamine, and ascorbic acid in a syrup having low vitamin content. Ismaiel and Ismaiel (282) reported that thiamine, riboflavin, and ascorbic acid in syrups could be stabilized by replacing sucrose, vanillin, and various aldehyde-rich essential oils with sorbitol, sodium saccharin, sodium edetate, and essence of banana or apple.

Bartilucci *et al.* (283) studied the stability of cyanocobalamin in aqueous solutions of sorbitol, glycerin, dextrose, and sucrose stored at 25 and 45° for up to 140 days. Cyanocobalamin was compatible with sorbitol and glycerin, but dextrose and sucrose caused losses of cyanocobalamin with dextrose being worse. Gerber *et al.* (284) found that the instability of cyanocobalamin and ascorbic acid in solution with ferrous sulfate could be largely overcome by using 70% sorbitol as the vehicle.

Capsules—Campbell and McLeod (39) determined the vitamin contents of three multivitamin capsule products purchased on the retail market in Canada. Pantothenic acid appeared to be markedly affected by shelf life and storage conditions. In a shelf storage test of one product, pantothenic acid showed high losses and vitamin A, thiamine, and ascorbic acid small losses, while riboflavin, pyridoxine, and niacinamide were stable.

Stability tests of soft gelatin capsules in the laboratory revealed riboflavin, pyridoxine, vitamin E, ascorbic acid, biotin, and niacinamide to have excellent stability. Vitamin A and cholecalciferol showed reasonably good stability, while cyanocobalamin and folic acid tended to vary from product to product. Calcium pantothenate generally had poor stability at pH levels < 3.5, and panthenol was more stable than calcium pantothenate at < pH 4.5; > pH 4 both forms have good stability. Thiamine hydrochloride suffered high losses at pH > 4, whereas thiamine mononitrate showed fair to good stability up to pH 6.4. Macek *et al.* (4) also found thiamine mononitrate to be more stable than the hydrochloride salt in soft gelatin capsules as well as in multivitamin and vitamin B complex, dry-filled capsules. Acidification of the contents of the vitamin B complex capsules was necessary to stabilize the hydrochloride, but

[6] 15% Kollidon 17.

the mononitrate was stable without acidification, which was very destructive of calcium pantothenate. With thiamine mononitrate and a higher pH, it was possible to achieve good stability of both thiamine and calcium pantothenate.

PREDICTION OF VITAMIN STABILITY FROM ACCELERATED AGING TESTS

Garrett and Carper (285) proposed a procedure for predicting degradation of vitamins in liquid multivitamin preparations at normal shelf-life temperatures by measurement of degradation at several elevated temperatures and application of the Arrhenius equation. Garrett (286–288) applied this method in studies of complex multivitamin liquids and demonstrated the validity of the stability predictions for vitamin A, thiamine, ascorbic acid, dexpanthenol, folic acid, and cyanocobalamin. Since a slight change in vehicle composition does not significantly change the heat of activation or the rate of change of degradation with temperature, it was suggested by Garrett (287) that it may be practical in such cases to predict stability on the basis of one elevated temperature once the behavior of the system has been established at several elevated temperatures. The Arrhenius approach to stability prediction is limited according to Garrett (287) to degradation mechanisms that have heats of activation of sufficiently high magnitude (*i.e.*, in excess of 10 kcal/mole), but too high a heat of activation of components (sugars, *etc.*) that trigger a degradation of other components may give unreliable estimates of stability.

McLeod *et al.* (289) confirmed the validity of Garrett's method of predicting degradation rates of liquid multivitamin preparations at room temperature and described a simple graphical method of extrapolation to avoid the time-consuming mathematical calculations. Tardif (17) verified the applicability of the graphical method to the prediction of stability of a multivitamin tablet. Pelletier (290) found that storage tests at 50, 60, and 70° were suitable to predict the stability of thiamine and ascorbic acid in several multivitamin tablets and capsules and verified the predictions by analysis of samples stored at room temperature for 3 years. A short preliminary test at 70° was suggested for guidance in the selection of the most appropriate elevated temperatures, the equations used for the calculations were summarized, and a computer program for handling the calculations listed.

BIOAVAILABILITY TESTING OF VITAMIN PRODUCTS

Bioavailability of a vitamin may be defined as the relative amount of that vitamin contained in a dosage form, which enters the systemic circulation in an active form after administration as compared to the amount after administration of a comparable standard dose of the vitamin in fully available form such as a solution.

Various biochemical laboratory techniques have been employed in assessing bioavailability (291). The majority of these techniques fall into the following categories: (*a*) measurement of the nutrient level in the blood; (*b*) measurement of the urinary excretion rate of the nutrient; (*c*) measurement of urinary metabolites of the nutrient; (*d*) measurement of changes in blood components or enzyme activities that can be related to intakes of the nutrient; and (*e*) load, saturation, and isotopic tests. In the human population one is limited to urine and blood samples. Generally, one is restricted to measuring the concentration of a particular nutrient which is either excreted in the urine or circulating in the vascular system. Blood samples do permit the investigator a slightly greater latitude than urine, since the former can be partitioned into whole blood, serum, plasma, and/or red blood cells if a refinement of technique is justified. It is important that appropriate guidelines are available for the interpretation of the meaning of these measurements. A serum vitamin A level or a urinary value for riboflavin has little meaning until it can be compared with standard levels which have been measured experimentally under comparable conditions.

Measurement of absorption of vitamins *in vivo* requires specific and sensitive methods for determining the concentration of the vitamins or their metabolic products in human body fluids. For the fat-soluble vitamins A and E and cholecalciferol, measurement of blood levels provides a means of assessing absorption directly. For the water-soluble vitamins, except cyanocobalamin, urinary excretion of the vitamins or vitamin metabolites (indirect measurements) are most commonly used. Since normal human subjects consuming average diets without additional supplementation with vitamin products vary widely in the degree of saturation of their tissues with vitamins, relatively uniform excretion patterns can only be achieved by saturating the subjects with the vitamin under test before administering the test doses of the vitamin and measuring urinary excretions. For example, little or no urinary excretion of ascorbic acid is frequently observed following a 500-mg dose of ascorbic acid to subjects not previously saturated, whereas ~50% of such a dose is excreted in the urine of subjects after a state of saturation is reached.

Absorption and Excretion Patterns of the Vitamins—The patterns of absorption, metabolism, and excretion of the individual vitamins that serve as a basis for the test procedures are as follows:

Vitamin A—For evaluation studies in humans, blood level measurements are the only practical means of assessing the absorption of this nutrient. There are, however, many factors that influence the absorption of vitamin A, including type and amount of fat in the diet; interfering substances in diet such as nitrites, ethanol, absorbants, and drugs; size of dose; emulsion (rapid absorption) *versus* oil solution (slower absorption); vitamin E adequacy of subject; zinc adequacy of subject; state of protein nutrition of subject; respiratory, intestinal, renal, or parasitic disease in subject.

By the use of normal subjects in a cross-over design, control of diet, size of dose and the vitamin E level accompanying the dose, and measurement at specific time intervals after dosing to establish a tolerance curve, it is possible to utilize blood levels in humans for assessment of bioavailability of vitamin A products. Although adequate analytical methods are available for measuring blood levels of vitamin A, the difficulties in withdrawing repeated samples of blood under proper supervision make this type of testing unattractive, even when the facilities for conducting such tests are available. In addition, large numbers of subjects are needed if results within a small error range are desired.

Cholecalciferol—The metabolism of cholecalciferol in humans involves conversion to hydroxylated derivatives. Measurement of cholecalciferol or its metabolites in the blood is possible but has not been applied for bioavailability testing. Since cholecalciferol is normally present in a multivitamin product in the same physical form as vitamin A (*i.e.*, as an oil solution, an emulsion, or a dry stabilized beadlet or powder), evidence for bioavailability of vitamin A is likely to be valid for cholecalciferol as well in the majority of products.

Vitamin E—Bioavailability of vitamin E can also be evaluated by determining blood level curves after loading doses. Methodology for determining blood levels of tocopherol has been reviewed by Bunnell (292). The factors listed earlier which influence the absorption of vitamin A may also affect absorption of vitamin E. In addition, the differing biopotencies of the *d*- and *dl*-forms of tocopherol, the differences in absorption between tocopherol, tocopheryl acetate, tocopheryl succinate, and tocopheryl polyethylene glycol 1000 succinate (293) and the possible need to measure specific tocopherols in blood are factors which must be considered in interpreting blood level data. The practical difficulties in conducting blood level studies, as noted under *Vitamin A*, apply also to such tests of vitamin E in blood.

Vitamin K—Increases in blood levels of vitamin K after loading doses have not been used as a measure of bioavailability of dosage forms since most supplemental vitamin K is given parenterally. Oral doses in the form of an oil solution or emulsion would not be expected to present significant problems with bioavailability. Water-dispersible beadlets containing phytonadione have been demonstrated to be biologically available *via* animal tests and are used as a reference standard for animal bioassays (294). Hence, if these are used in solid dosage forms, a rapid *in vitro* disintegration would be indicative of adequate bioavailability.

Thiamine—Of the various biochemical procedures which have been developed for assessing thiamine absorption, the most common one is the measurement of urinary levels. At levels in the range of the daily requirement, thiamine is absorbed fairly well. Thomson (295) reported that 54% of the 1-mg dose of ^{35}S-labeled thiamine hydrochloride given orally after an overnight fast was found in the urine within 24 hr by radioactivity measurements. Continuing the urine collection in one subject for 9 days gave only an additional 7.5% of the dose in the standard test. It appears that the absorption of thiamine in humans is mainly in the upper small intestine. Melnick *et al.* (296) and Jowett (297) also concluded that the absorption of thiamine seems to be confined largely to the upper intestinal tract.

As the oral dose of thiamine is increased (298, 299), the percentage absorption decreases progressively although the total amount absorbed increases. With a single oral dose of 5 mg given on successive days, Schultz *et al.* (300) reported recoveries in urine of 24–42% of the dose and levels of thiamine in the stools were increased. On giving 10-mg doses, only 19–21% was recovered in the urine. The percentage of a thiamine test dose excreted is increased slightly when the dose is given with food (298).

Riboflavin—Riboflavin is absorbed efficiently by humans in any practical dosage range. Friedemann *et al.* (298) state that at least several hundred milligrams of riboflavin is absorbed readily. Human urinary excretion studies by Morrison and Campbell (299) showed that the total extra excretion of riboflavin averaged close to 60% of the oral dose over the dosage range of 1–20 mg. The studies have shown also that 50% or more of an oral dose of riboflavin up to 30 mg is excreted in the urine within 24 hr when the dose was given after breakfast. Levy and Hewitt (301) obtained only 19.3% excretion of a dose of 41 mg of riboflavin phosphate given on an empty stomach as compared to 52.6% when the same dose was given after breakfast.

Niacin or Niacinamide—Hundreds of milligrams of niacin or niacinamide are absorbed readily after oral dosage. Approximately three-fourths of such large doses can be accounted for in the urine of human subjects within 72 hr as a combination of niacin, niacinamide, trigonelline, N^1-methylnicotinamide, and N^1-methyl-2-pyridone-5-carboxamide. The latter is the main metabolite appearing in the urine. If the rate of release of niacin is retarded, the metabolism proceeds to a somewhat greater extent to the end product, the pyridone.

Pyridoxine—Pyridoxine is absorbed readily from the intestinal tract by human subjects. Efficient absorption of several hundred milligrams is normal. Only a small fraction of ingested pyridoxine is excreted unchanged in the urine (only 4–8% of doses in the range of 8–100 mg). In humans, 4-pyridoxic acid accounts for 70–90% of the urinary excretion products of pyridoxine (302).

Calcium Pantothenate or Panthenol—Both pantothenic acid (or its calcium salt) and panthenol are absorbed readily from the intestinal tract. At RDA dose levels, only a small percentage of the dose is excreted in the urine as pantothenic acid but the excretion of the vitamin *per se* increases to ~16% after a 100-mg oral dose and to 36% of a 250-mg dose (303).

Biotin—One milligram of biotin was administered orally to a human male subject on each of two successive days. Urinary excretion of biotin, measured microbiologically with *Lactobacillus arabinosus* as the test organism, amounted to 20 and 48% of the dose in the 24-hr periods after the two doses (304). Although bioavailability of biotin in vitamin products has not been reported, urinary excretion measurements of biotin after oral dosage to saturated subjects may represent a possible approach to this problem.

Folic Acid—By saturating human subjects with high doses of folic acid (10 mg on the first day, 5 mg on the second day, 2 mg on the third and fourth days, and every second day thereafter while excretion tests are in progress), it is possible to obtain urinary excretion of a reasonable percentage of a normal test dose (305). In the author's trials, this technique yielded 5–13% excretion of an oral dose of 0.65 mg of folic acid in a group of five subjects.

Cyanocobalamin—Cyanocobalamin is absorbed maximally in the middle segment of the small intestine and is poorly absorbed in the distal segments (306–308). The size of the dose significantly influences the percent absorption (309). Conley *et al.* (310) reported that oral doses up to 10 mg of cyanocobalamin resulted in no detectable increase of the vitamin in urine within 48 hr. Normal subjects and patients with pernicious anemia behaved similarly. Thus, no bioavailability test for cyanocobalamin can be based on

urinary excretion measurements. Adams *et al.* (311) state that cyanocobalamin absorption can only be studied by using radioactive cyanocobalamin. These authors used a whole-body counting technique in their absorption studies on human subjects. Such techniques are beyond the capability of many laboratories. It is evident that there is no simple method of assessing the bioavailability of cyanocobalamin in pharmaceutical products which would permit testing of bioavailability.

Ascorbic Acid—Ascorbic acid is absorbed efficiently from the upper part of the small intestine. Nicholson and Chornock (312), using an intubation technique, found that 50% of an oral dose of 600 mg of ascorbic acid given in 600 ml of water or saline was absorbed in 1 hr. Saturation of the subjects by giving 500 mg of ascorbic acid daily in addition to the regular diet had no significant effect on their absorption of ascorbic acid. The authors state that the upper part of the small intestine has a capacity for absorption of ascorbic acid far in excess of the optimal daily requirement. Kübler and Gehler (313) studied blood serum levels and rates of urinary excretion of ascorbic acid in previously saturated subjects and concluded that the rate of absorption in the distal part of the absorbing intestine was about half the rate in the proximal portion. These authors found that with increasing doses of ascorbic acid the percent absorption decreases. They reported 71.5% absorption of a dose of 180 mg, 49.5% for 1.5 g, and 16.1% for 12 g. In the dose range of 1.5–12 g, the mean urinary excretion was 62 ± 3.7% of the absorbed ascorbic acid. Proportionality between ingestion and intestinal absorption was claimed up to a single dose of ~180 mg.

Stewart and Booth (314) gave one subject single doses of ascorbic acid at 4-day intervals of 0.2, 0.4, 0.5, 0.7, 1.0, 1.5, 2, and 3 g. Total urinary excretion in up to 30 hr rose steeply up to the 0.5-g dose where ~50% was excreted, and then much less steeply with increasing doses, with an excretion of ~16.7% of the 3-g dose. At the lowest dosage in the range (200 mg) there is practically no excretion of the ascorbic acid dose in the feces. Chieffi and Kirk (315) reported fecal excretion averaging 1.2 mg of ascorbic acid daily by 13 elderly men on an ordinary diet. With a 200-mg supplement of ascorbic acid in the form of a tablet, the fecal excretion averaged 1.5 mg/day, indicating no significant excretion of the supplement in the feces.

Absorption Tests—*Fat-Soluble Vitamins (Blood Level Tolerance Test): Vitamin A*—The application of the blood level tolerance test to determination of the biological availability of vitamin A in three different multivitamin capsules was reported by Sobel and Rosenberg (316). Either 1000 or 2000 IU of vitamin/kg of body weight was administered to eight normal adults in the postabsorptive state. Blood specimens were taken prior to the test and 3, 6, 9, and 24 hr posttreatment. The capsules were swallowed without chewing. As a reference of absorption, oleovitamins A and D was used. Intervals betweeen doses were 2–7 days. If the response as serum vitamin A for the oil dose is taken as 100%, the three capsules showed comparative responses of 198, 185, and 70% based on the mean maximal rise and ~150, 170, and 50% based on the area within the tolerance curves for the 24-hr period.

The authors mention three factors that influence the physiological availability of fat-soluble vitamins: (*a*) the site at which the capsule releases its contents, (*b*) the stability of vitamin A in the GI contents, (*c*) the state of dispersion of the vitamin A released to the GI content (finely dispersed particles are absorbed more readily). The study of Sobel and Rosenberg illustrates some of the difficulties in assessing bioavailability in humans by measuring blood levels of a fat-soluble vitamin such as vitamin A. Such tests have not been utilized to any great extent for determining bioavailability of pharmaceutical products.

Vitamin E—Increases in blood plasma levels of tocopherol in response to oral doses have been reported. For example, Filer *et al.* (317) gave premature and full-term infants oral doses of α-tocopherol and α-tocopheryl esters of 20 mg/kg of body weight and measured blood level increases after 3, 6, 9, 12, and 24 hr. No significant differences in plasma tolerance curves were found between tocopherol and its esters in healthy infants. In disease states diagnosed as fibrocystic disease of the pancreas, diarrhea, and cirrhosis, low vitamin E tolerance curves were observed. Metabolic disorders associated with hypercholesterolemia gave abnormally high values for the areas under the tolerance curve.

Week *et al.* (318) gave oral doses of 1000 mg of *dl*-α-tocopherol or an equivalent amount of *dl*-α-tocopheryl acetate in corn oil in capsules to 10 male and 10 female adult subjects. Blood levels of tocopherol were measured at 0, 2, 4, 5, 6, 8, 12, and 24 hr. The average area under the tolerance curve was 35% higher for free tocopherol than for the acetate ester.

Overman *et al.* (319) found higher blood levels after oral doses of *dl*-α-tocopheryl acetate to humans in the form of aqueous emulsion than after a capsule dosage. They concluded that the amount of vitamin E given and the vehicle are important in determining the effectiveness of vitamin E preparations in increasing the plasma levels of free tocopherol.

Water-Soluble Vitamins (Urinary Excretion Tests)—The human bioassay technique for determining bioavailability of water-soluble vitamins was introduced by Melnick *et al.* in 1945 (320, 321). The principle of this method was summarized as follows: "In normal human subjects the urinary excretion of the water-soluble vitamins, as such or as their derivatives, is directly proportionial to the quantity consumed, provided that at the time of the tests the subjects are subsisting on an adequate diet. The linear dose–response relationship established by feeding the vitamins in pure solution, *i.e.*, in their most completely available form, constitutes the basis of the bioassay."

Outline of the Testing Program—A group of at least five nutritionally normal subjects is chosen. If possible, the subjects should keep their diet the same on two consecutive urine collection days and avoid vitamin supplements and foods unusually high in vitamins such as liver. Urine is collected over a 24-hr period for each of these 2 days. Tuesday and Wednesday are the days of choice for conducting the experiment. Thus, urine collections are made from Tuesday morning after the first voiding and continued until after the first voiding the following morning and repeated the second day similarly. The two 24-hr samples are collected in amber bottles containing a total of 20 ml of 3.5 *N* H_2SO_4 or 2 g of thymol and kept in the refrigerator whenever feasible. On the first day the subjects ingest nothing but their usual diet; the urinary

excretion for this period represents the basal urinary excretion. At the beginning of the second day the subjects take either a standard control dose or a test dose which is reversed to each subject the following week. The control dose contains known amounts of the pure vitamins on test in aqueous solution or suspension. By subtracting the basal excretion from the excretion after the control or test dose, the extra urinary excretion due to dose is determined. The results for each subject are expressed in terms of percent of dose. These tests can be run weekly. The control dose and the test dose should contain similar concentrations of the vitamins.

Saturation Dosage—To ensure maximum and uniform urinary excretion of the extra vitamins fed, it is necessary to saturate the subjects with the vitamins under test. Since the excretion of an individual vitamin is relatively independent of the other vitamins, a high potency multiple vitamin preparation can be used, provided it contains at least the following quantities of the vitamins under test: thiamine (5 mg); riboflavin (5 mg); ascorbic acid (500 mg); calcium pantothenate (50 mg); niacinamide (50 mg); pyridoxine (5 mg). The saturating dose is taken for 4 days and 1 day without this dosage is allowed before the day on which the basal urine is collected. (The saturating dosage schedule for folic acid was mentioned previously.)

Control and Test Doses—The standard control dose of pure vitamins is given in water. The amount of the control dose of each vitamin should be as nearly equal as possible to the amount of each vitamin in the pharmaceutical product to be tested. Following are suggested dosing ranges of the vitamins for use in bioavailability tests:

Thiamine	5–15 mg
Riboflavin	5–30 mg
Niacinamide	50–100 mg
Ascorbic acid	500–1000 mg
Calcium pantothenate or panthenol	25–100 mg
Pyridoxine	5–15 mg
Folic acid	0.5–1 mg

Urine Assay Methods—The following methods may be used for measuring excretions of the vitamins or metabolites:

Thiamine	—fluorometric assay by the thiochrome method of Mawson and Thompson (322)
Riboflavin	—direct dilution with pH 6 acetate buffer in final solution for fluorometric measurement
Niacinamide	—assay of the metabolite, N^1-methylnicotinamide, by the method of Pelletier and Campbell (323)
Calcium pantothenate	—microbiologically with *L. plantarum* as test organism and the medium of Skeggs and Wright (324)
Pyridoxine	—assay of the metabolite, 4-pyridoxic acid, by the method of Pearson (325)
Folic acid	—microbiologically with *Streptococcus faecalis* as test organism and the medium of Rabinowitz and Snell (326) modified by the omission of folic acid and the addition of 2 μg of pyridoxamine/tube
Ascorbic acid	—colorimetrically, using the dichlorophenolindophenol–xylene technique of Rubin *et al.* (327)

Basal and Extra Excretions—In Table VI the average basal excretions and average extra urinary excretions for saturated subjects receiving the above doses are presented.

Availability of a Test Dose—The physiological availability of a test preparation is ascertained by the following calculation:

$$\frac{\text{Percent recovery of test dose}}{\text{Percent recovery of control dose}} \times 100 = \text{Percent availability}$$

Due to the variability of physiological responses in different subjects and in the same subject from time to time, the analytical errors, and the fact that availability is measured as the ratio of two recoveries, which in themselves are the difference between two excretion values, an error of ±20% is not thought excessive. Therefore, a value ≥80% is considered indicative of satisfactory availability. Oser *et al.* (321) present data obtained by this technique on various multivitamin tablets and discuss some factors that might lead to low availabilities.

Rate of Excretion of Dose—According to Johnson *et al.* (328) urinary excretion of oral vitamin doses dissolved in water is practically complete in 8–10 hr. Results obtained in the author's laboratory using the sodium salt of riboflavin phosphate as the test material indicate that this is essentially correct. In these studies a dose of the phosphate, equivalent to 30 mg of riboflavin, was dissolved in water and taken by the test subjects. The hourly excretion (as percent of dose) is summarized below.

Hours after Dose	Average Excretion of Dose, %
0–1	6.5
1–2	12.9
2–3	9.4
3–4	6.0
4–5	3.8
5–6	2.8
6–7	2.2
7–8	1.3
Total percentage of dose excreted in 8 hr	44.9

From the data, one would not expect much excretion beyond a 10-hr collection period. Excretion data after oral dosage of thiamine, ascorbic acid, and niacinamide show that the bulk of the urinary output occurs in the first 12 hr postdose.

However, when the dose is taken in capsule or tablet form, significant excretion may occur in the 12–24-hr period postdose. Therefore, as a standard procedure, it is advisable to run both test and control collections for 24 hr.

Correlation of Excretion Tests with In Vitro *Disintegration and Dissolution Tests*—There is considerable evidence that the pharmaceutical form has a marked influence on the availability of vitamins to the body. Chap-

Table VI—Average Basal Urinary Excretions and Average Extra Excretions of Vitamins or Metabolites After Control Doses

Vitamin	Vitamin or Metabolite Measured	Basal Excretion (on normal diets), mg	Approximate Extra Excretion Due to Control Dose[a]
			Percent of dose
Riboflavin	Riboflavin	0.5–2.5	45–70
Thiamine	Thiamine	0.3–0.5	5–20[b]
Ascorbic acid	Ascorbic acid	30–60	40–60
Calcium pantothenate	Pantothenic acid	4–9[c]	10–20
Panthenol	Pantothenic acid	4–9[c]	10–20
Niacinamide	N^1-Methylnicotinamide[d]	6–12	10–20
Pyridoxine	4-Pyridoxic acid[e]	0.6–1.2	10–15
Folic acid	Folic acid	0.003–0.006	10–15

[a] If the test product is completely available, the percent excretion of the test dose will be equivalent within experimental limits to the percent excretion of the control dose given in aqueous solution. [b] In the case of thiamine, the percent of dose excreted diminishes markedly as the dose is increased; in the case of a 30-mg dose, the excretion may be only ~2–5% of the dose. [c] Expressed in terms of pantothenic acid. [d] When N^1-methylnicotinamide hydrochloride is used as the standard, multiplication of the urinary results by 0.707 converts them to the equivalent niacinamide levels. [e] To convert milligrams of 4-pyridoxic acid to pyridoxine hydrochloride, multiply by 1.123. At higher doses the percent of dose excreted as 4-pyridoxic acid will be higher.

man *et al.* (252) found by a specified *in vitro* test that vitamin tablets that did not disintegrate in 1 hr were not fully available to the body, as judged by the amount of riboflavin excreted in the urine. This was confirmed by Morrison *et al.* (253) and has been the basis for a regulation promulgated under the Canadian Food and Drugs Act setting a 1-hr time limit for the disintegration of ordinary tablets. Middleton *et al.* (329) examined the relationships between *in vitro* dissolution rate, disintegration time, and physiological availability of riboflavin in sugar-coated tablets. They found a close relationship between disintegration time and dissolution rate, and both *in vitro* procedures correlated reasonably well with physiological availablity as measured by urinary excretion of riboflavin. It was concluded that either of these two *in vitro* procedures can provide a useful estimate of the availability to the body of riboflavin in sugar-coated tablets.

Libby *et al.* (330) studied riboflavin excretion by human subjects ingesting sustained-release capsules or tablets which did not pass the official USP disintegration test. Urinary recoveries of riboflavin from these preparations were extremely low in comparison to those obtained with an equal dose of riboflavin standard. These authors also showed reduced urinary excretion of riboflavin when six tablets containing calcium, ascorbic acid and cholecalciferol were fed together with fully available riboflavin tablets. Ida *et al.* (331) reported a lower rate of excretion in the first 6 hr after dose of compressed film-coated tablets, even though these disintegrated in 4 min in water and in 5–10 min in simulated gastric juice by the USP XVI method. These workers, however, did not measure excretions in later hours, which may have indicated complete absorption of the riboflavin over a longer period.

Evaluation of the absorption of most of the vitamins in a multivitamin preparation is a very time-consuming and expensive operation, involving skilled staff and analytical methodology for vitamin metabolites which is not normally used in vitamin assay laboratories. Emphasis in various studies of vitamin absorption has been placed on the excretion of riboflavin, primarily because of its relative insolubility compared with most other water-soluble vitamins. With the exception of biotin, the solubility of which is close to that of riboflavin in water, and folic acid, the solubility of which is strongly dependent on pH, all other vitamins are many times more soluble than riboflavin. Thus, if a tablet granulation is made with a uniform mixture of the water-soluble vitamins and water-dispersible beadlets or powders containing the fat-soluble vitamins (a usual procedure), the finding in a urinary excretion test that the riboflavin from the finished tablet is fully available to the body makes it evident that the tablet has disintegrated suitably. Since the riboflavin has then obviously dissolved in order to have been absorbed in the upper part of the intestinal tract, it is most likely that the more soluble vitamins and the readily dispersible powders have also been readily available for absorption. If, on the other hand, any of the other individual vitamins are coated or added to the tablet mix in a different manner for a special solid dosage form than the riboflavin component, then it becomes desirable to establish the correlation of disintegration and/or dissolution tests with an *in vivo* absorption test for such vitamins. Once a relationship of *in vivo* tests to *in vitro* tests has been established, the *in vitro* tests should be an adequate means of control for ensuring suitable absorption characteristics of solid pharmaceutical dosage forms in routine pharmaceutical manufacturing operations.

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RESEARCH ARTICLES

Solid-State Stability of Aspirin in the Presence of Excipients: Kinetic Interpretation, Modeling, and Prediction

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Abstract □ Salicylsalicylic acid and acetylsalicylsalicylic acid were identified as decomposition products of aspirin when mixtures of the drug, with magnesium stearate, were stored in the solid state at 60° and 75% relative humidity. The effect of increasing the concentration of magnesium stearate and the addition of other alkali stearates on the rate of decomposition of aspirin were studied. The validity of the theory that pH changes induced by the alkali stearates account for the catalytic effect of the lubricants on the decomposition was tested. The changes observed were modeled and the mechanism involved elucidated. The potential use of the melting points of aspirin mixtures in predicting the stability of the drug in such drug–excipient mixtures is demonstrated.

Keyphrases □ Aspirin—solid-state stability in presence of excipients, kinetic interpretation, modeling and prediction, decomposition □ Decomposition—solid-state stability, aspirin, excipients, kinetic interpretation, modeling and prediction □ Stability—solid state, aspirin, in presence of excipients, kinetic interpretation, modeling and prediction

The mechanisms of decomposition of drugs in the solid state are complex and difficult to unravel (1–3). The problems are compounded by the fact that most drugs are formulated with excipients, and decomposition in such systems is even more complicated. This, together with the usually slow rates of decomposition in the solid state relative to solutions, may explain the comparatively small number of reports on the quantitation of decomposition of drugs in formulated solid-dosage forms. Many of the reports that have appeared have tended to be semiquantitative, although a few detailed studies have been reported (4–9). To overcome the time constraints, some workers have resorted to the prediction of the solid-state stability of hydrolabile drugs by studying their decomposition in suspension systems. Kornblum and Zoglio (10) for example attempted to predict the stability of aspirin in the presence of tablet lubricants in the solid state by this approach. Although the method described is attractive, the mechanisms of decomposition in solid dosage systems may be different from those observed in systems containing a higher proportion of water.

More recent studies have shown that in addition to

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