A STUDY OF VITAMIN B_6 IN TOTAL PARENTERAL NUTRITION

N M BARNES

MASTER OF PHILOSOPHY

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Summary

Two novel reverse phase high performance liquid chromatography methods for the analysis of pyridoxine have been developed, capable of detecting pyridoxine below concentrations of 500 microgram/L. One assay involves a mobile phase of pH 2.5 with the detection of pyridoxine by UV at 291nm. It is used for measuring pyridoxine in Total Parenteral Nutrition (TPN) mixtures containing Vamin 9 and Vamin 9 glucose. The second assay has a mobile phase of pH 6.75 with UV detection at 324 nm, and is used for measuring pyridoxine concentration in all TPN solutions except those containing Vamin 9 or Vamin 9 glucose. Both assays show a linear response to concentration with corellation coefficients of 0.99 for both assays, and coefficients of variation of 2.6% for the first assay and 2.4% for the second assay.

The stability of pyridoxine was followed in various constituent TPN solutions and in complete TPN regimen solutions. Pyridoxine was shown to be stable in all constituent solutions, in the absence of water soluble vitamins, in the light and dark, except in Intralipid solutions. In solutions containing water soluble vitamins (Solivito), pyridoxine was stable in the dark but rapidly degraded in the light in dextrose 10% and saline 0.9% infusion solutions. The addition of trace element solution (Addamel) statistically significantly stabilised pyridoxine to the effect of light ($P < 0.001$ by student's t-test). In amino acid solutions, pyridoxine degraded in the light in solutions of low amino acid content (Vamin 9 Glucose, Aminoplex 12) but was statistically significantly more stable in solutions of high amino acid content (Aminoplex 24, Vamin 18) ($P<0.001$) by student's t-test. In Intralipid solutions, pyridoxine showed a slow degradation in both the light and the dark. This was statistically significantly increased on dilution ($P < 0.001$ by student's t-test) and in diffused sunlight ($P < 0.001$ by students t-test). In complete TPN regimen solutions, pyridoxine was statistically significantly more stable in the light than in diffused sunlight ($P < 0.001$ by student's t-test). Pyridoxine was stable for seven days when the TPN solution was protected from light by an opaque outer cover, and when stored in a refrigerator.

The stability of pyridoxine was followed in solutions containing riboflavin and found to degrade. The rate of degradation was dependent on riboflavin concentration.

The majority of patients referred for TPN were found to have serum pyridoxal-5-phosphate (PLP) concentrations below the stated reference range (20-80 nmol/L) before starting TPN. After one week of receiving 2mg pyridoxine daily via continuous TPN infusion, the majority of patients had serum PLP concentrations in the lower half of the normal range. It is, therefore, suggested that patients receive a dose of 4mg daily or higher or a 'stat' dose of 10-20mg pyridoxine hydrochloride at the start of TPN, or both.

KEY WORDS: Vitamin B_6, Pyridoxine, Parenteral Hyperalimentation, Pyridoxine Stability, High Performance Liquid Chromatography.

ACKNOWLEDGEMENTS

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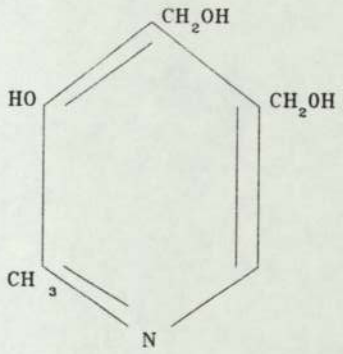
CHAPTER ONE

INTRODUCTION

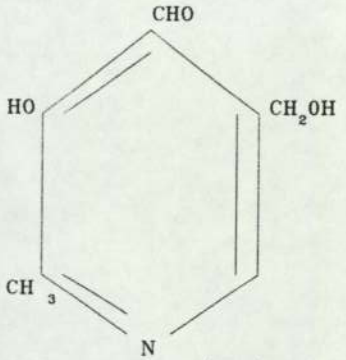
Vitamin B_6 was first recognised in 1934 when it was shown to prevent acrodynia in rats. It was later shown in the 1940's , that several compounds possessed Vitamin B_6 activity - these being Pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM). (1). Most of the Vitamin B_6 present in plant and animal materials is present as the phosphorylated derivatives of these three compounds. Thus there are six biologically active forms of Vitamin B_6 - see Fig 1.1, each of these being termed a vitamer.

All six B_6 vitamers show similar activity in supporting bacterial and animal growth and are interconvertible as shown in fig 1.2. However, Pyridoxal-5-phosphate (PLP) and Pyridoxamine-5-Phosphate are the only vitamers showing coenzyme activity. all other vitamers need to be converted to one of these coenzymes before utilisation (2)

4-pyridoxic acid (4-PA) cannot be considered a vitamer as it is not convertible to PLP or PMP and can, therefore, have no biological activity. It is the main form in which vitamin B_6 is excreted from the body. Vitamin B_6 is required for many metabolic processes within the body, though it is principally involved with amino acid metabolism, for example it acts as a coenzyme with transaminases whereby an amino group is transferred from one amino acid to an oxo-acid to form a new amino acid. This reaction occurs in two distinct steps. The first is the transfer of the amino group on the amino acid to PLP (which is bound to the transaminase enzyme) to form PMP. The second is the transfer of the amino group from PMP to the oxo acid to form the new amino acid, regenerating PLP - see fig 1.3. With the exception of lysine, all amino acids undergo reversible transamination, this forms the basis of all amino acid metabolism, being a preliminary step leading to more complete catabolism. It is also an essential step in the biosynthesis of non-essential amino acids from other metabolites.



Pyridoxine (PN)



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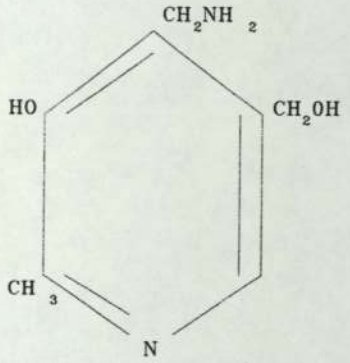
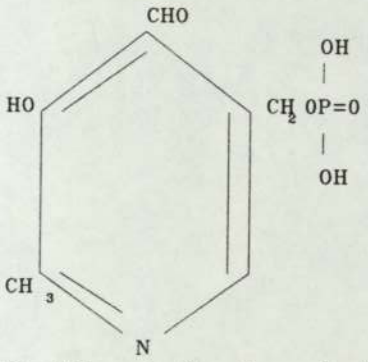
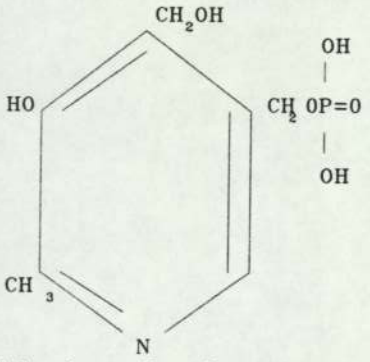


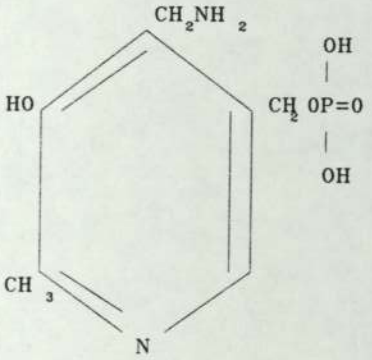
Pyridoxamine (PM)



Pyridoxal-5-phosphate
(PLP)



Pyridoxine-5-phosphate
(PNP)



Pyridoxamine-5-phosphate
(PMP)

Figure 1.1 The B6 Vitamers

Figure 1.2 - The Interconversion of Vitamin B_6

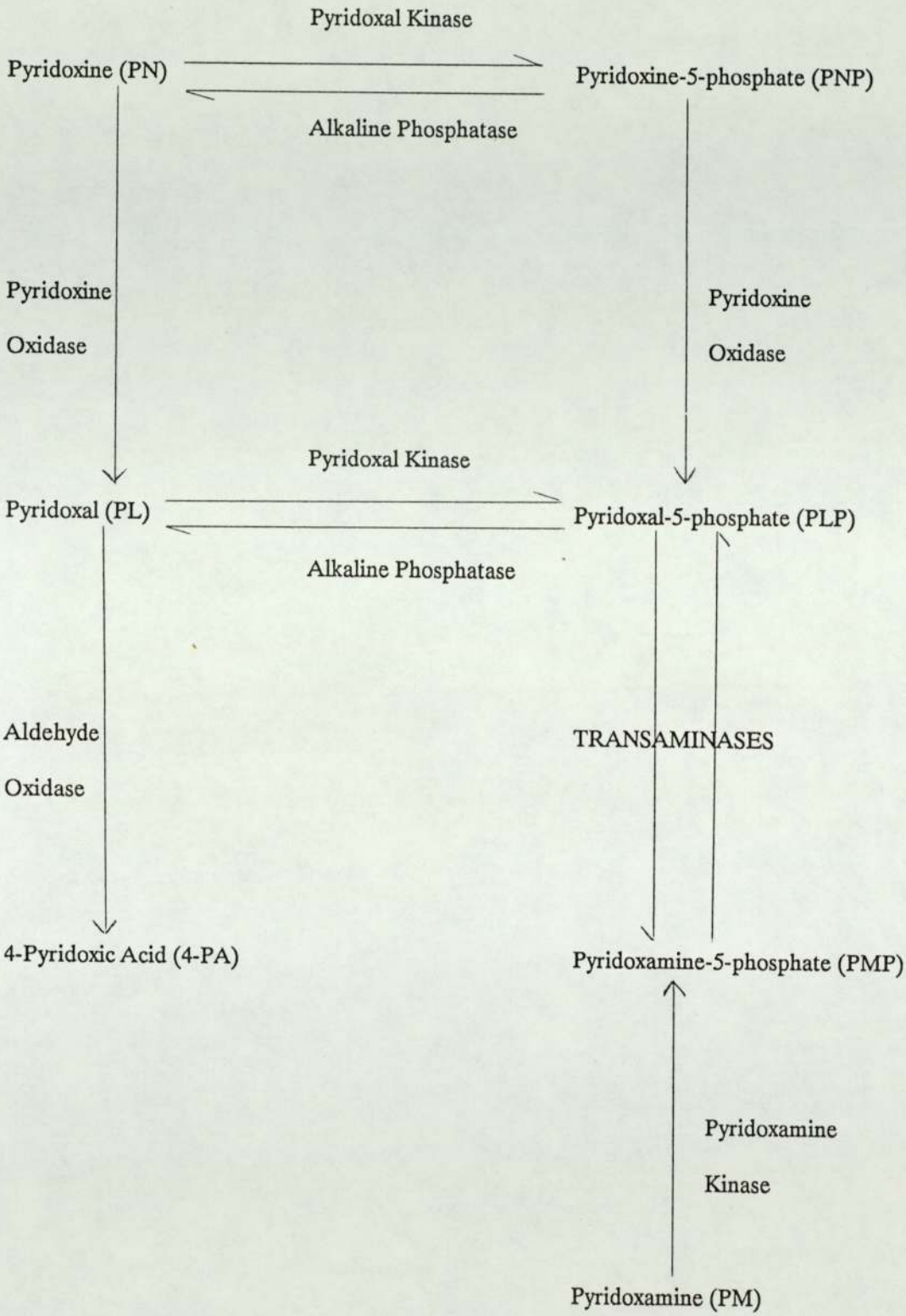
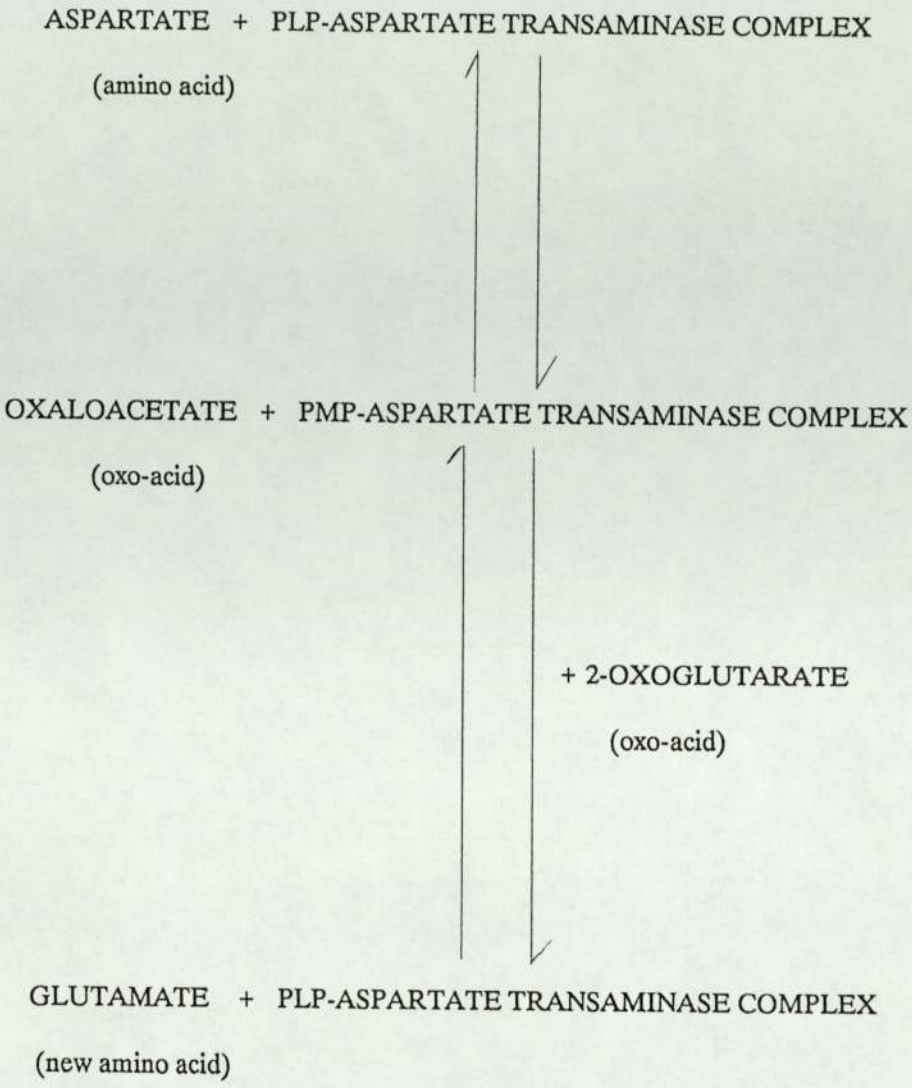


Figure 1.3 The Role of PLP in Enzymatic Transamination



Other enzymes involved in amino acid metabolism and requiring PLP to function are the amino acid decarboxylases - these are involved in some of the syntheses of neurotransmitters eg catecholamines, gamma-aminobutyric acid. There are also deaminases which split amino groups from amino acids to form oxo acids, with PMP being formed from PLP. PLP is also involved in sulphur amino acid metabolism (3) ie methionine and cysteine. Enzymes involved include cystathionine synthetase and gamma-cystathioninease. In vitamin B_6 deficiency, these enzymes show reduced activity, which affects some structural proteins involving disulphide bridges.

There are some enzymes not involved in amino acid metabolism which also require PLP to function. Glycogen Phosphorylase binds PLP (4). However, it appears to play a role in retaining the quaternary structure of the enzyme rather than exhibiting a coenzyme function. PLP is also required in the synthesis of porphyrins, eg in the synthesis of delta-amino-laevolinic acid, an initial stage in the formation of haem.

Of the two coenzyme forms of the vitamin, PLP is the most abundant (5). It is tightly bound to protein via a Schiff's base, eg in plasma it is bound to albumin (6). This binding retards the transport of PLP into cells and also retards it degradation by alkaline phosphatase.

On injection, PLP is rapidly cleared from plasma (5). As very little PLP is excreted in the urine, its clearance must be explained by uptake into tissues, directly or indirectly; or by degradation. As it is almost entirely bound to plasma albumin, it is protected from degradation. Evidence for this is given by Lumeng et al (6). Uptake by cells may be direct - cells must possess proteins of higher affinity for PLP than albumin and 'capture' PLP from plasma albumin. Cells may also be capable of assimilating albumin and, therefore, PLP. This mechanism alone may account for 30-40% of PLP clearance. Uptake by cells may also be indirect, hydrolysis to PL which is then transported across the cell membrane followed by rephosphorylation to PLP.

Vitamin B_6 is ingested in all six forms in diet, most of this being converted to PLP. This chiefly takes place in the liver. Conversion of PN to PL has been shown to occur in erythrocytes, PL then being returned to serum. Most of the vitamin B_6 found in plasma is in the form of PL or PLP along with the metabolite 4-PA. (7). However only the liver is capable of converting the B_6 vitamers into PLP or 4-PA, the inactive metabolite. This has been shown by various organectomies (6). Nephrectomy, splenectomy or resection of the gut do not significantly alter the rise of PLP folowing the injection of PN or PL. However, following hepatectomy, plasma PLP fails to rise on injection of these two vitamers, indicating that the liver is the principal site of conversion of these B_6 vitamers to PLP

The clearance of plasma PLP is dependent on the vitamin status of the individual (5). the higher the vitamin status, the slower the clearance of PLP from plasma. This is due to decreased uptake of PLP by body tissues. Thus the greater the stores of vitamin B_6 in the individual, the greater the plasma concentration.

Plasma PLP concentration has been shown to be proportional to the muscle content of PLP and it is said to be indicative of vitamin B_6 nutritional status (8). Plasma PLP concentration rises steadily with increased uptake of vitamin B_6 (9) and will, therefore, reflect the amount of vitamin B_6 in the body and, thus, undernutrition of the vitamin.

Excretion of vitamin B_6 occurs following metabolism of PLP to 4-PA in the liver, 4-PA rises in proportion to the intake of vitamin B_6 (10). The urine has been shown to be the main route of excretion, biliary excretion accounting for less than 3% of the total amount excreted (11).

Vitamin B_6 Deficiency And Requirements

Vitamin B_6 is ubiquitous in both plant and animal foodstuffs, and thus deficiency is rare, (2) especially as the body has large stores of the vitamin. The normal intestinal flora provide an extra source of the vitamin (12).

The most commonly cited result of vitamin B_6 deficiency is convulsions (1,2), for example in young children fed on milk substitutes deficient in vitamin B_6. This may be due to changes in neurotransmitters in the brain. Glutamic acid decarboxylase requires PLP as a coenzyme and is necessary in the synthesis of gamma-aminobutyric acid (GABA) a neurotransmitter raising the seizure threshold in animal models.

Peripheral nervous system changes have also been described, eg. peripheral neuritis. This may often be seen after administration of isoniazid which inactivates PLP. It hs been shown to be alleviated by administration of pyridoxine concurrent with that of isoniazid.

Sideroblastic anaemia is another manifestation of B_6 deficiency due to decreased porphyrin synthesis. This may be overlooked because of the rarity of vitamin B_6 deficiency.

Other deficiency syndromes have been seen in animals, but not yet demonstrated in man. Gyorgy (13) found that vitamin B_6 would cure the signs of acrodynia (14), a dermatitis affecting the paws, ears and nose in rats. Abnormal lipid metabolism has also been shown in vitamin B_6 deficient rats, these rats show increased plasma cholesterol levels and a reduced total body fat. These imbalances can be corrected by administration of vitamin B_6.

Oxaluria has been seen in cats and rats on B_6 free diets. This is due to the production of excess glycine which does not enter the transaminase pathways and is metabolised to oxalate. However, methods used to induce deficiency in animals are unlikely to be paralleled in man because of the ubiquity of the vitamin in foods.

Human requirements for vitamin B_6 are the subject of much controversy. The United Nations Food and Agriculture Organisation tables of Recommended Daily Intake and the DHSS tables do not list the vitamin (2). Nevertheless, there is no known metabolic pathway for its synthesis in man and it is intimately involved in amino acid metabolism. Thus it is an obvious dietary essential. The National Academy of Sciences of the United States reviews the literature on vitamin B_6 requirements and currently recommends a daily intake of 2.2mg for young adult males and 2.0mg for young adult females (1).

However, requirements vary with age and situation. Requirements are dependent on the amount of protein ingested (14). Patients receiving Parenteral Nutrition may also require vitamin supplementation. The more protein ingested in the diet, then the greater the metabolism of amino acids in the liver, enzymes being induced by a high protein diet. Requirements for PLP must come from the diet otherwise body stores for PLP will be depleted, which will lower the concentration of plasma PLP. A study (14) investigating the effect of dietary protein on the metabolism of vitamin B_6 showed that 4-PA excretion decreased with increased protein intake as did plasma PLP concentration, suggesting that requirements are met both from the diet and from body stores (14).

Paradoxically, at an inadequate level of protein intake, vitamin B_6 requirements may be increased. Rats fed very low protein diets (5% dietary protein content) had a plasma PLP concentration lower than rats fed high protein diets (20% or

50% dietary protein content). Diets of low quality protein may also affect the requirement for vitamin B_6 (15). In both cases, one would expect increased transamination in the liver to correct for deficiencies in certain amino acids.

Although guidelines for the maintenance of adequate vitamin status for the general population are readily available, there is only a limited amount of information regarding the requirements for parenteral vitamins. This is in contrast to other nutrients such as water, carbohydrates and nitrogen, where data is plentiful and consistent. Many of the current commercial multi-vitamin preparations used in Parenteral Nutrition are based on the requirements of healthy adults. Published work has shown that circulatory levels of several vitamins may fall in patients on TPN where these preparations are used eg. vitamin A (16), vitamin D (17), folate (18). This may be due to underlying disease, stress, trauma, all of which alter the metabolic rate, as well as simple malnourishment.

Most of the published work on parenteral vitamin requirements has investigated the fat soluble vitamins where there are fears of inducing toxicity states with infusion of high doses of these vitamins. Work done on parenteral requirements for water soluble vitamins is considerably less and much of the published work has concentrated on vitamin C and folate (18, 19, 20, 21). The findings of such studies are more controversial than those concerning fat soluble vitamins. This may be due to the large difference in nutritional status of patients referred for Parenteral Nutrition (22) as well as differing pathological status. Patients referred for TPN range from those with severe burns or head injuries to surgical patients and those with cancer. Their nutritional requirements may be expected to vary considerably.

Studies that have investigated vitamin B_6 in TPN have suggested various amounts to be infused, ranging from 2mg daily (23), 3mg daily (24) to an excess of 4mg daily (25). The patients in each of these studies ranged from post-surgical patients

to patients with cancer or gastro-intestinal problems, including post-surgical patients or patients with gastro-intestinal problems who had undergone surgery. These groups of patients may be expected to have widely differing nutritional requirements including that of vitamins. A review of vitamins in TPN (22) stated that the daily requirement of vitamin B_6 in TPN are not yet established. It suggested an intake of 4-8mg daily except where there were possible pharmacological interferences where intakes of 10-15mg may be more appropriate. One can, therefore, appreciate the controversy surrounding vitamin B_6 intake in TPN.

Before referral for TPN, patients have usually undergone some kind of injury or trauma eg burns, road traffic accidents or surgery. After surgery, Nitrogen excretion increases due to an increased breakdown of body protein (26). Up to 7% of body protein may be lost assuming there are no complications such as infection. Following a road traffic accident or burns, this catabolic phase can be so vast, it cannot be counteracted by feeding alone (27).

Following the catabolic phase, nitrogen balance becomes markedly positive, the body quickly restoring tissue lost after this trauma. The onset of this anabolic phase is determined by the extent of the injury and can be delayed for several weeks in seriously injured patients.

Amino acid metabolism is, therefore, severely increased during both catabolic and anabolic phases, increasing vitamin B_6 requirements and consequently lowering the plasma vitamin B_6 concentration.

The aim of TPN is to maintain and improve nutritional status. Nutritional status for many nutrients have been monitored for many years such as nitrogen balance, electrolytes, some vitamins, and positive balances where possible

maintained throughout treatment with TPN. Although much less is known about micronutrients, such as vitamins and trace elements, one should be aiming to at least maintain adequate levels of these micronutrients.

Vitamin B_6 Nutritional Status Measurement

Plasma PLP is said to be indicative of vitamin B_6 nutritional status (8), being the major vitamer in the blood stream, bound to albumin and reflecting the amount of PLP stored in the body (5). Evidence for this has already been discussed in a previous section.

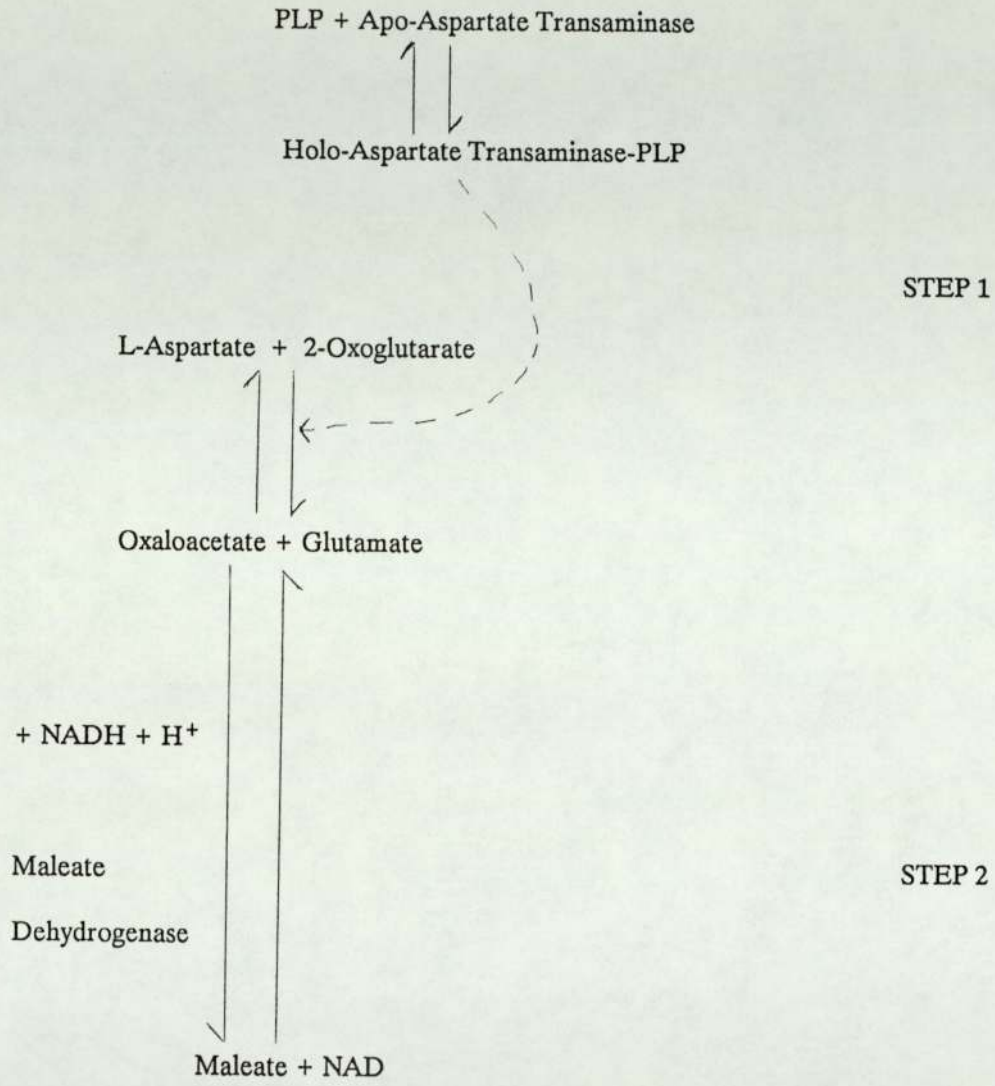
Many methods have been developed to measure vitamin B_6 status, ranging from functional measurements such as the Tryptophan Load test (28) or the Red Cell Activation Test (29) including the Erythrocyte Glutamic Oxalacetic Transaminase Index or EGOT index, to direct assays measuring plasma PLP using PLP dependent enzyme assays (30, 31). Studies looking at vitamin B_6 status in patients referred for TPN have usually used the EGOT index. This is not a direct measurement of vitamin B_6 status, the saturation of all red cell enzymes with PLP being assumed to be indicative of vitamin B_6 status. There is also evidence suggesting that PLP dissociates from red cell enzymes during frozen storage giving rise to artifactual results where status is measured by red cell activation tests (20).

As circulating plasma PLP concentration is indicative of vitamin B_6 status, it seems appropriate to measure this directly. A method developed by Sinkamani et al (32) using the PLP dependent enzyme Glutamic Oxalacetic Transaminase (GOT) has been adapted to measure vitamin B_6 status in patients referred for TPN. The reaction utilised in this method can either be followed manually using a simple spectrophotometer or can be automated using a clinical analyser. A diagramatic summary

of the reaction is given in fig 1.4. The reaction is followed by the decrease in NADH in the reaction solution, this being proportional to the rate of reaction (1) which is dependent on the amount of PLP present, bound to GOT.

It has been suggested that a plasma PLP concentration of 20 nmol/L (32,33) is the lower limit of the normal range. This assay has been shown to be sensitive to 5nmol/l and thus should indicate vitamin B_6 deficiency.

Figure 1.4 Enzymatic Reactions Utilised in The Assay of PLP



Vitamin B_6 in Total Parenteral Nutrition

Vitamins may be infused parenterally, either separately from other nutrients, or as part of a mixture of other nutrients eg in a 3L bag. Even when given in a pre-mixed system they may be given once or twice weekly, or each day. At Dudley Road Hospital, Birmingham, all micronutrients including vitamin B_6 are given on a daily basis. This poses several problems for a project of this kind.

The normal range quoted in the previous section is from fasting subjects. Patients receiving Parenteral Nutrition are constantly being 'fed', and one may expect measured nutritional status to be slightly elevated, and results at the lower end of the normal range may need to be interpreted carefully.

One must also be concerned about the stability of vitamin B_6 in pre-mixed Parenteral Nutrition Fluids, and the conditions, if any, in which vitamin B_6 becomes unstable, so that one is confident about the amount of vitamin being delivered to the patient. In this report, a detailed investigation into the stability of vitamin B_6 is described. Assays of vitamin B_6 were developed which were capable of measuring low concentrations of pyridoxine in pharmaceuticals.

The Assay Of Vitamin B_6 In Infusion Fluids

Pyridoxine is the only B_6 vitamer used in pharmaceuticals as it is cheap, abundant, and rather more stable than other B_6 vitamers. A number of methods have been reported in the literature for the analysis of pyridoxine, either in pharmaceuticals, or in foods and body tissues, where other B_6 vitamers are also present. Techniques utilised in these assays range from microbiological (34, 35), to spectrophotometric (36) and colourimetric to chromatographic including Gas chromatography (37, 38) and Liquid Chromatography (39,40,41,42,43).

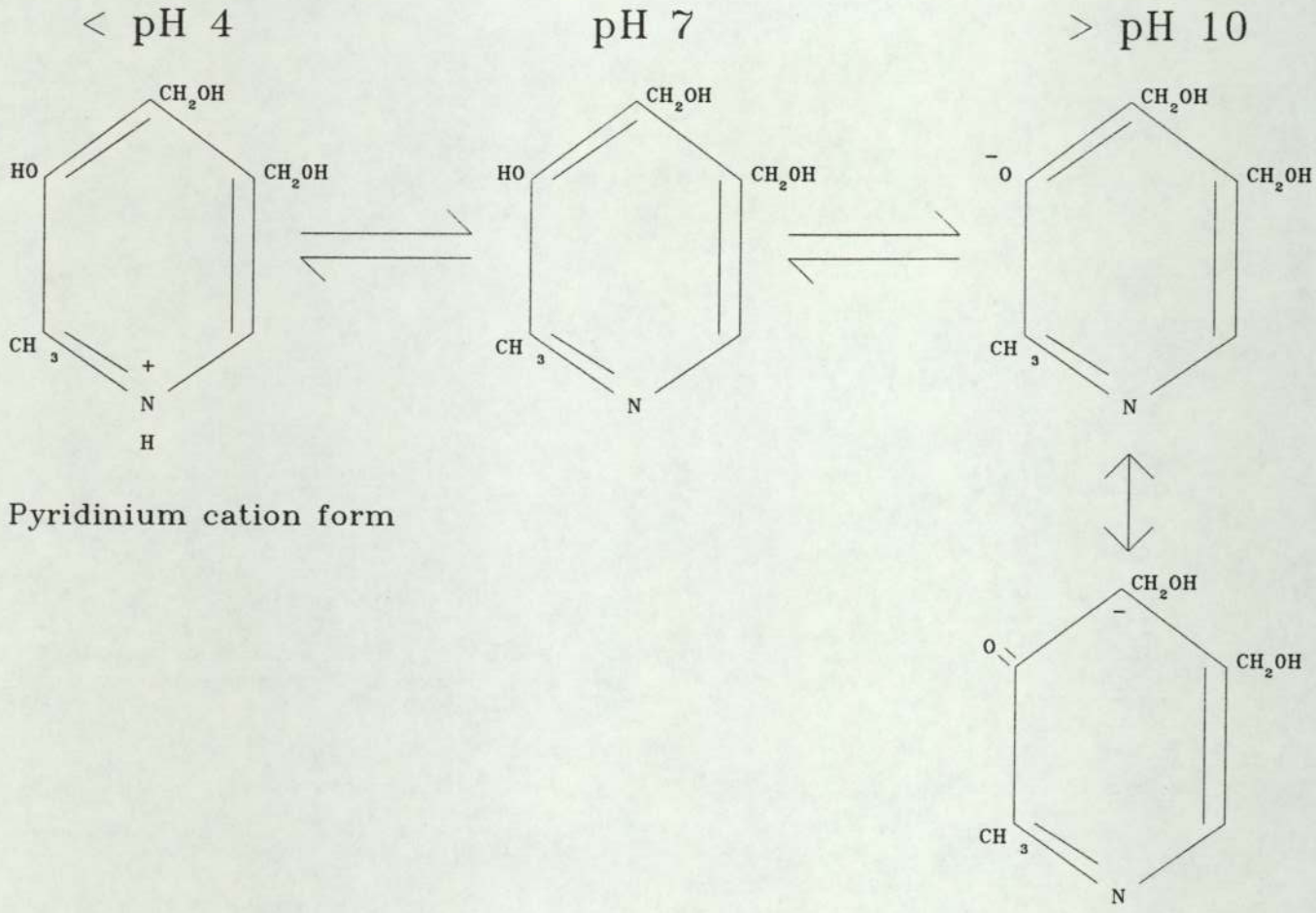
Only the microbiological methods published to date appear to give the sensitivity and specificity required to measure pyridoxine in infusion fluids including Parenteral Nutrition fluids. However, these assays suffer from being tedious and time consuming. Many High Performance Liquid Chromatography methods have been published but some are only suitable for pharmaceuticals (40, 41) such as tablets and capsules or oral liquids, because of their low sensitivity, or they utilised complex systems designed to separate the B_6 vitamers with pyridoxine often having a long retention time (42, 43).

In this thesis, two rugged HPLC assays will be described suitable for the quantitative analysis of pyridoxine levels below 1 microgram per litre, if necessary, in various pharmaceutical preparations. The methods have been developed for use with simple HPLC apparatus and using readily available materials.

Initial inspection of the physical chemistry of pyridoxine (44,45) shows the molecule to have a characteristic UV spectrum with absorbance maxima at 241nm in 0.1N HCl (E1% =422) whilst at pH 7 showed a maximum at 324nm (E1% =350). Pyridoxine hydrochloride showes pKa's of 5.0 and 9.0, thus its retention times would not be expected to change much below pH 3 or around pH 7 where pyridoxine is essentially in one form. Below pH 4 pyridoxine exists as the pyridinium cationic form but it exists almost completely as the neutral molecule at pH 7. This is shown in fig 5. The retention time would not be expected to change significantly around pH 7.

This forms the basis for the development of the two HPLC methods discussed for the assay of pyridoxine. Two methods are necessary due to the fact that the assay utilised for most Vamin and Aminoplex amino acid solutions is not suitable for Vamin 9 solutions. This is due to the considerable interference by constituents of Vamin 9 solution. The assay developed for Vamin 9 preparations is unsuitable for other amino acid solutions, as there is no significant peak detected when these

solutions are used. This method utilises a mobile phase of pH 2.85 whereas the other method utilises a mobile phase of pH 6.85, and is based on a spectrophotometric method. This ensures the absorbance of pyridoxine at pH 7.0 and compares it to the absorbance at pH 1.0 (36). These methods will be used to investigate the stability profile in infusion fluids and TPN fluids.



Pyridinium cation form

Figure 1.5 Variation in Ionic Form Of Pyridoxine With pH

There are many reports of stability studies of pyridoxine. It is destroyed by direct sunlight in phosphate buffer pH 6.8 within one hour. Diffuse sunlight and artificial light destroy the vitamin within several hours (46). The major decomposition product has been stated to be the pyridoxine dimer. (47)

Photodecomposition is dependent on pH (46,48). At low pH, pyridoxine is virtually unaffected by light. In neutral and alkaline media, pyridoxine is rapidly destroyed by light. In the dark, pyridoxine is unaffected by acids and alkalis.

Pyridoxine is affected by oxidising agents. Major decomposition are stated to be (47)

a) Pyridoxal - a B_6 vitamer

b) 4 Pyridoxic acid

Oxidation occurs in acidic media rather than alkaline media eg permanganate, manganese dioxide. Nitric acid also oxidises pyridoxine.

Many of the stability studies of pyridoxine have investigated its stability in foods, both fortified and unfortified, under a variety of food processings. The stability of pyridoxine has been found to be good in fortified dry foods over a period of several months (49,50). Pyridoxine, however is rapidly destroyed during thermal procedures. In liquid food systems, pyridoxine shows similar stability to that in dry systems (51).

Of its stability in pharmaceuticals, pyridoxine is stable to light, but on dilution by either distilled water or in infusion fluids it became unstable to light (52). Minerals may also affect the stability of pyridoxine formulations unfavourably eg copper, iron. The stability of pyridoxine is enhanced by disodium edtate, suggesting that metal ions affect the stability of pyridoxine (48).

There is very little work published on the stability of pyridoxine in infusion fluids especially Parenteral Nutrition fluids. A paper published by M. F. Chen et al (53) studied the stability of the B_6 vitamers in TPN fluids without lipid emulsions in varying degrees of light intensity. Pyridoxine was found to be unstable only in direct sunlight being stable to flourescent light and diffused sunlight over an 8 hour period. In this thesis, the effects of ambient light are described. Fluorescent light and diffused sunlight are compared. The influence of trace elements, amino acids and fat emulsion may all affect photodegradation of pyridoxine in TPN solutions. The quality of light may affect the rate of degradation of pyridoxine. Allwood and Plane (53a) investigated the stability of vitamin A in light. They found that the degradation of vitamin A is dependent on both the wavelength of light received and its intensity. In these experiments described, the effects of specific wavelengths of light and light intensity were not investigated. However, the effect of fluorescent light was compared to diffused sunlight. The effect of trace elements, amino acids, fat emulsion, other vitamins on the stability of pyridoxine were all investigated.

CHAPTER TWO

MATERIALS AND METHODS

The Assay of Pyridoxine in Parenteral Nutrition Fluids Containing Vamin 9, Vamin 9 Glucose (Assay Method One)

Materials

Both methanol and sodium heptanesulphonate were of HPLC grade obtained from BDH. Glacial acetic acid was of Analar grade (BDH). All water used in mobile phases was of water for injections standard and obtained from the Parkfields Regional Sterile Supply Unit, Wolverhampton. Solivito, Vamin 9 Glucose and Vamin 9 were purchased from Kabi Vitrum. Details of commercial products are given in appendix 1. Pyridoxine Hydrochloride standard (Analar grade) was obtained from B.D.H.

Instrumentation

The HPLC system utilised consisted of an Altex pump, model 110A, connected to a Rheodyne model 7125 syringe loading injector, fitted with a 20 microlitre loop, a Hichrom 5 micron 10cm long column of 4mm internal diameter. Eluate was monitored using a Pye Unicam Variable wavelength detector. Recordings were made on a Tekman chart recorder and/or Shimadzu CR-3 integrator.

Chromatographic Conditions

Chromatography was performed at ambient temperatures. The mobile phase consisted of methanol:water:glacial acetic acid in the volume ratio 15:83:2 with sodium heptane sulphonate to a mobile phase concentration of 0.01M. The pH of the mobile phase was around 2.85. All mobile phases were degassed by agitation under vacuum and filtered through a 0.45 micron filter before use. The flow rate was 1.0ml/min and the detector was set at 291nm with an attenuation of 0.01 or 0.005 AUfs.

When samples were derived from fluids containing fat emulsion, a 5cm guard column packed with pellicular ODS (Hichrom) was used.

Amendment – end of first paragraph:

The peak response to pyridoxine concentration at 324nm was calibrated each day using standard solutions. The calibration was then periodically checked throughout the day.

of the lipid layer.

The Assay of Pyridoxine in Infusion Liquids and Parenteral Nutrition Fluids not Containing Vamin 9 Preparations (Assay Method Two)

Materials

Both methanol and cetyltrimethylammonium bromide were HPLC grade and obtained from BDH. Potassium hydrogen phosphate and sodium hydroxide were of Analar grade (BDH). The commercial multivitamin preparation used was Solivito (Kabi Vitrum). Amino acid infusion fluids used were Vamin 14 and Vamin 18 solutions (Kabi Vitrum) or Aminoplex 12 and Aminoplex 24 (Geistlich). Injectable trace element solution used was Addamel (Kabi Vitrum) and fat emulsion was either Intralipid 10% or Intralipid 20% (Kabi Vitrum). Dextrose 10% and saline 0.9% infusion fluids were purchased from Travenol. Details of commercial injectables are given in appendix 1.

When samples were derived from fluids containing fat emulsion, a 5cm guard column packed with pellicular ODS (Hichrom) was used.

Sample Preparation

Samples not containing fat emulsion were injected directly onto the column and required no preparation beforehand. Parenteral Nutrition fluids containing fat emulsion were centrifuged at 100,000g for 30 mins to aggregate the lipid phase. The aqueous phase was then recovered with a hypodermic syringe following careful puncture of the lipid layer.

The Assay of Pyridoxine in Infusion Liquids and Parenteral Nutrition Fluids not Containing Vamin 9 Preparations (Assay Method Two)

Materials

Both methanol and cetyltrimethylammonium bromide were HPLC grade and obtained from BDH. Potassium hydrogen phosphate and sodium hydroxide were of Analar grade (BDH). The commercial multivitamin preparation used was Solivito (Kabi Vitrum). Amino acid infusion fluids used were Vamin 14 and Vamin 18 solutions (Kabi Vitrum) or Aminoplex 12 and Aminoplex 24 (Geistlich). Injectable trace element solution used was Addamel (Kabi Vitrum) and fat emulsion was either Intralipid 10% or Intralipid 20% (Kabi Vitrum). Dextrose 10% and saline 0.9% infusion fluids were purchased from Travenol. Details of commercial injectables are given in appendix 1.

Chromatographic Conditions

Chromatography was performed at ambient temperatures, using the same HPLC apparatus as before. In this assay, however, a 25cm Hichrom 5 micron ODS column of internal diameter 4mm was used. Where samples were derived from fluids containing lipid emulsion then a 5cm pellicular ODS guard column was used as before. The mobile phase consisted of methanol:0.05M phosphate buffer pH 6.85 in a ratio of 5:95 with cetyltrimethylammonium bromide to a mobile phase concentration of 0.01M. All mobile phases were degassed and filtered before use as previously described.

The flow rate was 1.0ml/min and the detector set to 324nm with an attenuation of 0.04AUfs except for samples with a pyridoxine concentration below 1microgram/ml when a setting of 0.01AUfs was used.

Amendment – end of second paragraph:
The peak response to pyridoxine concentration at 324nm was calibrated each day using standard solutions. The calibration was then periodically checked throughout the day.

Stability In Saline 0.9%, Dextrose Infusion Fluids

Pyridoxine was added, either on its own or with other soluble vitamins in a commercial multivitamin injectable preparation, Solivito, to saline 0.9% and dextrose 10% infusion fluids. The stability of pyridoxine in each solution was then

Chromatographic Conditions

Chromatography was performed at ambient temperatures, using the same HPLC apparatus as before. In this assay, however, a 25cm Hichrom 5 micron ODS column of internal diameter 4mm was used. Where samples were derived from fluids containing lipid emulsion then a 5cm pellicular ODS guard column was used as before. The mobile phase consisted of methanol:0.05M phosphate buffer pH 6.85 in a ratio of 5:95 with cetyltrimethylammonium bromide to a mobile phase concentration of 0.01M. All mobile phases were degassed and filtered before use as previously described.

The flow rate was 1.0ml/min and the detector set to 324nm with an attenuation of 0.04AUfs except for samples with a pyridoxine concentration below 1microgram/ml when a setting of 0.01AUfs was used.

Sample Preparation

Samples containing fat emulsion were centrifuged as previously described, and the aqueous phase injected onto the column. For other infusion fluids, no sample preparation was required.

The Stability Of Pyridoxine In Infusion Fluids

Stability In Saline 0.9%, Dextrose Infusion Fluids

Pyridoxine was added, either on its own or with other soluble vitamins in a commercial multivitamin injectable preparation, Solivito, to saline 0.9% and dextrose 10% infusion fluids. The stability of pyridoxine in each solution was then

assessed over a period of 10-20 days, in both fluorescent light conditions and in the dark at ambient room temperature (18-22°C). Pyridoxine concentrations were assayed by HPLC using the second assay. The solutions were approximately 1.5m from the fluorescent light source, a Polylux 4000 (Thorn Lighting).

The Effect of Trace Elements On Pyridoxine Stability

The above experiment was repeated, except that to each infusion solution was added 10ml (one ampoule) of commercial trace element solution, Addamel. The stability of pyridoxine was assessed over 15 days, both in fluorescent light conditions and in the dark, pyridoxine content being measured as before.

The experiment was repeated but the amount of trace element solution added to each infusion solution varied, 10, 20, or 30 ml were added to saline 0.9% or dextrose 5% infusion solutions containing 1 vial of Solivito. The affect of trace element concentration on pyridoxine stability was assessed over a 20 day period.

The Effect Of Amino Acid Solutions On Pyridoxine Stability

Commercial amino acid infusion fluids were diluted 40 parts to 60 parts with saline 0.9% infusion fluid. Pyridoxine was then added, either on its own or with other water soluble vitamins in Solivito. The stability of pyridoxine in each solution was followed over a period of 15-20 days in fluorescent light conditions and in the dark whilst in the manufacturers original glass bottle. Pyridoxine concentration was measured using the second assay (mobile phase pH 6.65, detector set at 324 nm), except solutions containing Vamin 9 glucose, where the first assay was used (mobile phase pH 2.85, detector set at 291 nm).

The experiment with Aminoplex 24 was repeated using dextrose 10% solution as the diluent rather than saline 0.9% solution to assess the effect if any of dextrose infusion solution had on stability. One solution was left under fluorescent light, and an identical one left by the window in diffused sunlight, to investigate any increased degradation.

Amendment – end of first paragraph:

The effect of the type of container of the infusion fluid was investigated. There was no significant difference in the rate of degradation of pyridoxine in either PVC 'Viaflex' containers or glass.

added to 500ml of Intralipid 10% containing 10 ml of a commercial fat soluble vitamin preparation, Vitlipid. The stability of pyridoxine was then assessed in both fluorescent light conditions and in the dark over a period of 20 days. The effect of fat soluble vitamins on the stability of pyridoxine in Solivito was checked by repeating the experiment with Solivito but omitting the Vitlipid.

The stability of pyridoxine in fluorescent light conditions in dilute Intralipid (diluted to 15%v/v with saline 0.9% solution) was also assessed over a 20 day period.

The effect of diffused sunlight was investigated by repeating the experiments in front of a window receiving sunlight. The effect of diffused sunlight on Pyridoxine on its own and in Solivito was studied over 20 days. The effect of adding 10 ml trace element solution to Intralipid 10% on the stability of pyridoxine in Solivito was also assessed in the same manner.

The experiment with Aminoplex 24 was repeated using dextrose 10% solution as the diluent rather than saline 0.9% solution to assess the effect if any of dextrose infusion solution had on stability. One solution was left under fluorescent light, and an identical one left by the window in diffused sunlight, to investigate any increased degradation.

The Effect Of Fat Emulsion on Pyridoxine Stability

(i) Intralipid 10%

Pyridoxine, either on its own, or with other water soluble vitamins was added to 500ml of Intralipid 10% containing 10 ml of a commercial fat soluble vitamin preparation, Vitlipid. The stability of pyridoxine was then assessed in both fluorescent light conditions and in the dark over a period of 20 days. The effect of fat soluble vitamins on the stability of pyridoxine in Solivito was checked by repeating the experiment with Solivito but omitting the Vitlipid.

The stability of pyridoxine in fluorescent light conditions in dilute Intralipid (diluted to 15%v/v with saline 0.9% solution) was also assessed over a 20 day period.

The effect of diffused sunlight was investigated by repeating the experiments in front of a window receiving sunlight. The effect of diffused sunlight on Pyridoxine on its own and in Solivito was studied over 20 days. The effect of adding 10 ml trace element solution to Intralipid 10% on the stability of pyridoxine in Solivito was also assessed in the same manner.

(ii) Stability in Intralipid 20%

The stability of pyridoxine in Intralipid 20% was investigated in the same manner in fluorescent light conditions. The experiment was then repeated in dilute Intralipid (15%v/v in saline 0.9% solution) both under fluorescent light conditions and diffused sunlight. The effect of addition of 10ml trace element solution to Intralipid 20% on the stability of pyridoxine in Solivito was also investigated in diffused sunlight.

The Stability Of Pyridoxine In Ready Mixed TPN Fluid

Standard TPN mixtures were made up with and without Intralipid 20%. One of each was then left either in fluorescent light conditions or diffused sunlight. Amino acid solutions used were Aminoplex 12, Vamin 14 which were assayed by method 2 for pyridoxine concentration. Pyridoxine stability was followed for one week in the light at room temperature. A further TPN mixture containing Vamin 14 was left in the refrigerator and stability followed for two weeks.

The composition of the mixture was as follows :

Amino acid solution	1000 ml
Dextrose 10%	1000 ml
Intralipid 20% or Dextrose 10% *	500 ml
Addamel	10 ml
Addiphos	20 ml
Solivito	1 vial
Vitlipid	10 ml
Total Volume	2540 ml

* Dependent upon whether mixture contained fat emulsion.

The Investigation Of The Effect Of Riboflavin On Pyridoxine Stability

Varying amounts of Riboflavin were added to saline solution containing pyridoxine and the stability followed over a period of seven days. Riboflavin concentrations were 1 microgram/ml, 5 microgram/ml, 10 microgram/ml. Pyridoxine concentrations were approximately 2 microgram/ml and assayed using HPLC method 2.

The Measurement of Vitamin B_6 Nutritional Status

Materials

109 mmol/l Tris-EDTA buffers pH 6.0 7.0 7.4 7.8

These were prepared containing 17.2g Tris(hydroxymethyl)aminomethane hydrochloride (Tris HCl) and 2.54g disodium acetate. pH was then adjusted by addition of 1N sodium hydroxide and made up to 1L with distilled water.

545 mmol/l Tris-EDTA buffer pH 6.2

This was prepared containing 86g Tris HCl and 2.45g disodium edetate adjusted to pH 6.2 with 1N sodium hydroxide and diluted to 1L with distilled water.

2-oxoglutarate 300 mmol/l

2.48g sodium hydrogen 2-oxoglutarate was dissolved in 50ml 109mmol/l Tris-EDTA buffer pH 7.8 and the pH readjusted to 7.8 with 1N Sodium hydroxide.

Potassium Glutamate 200 mmol/l

1.47g L-glutamic acid and 0.56g potassium hydroxide were dissolved in distilled water and the pH adjusted to 8.3 with 1N potassium hydroxide and made up to 100 ml.

Substrate mixture

4.5g potassium L-aspartate, 16mg sodium NADH and 50 units malic dehydrogenase were dissolved in 100 ml Tris-EDTA buffer pH 7.4. This was prepared freshly each day.

Standard PLP 1.5 - 2 micromol/l

20 microlitres of saturated PLP solution in distilled water was chromatographed on silica gel 60F-254 5x10 cm Thin layer chromatography plates (BDH) using MeOH:$CHCl_3$:iso-amyl alcohol:diethylamine, 10:10:1.5:1.5. The PLP band, Rf 0.4 was extracted with 3ml Tris EDTA buffer pH 7.0. The concentration was then determined using the molar extinction coefficient of 4.9×10^3 at 388nm. This solution was then diluted to give a solution of approximate concentration 1.5 - 2 micromol/l and stored in 1ml units at -20 °C until ready for use.

Methods

Preparation of Apo Aspartate Transaminase

500 units Aspartate Amino Transferase (Sigma lyophilised form type II-A) was dissolved in 0.2 ml Tris-EDTA buffer pH 7.8 and 1.0 ml 200 mmol/l L-Glutamate pH 8.3 added. The solution was incubated at 37 °C for 30 mins and then 1.0 ml of

150mmol/L phosphate buffer pH 4.8 added and incubated for a further 30 mins. 7.5 ml of saturated ice cold ammonium sulphate was added and the solution left in an ice bath for 30 mins to allow the enzyme to precipitate, further crystals of ammonium sulphate were added to keep the solution saturated. After centrifgation at 2000xg the precipitate was dissolved in 0.2ml Tris EDTA buffer and the above procedure repeated twice. Finally the protein precipitate was dissolved in 1.0ml 109mmol Tris-EDTA buffer and dialysed against the same buffer for 24 hours on a cold room, changing the buffer after 6 hrs. The enzyme solution was finally stored in 0.2 ml units at -20 °C until ready for use.

Prepatation Of Samples With and Without added PLP Standard

Blood specimens were collected using potassium EDTA specimen tubes. 25 microlitres of standard PLP (200nmol/l) was added to one of two tubes, each containing 0.5ml of plasma and 50 microlitres of 6N perchloric acid (BDH) added whilst mixing thoroughly. After centrifugation at 2000xg for 20 minutes, 0.4 ml of the supernatant was removed and neutralised with approximately 150 microlitres 2N potassium hydroxide to precipitate excess perchloric acid and the mixture left in an ice bath for 45 mins to complete precipitation. After centrifugation at 2000xg for 5 mins, the supernatant was removed and stored at -20 °C until ready for assay.

Enzymatic assay

(i) Manual assay

Two steps are required for the enzyme assay.

Step 1 - binding of PLP to Apo Aspartate Transaminase

To 0.5 ml of 545 mmol/l Tris EDTA buffer pH 6.2 was added 0.2 ml of standard solution or extract containing PLP and then 0.01 ml of apo-aspartate-transaminase. The mixture was then incubated for 90 mins at 37 °C.

Step 2 - Enzymatic determination

After incubation as above to prepare the holoenzyme, 2.5 ml of a mixture of the substrate mixture and 2-oxoglutarate solution, in the ratio of 50:1, was added and the resultant mixture transferred to a cuvette. The reaction was followed by monitoring the decrease in the optical density of NADH at 340nm for 2 minutes

(ii) Automated procedure

The binding step was as in the manual method, incubating at 37 °C for 90 mins. The enzymatic determination was then automated on the Cobas Bio Clinical Analyser. 0.25 ml of the incubated mixture was transferred to a Cobas Bio sample pot and placed in situ in the analyser. 50 microlitres was automatically transferred to a cuvette and mixed with 250 microlitres of substrate/2-oxoglutarate mixture (mixed in ratio of 50:1) and distilled water. The mixture was mixed for 80sec at 37 °C and the reaction monitored by the machine at 10 sec intervals for 2 mins, as for the manual procedure.

The analyser gave a printout in terms of holoenzyme concentration from which PLP could be determined.

Blood samples were obtained from patients on the day prior to starting Parenteral Nutrition and then again on the seventh day of the Parenteral Nutrition regime. A fixed daily amount of pyridoxine, 2mg of the base, was infused daily over 24 hrs via the TPN infusion.

Samples were collected from Pathology within 72 hrs of being taken and the plasma stored at -20 °C until ready for analysis. Two plasma samples were taken from each blood sample and two determinations performed on each sample. Plasma PLP concentration was taken as the mean of the four determinations.

CHAPTER THREE

THE ASSAY OF PYRIDOXINE IN SOLUTIONS FOR TOTAL PARENTERAL NUTRITION

Assay Of Pyridoxine Solutions Containing Vamin 9 Preparations (Assay Method One)

Chromatography

Under the chromatographic conditions, pyridoxine was the only vitamin detected, other vitamins not being detected even when present in high concentration. Pyridoxine eluted after 10.5 minutes. Many of the constituents of Vamin 9 appeared in the chromatogram but they were sufficiently resolved to allow the assay of pyridoxine. (See Fig 3.1)

There was a large peak not shown in the sample chromatogram that eluted from the column after 45 minutes. This took 5 to 10 minutes to elute completely.

The assay has been shown to be stability indicating. When an alkaline solution of pyridoxine was subjected to diffuse sunlight, the pyridoxine peak disappeared from the chromatogram within one hour.

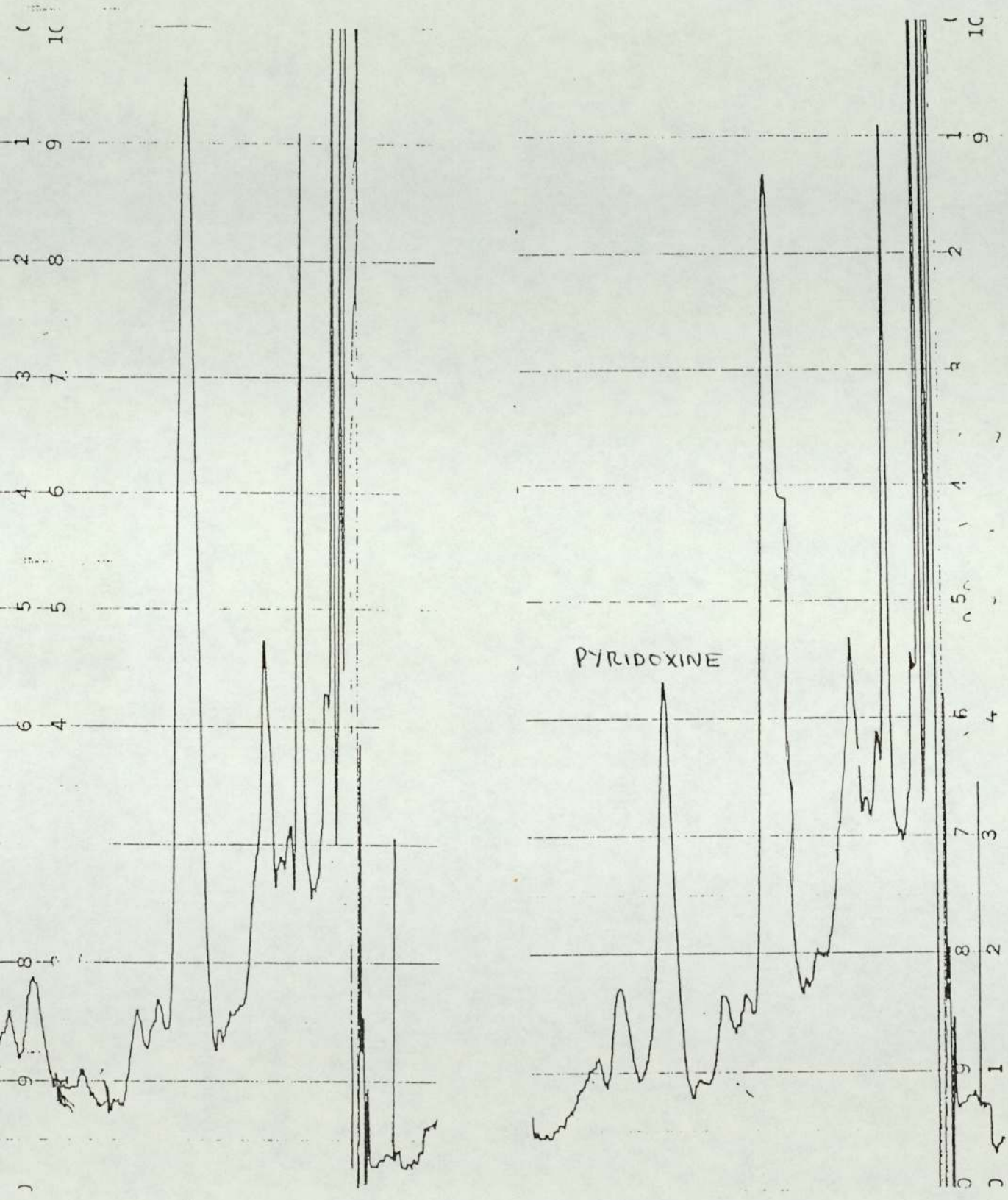
Linearity

Calibration curves were determined with four concentrations of pyridoxine hydrochloride and were found to be linear over the concentration range 0.5 microgram/ml to 2.0 microgram/ml with correlation cofficients of 0.99 for TPN mixtures containing Vamin 9 or Vamin 9 Glucose and 0.99 for solutions of pyridoxine hydrochloride in distilled water. (See Fig 3.2)

Precision and Accuracy

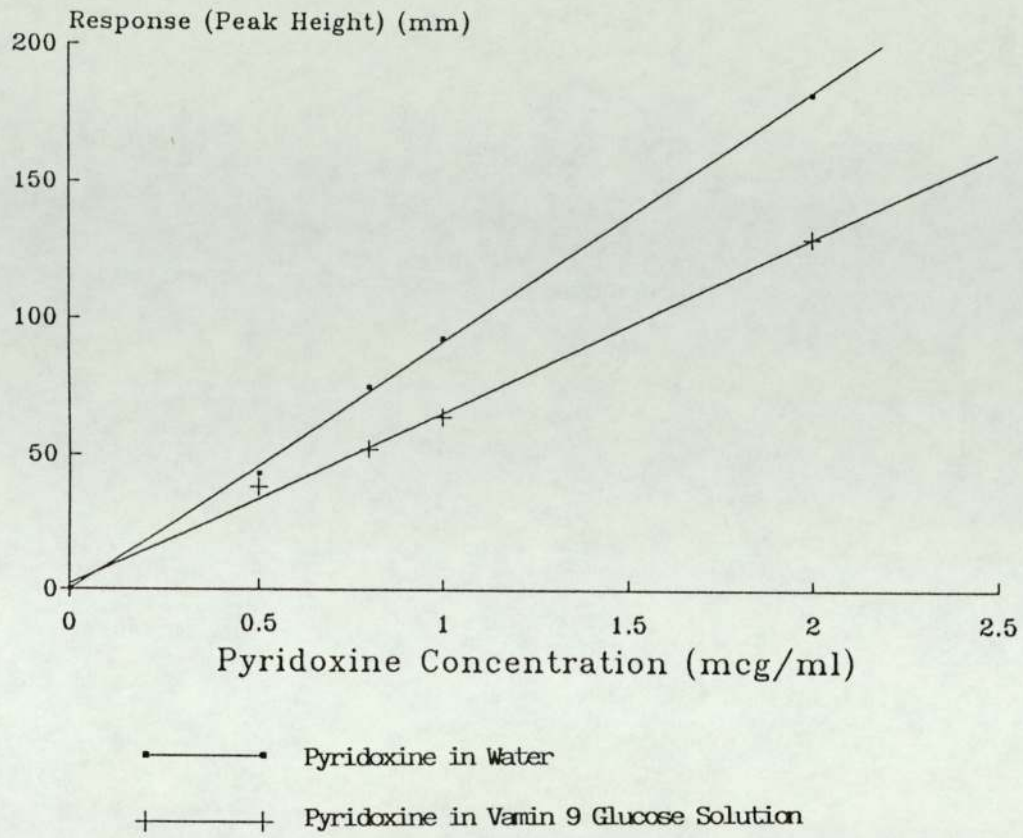
Intra-batch precision was assessed by the analysis of 5 aliquots containing 1 microgram/ml pyridoxine hydrochloride, a coefficient of variation of 2.6% being acheived. Inter-batch precision gave a coefficient of variation of 3.4%

Figure 3.1 Chromatogram Showing The Separation of Pyridoxine From the Constituents of Vamin 9 Glucose Using Assay Method One



Pyridoxine elutes after 10.5 minutes and is adequately separated from the other constituents in the chromatogram to allow quantitative work.

Figure 3.2 Calibration Curve of Response (Peak Height) to Concentration of Pyridoxine in Water and in Vamin 9 Glucose Solution Using Assay Method One



Discussion

This method for measuring pyridoxine in Parenteral Nutrition infusions containing Vamin 9 preparations has been shown to be sensitive, accurate and precise. The assay is free from interference by other vitamins and is sufficiently resolved from the constituents of the amino acid solutions for quantitative analysis.

Although linearity has been shown between 0.5 microgram/ml and 2.0 microgram/ml the assay is sensitive down to 0.1 microg/ml (Taking a signal to noise ratio of 5:1), well below the reported sensitivity limits of other published HPLC assays for pyridoxine utilising UV detection.

However, this assay has several disadvantages. It is time consuming, a full chromatogram taking 50 minutes to complete, extending the assay time considerably despite the elution of pyridoxine after just 10 minutes. The assay is therefore very expensive, especially in terms of material costs and operator time, compared to other assays of pyridoxine in pharmaceuticals.

The assay is only suitable for TPN solutions containing Vamin 9 preparations. For samples from Parenteral Nutrition mixtures containing higher amino acid content for example Vamin 14, Vamin 18 both the sensitivity and resolution are much reduced. For example in preparations containing Vamin 14 solution a sensitivity limit of around 2 microgram/ml is obtained with a very broad peak in the chromatogram. This therefore, renders the assay unsuitable for Parenteral Nutrition mixtures of high amino acid content.

The reason for this is unclear though it is possible that at this pH, some of the amino acids complex pyridoxine, ie act similar to an ion pair reagent, causing the broadening of the pyridoxine peak. The retention time also changed with solutions of high amino acid content, supporting this hypothesis.

Assay Of Pyridoxine In Solutions Containing Other Amino Acid Solutions (Assay Method Two)

Chromatography

Under the chromatographic conditions used, pyridoxine was the only vitamin detected with a retention time of 5.8 minutes (See Fig 3.3). The assay has been shown to be free from interference from the constituents of multivitamin injections (Solivito), fat emulsions and amino acid infusion solutions. These tended to appear very much earlier in the chromatogram. Dextrose appeared in the chromatogram at around 7.0 minutes (See fig 3.4,3.5).

Other B_6 vitamers did not interfere with the assay, pyridoxal eluting after 7.5 minutes and pyridoxamine eluted with the solvent front (See fig 3.6). 4-pyridoxic acid is not eluted from the column after 1 hr. The assay was shown to be stability indicating for pyridoxine, When an alkaline aqueous solution was subjected to diffuse sunlight, the pyridoxine peak in the chromatogram quickly dissappeared. The detection limit based on a signal to noise ratio of 5:1 was 0.20 microgram/ml for pyridoxine hydrochloride

Linearity

Calibration curves, determined with five different concentrations of pyridoxine hydrochloride, were obtained and were linear over the concentration range 0.5 microgram/ml to 2.0 microgram/ml with correlation cofficients of 0.99 in infusion fluids including TPN mixtures (See Fig 3.7). The y intercept obtained with different amino acid solutions varied slightly due to the presence of small peaks around the base of the pyridoxine peak. These did not affect the linearity in any way.

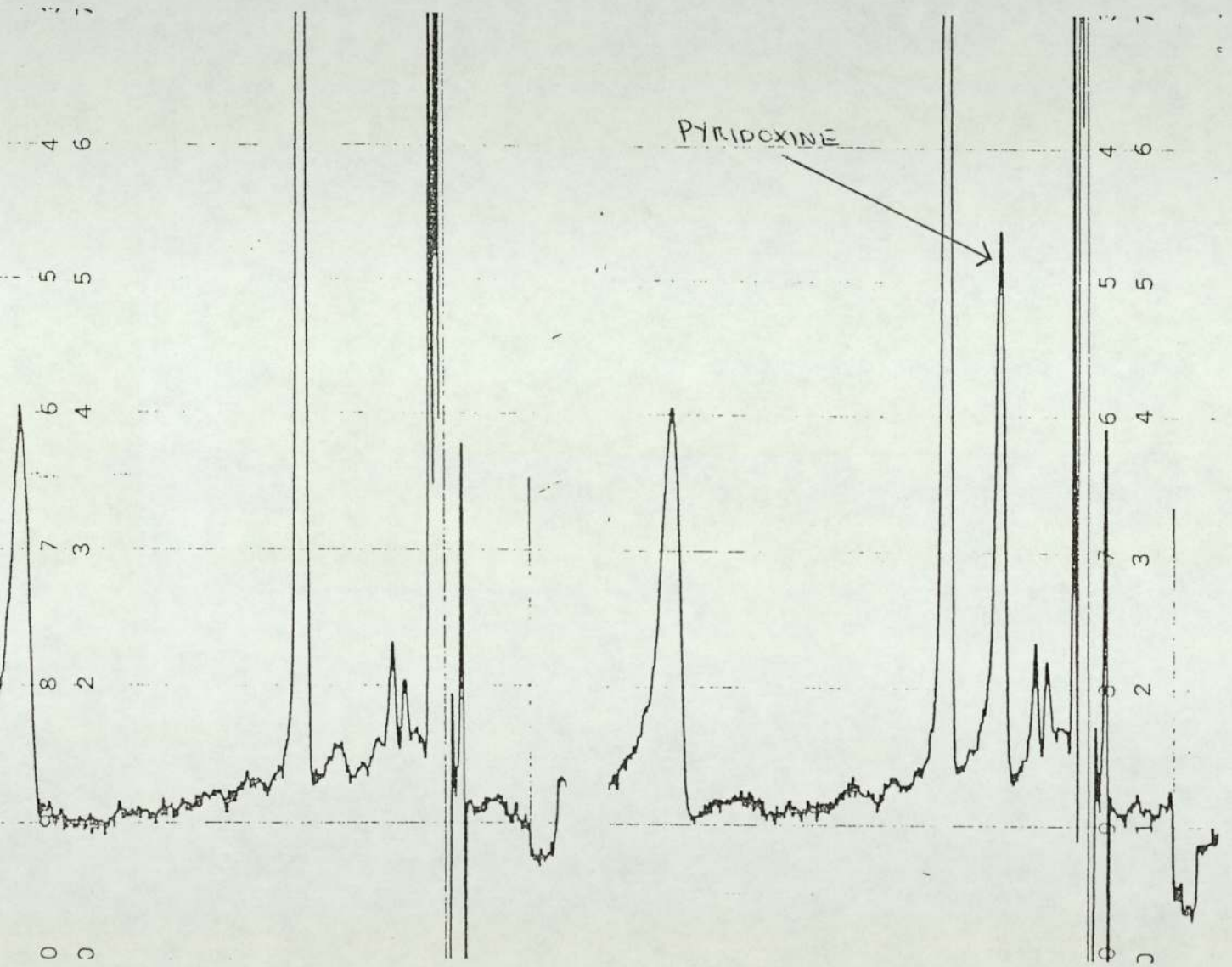
Precision and Accuracy

Intra-batch precision was assessed by the analysis of ten aliquots from a single TPN infusion mixture containing 0.8 microgram/ml pyridoxine hydrochloride giving a coefficient of variation of 2.4%. Samples were found to be stable for several weeks when stored in the refrigerator either in infusion solutions or in aqueous solution. Inter-batch precision was found to be 3.2% throughout the project.

Extraction efficiency

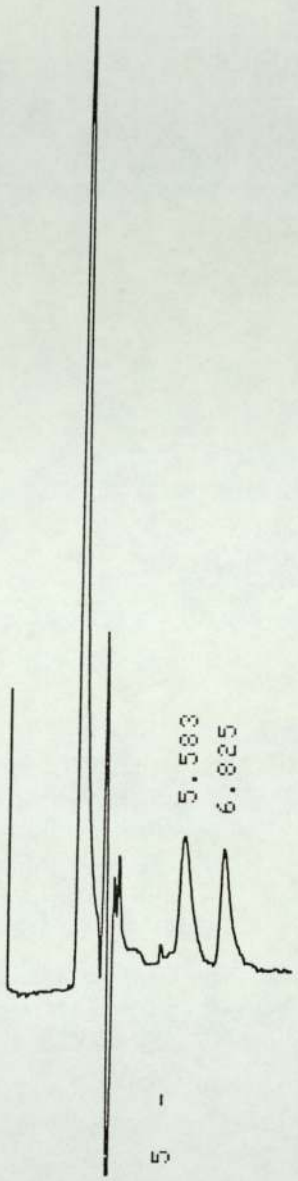
The extraction efficiency of pyridoxine from lipid emulsions gave recoveries varying between 98% and 102% when compared to that of aqueous solution. This indicated that all the pyridoxine was present in the aqueous phase of the lipid emulsion. The lipid phase was found to occupy only a small fraction, below 5% of the total volume of a mixture. This could not be determined exactly as centrifugation did not crack the emulsion, only aggregating lipid particles. No corrections to results were therefore made for this.

Figure 3.3 Chromatogram of a Solution of Solivito in Water Using Assay Method Two



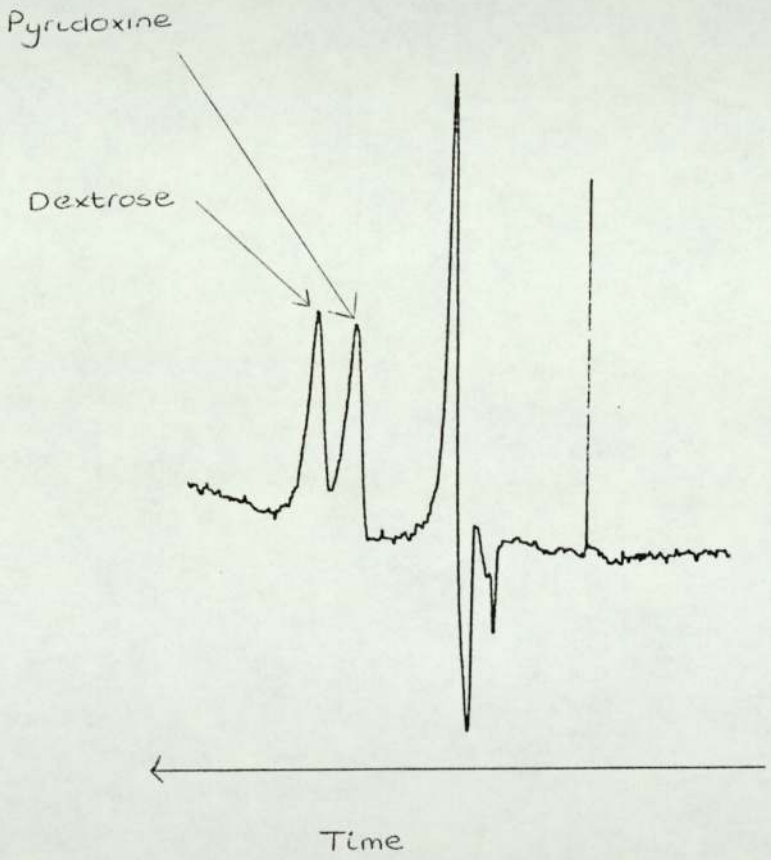
Pyridoxine was the only compound detected out of the vitamins present, eluting after 5.8 minutes.

Figure 3.4 Chromatogram of Pyridoxine in Aminoplex 24 Solution Diluted with Dextrose 10% Solution Using Assay Method Two



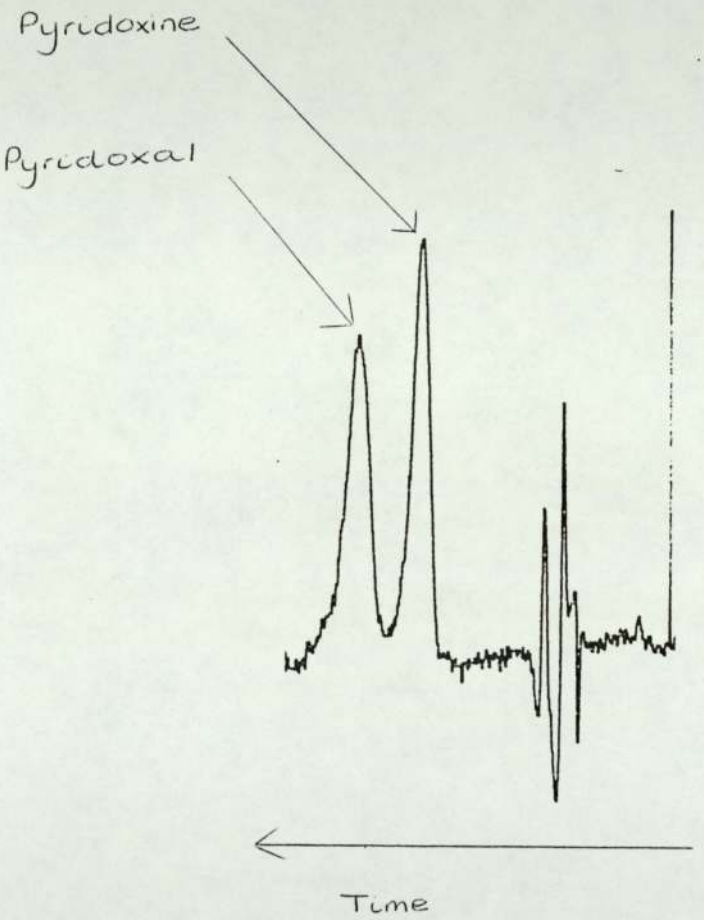
Pyridoxine was separated from the other constituents in the solution, eluting after 5.6 minutes. Constituents from Aminoplex 24 eluted close to the solvent front, and dextrose eluted after 6.8 minutes.

Figure 3.5 Chromatogram of Solivito in Dextrose 10% Solution Using Assay Method Two



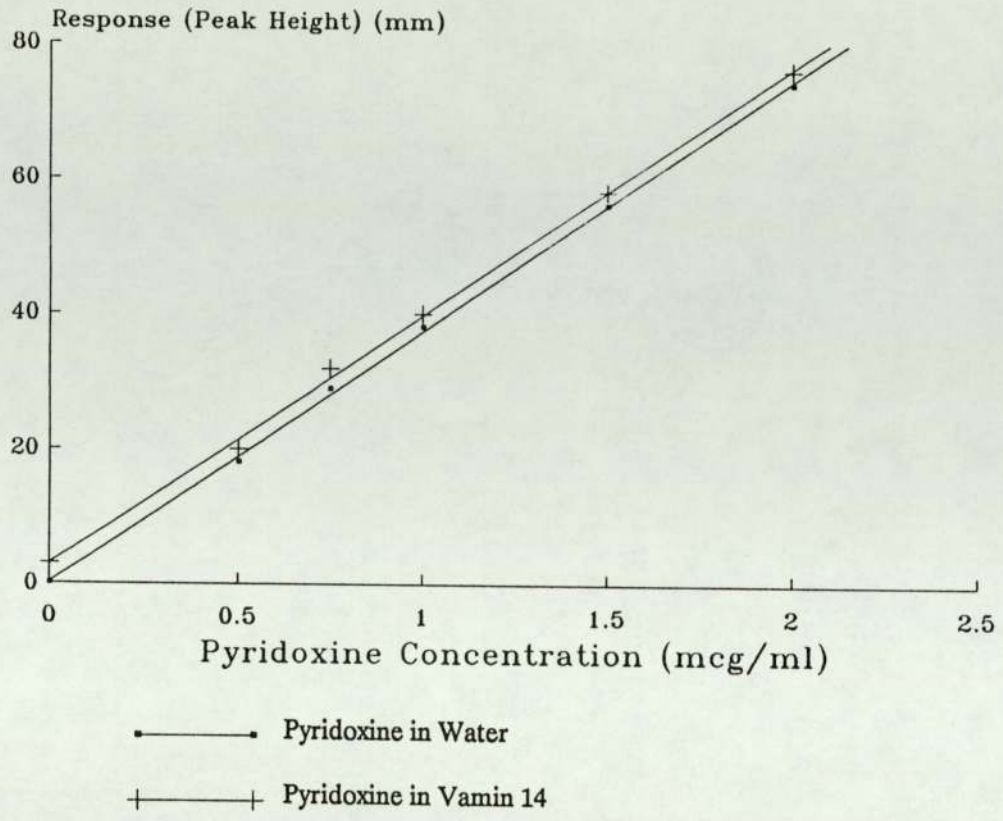
Pyridoxine eluted after 5.9 minutes and was adequately separated from dextrose which eluted after 7.0 minutes.

Figure 3.6 Separation of Pyridoxal and Pyridoxine Using Assay Method Two



Pyridoxal eluted after 7.5 minutes and did not interfere with the assay of pyridoxine.

Figure 3.7 Calibration Curve of Response (Peak Height) to Concentration of Pyridoxine in Water and in Vamin 14 Solution Using Assay Method Two



Discussion

The analytical method discussed is rapid, sensitive, accurate and reproducible, for the assay of pyridoxine in infusion fluids and TPN mixtures. The assay has been shown to be specific to pyridoxine, with no interference from the constituents of infusion solutions or TPN mixtures, with the exception of the slight interference from amino acid infusion solutions already mentioned. However, linearity, precision and accuracy were not affected.

The assay utilises the absorption maximum of 324 nm for pyridoxine at pH7 rather than 280nm or 291nm as in other published assays. A pH of 6.85 was chosen for the assay so as to protect the column from hydrolysis (54). Cetyltrimethylammonium bromide in this system appears to act as a modifier rather than an ion pair reagent for pyridoxine, its presence in the mobile phase giving a much narrower peak for pyridoxine, enhancing the resolution from amino acids for TPN samples and improving the sensitivity fivefold, compared to the sensitivity in the absence of cetyltrimethylammonium bromide

In comparison to the previous assay, whilst being of similar accuracy and precision, for normal infusion fluids, it is much less time consuming, taking around 10 minutes for multivitamin infusion fluids, as opposed to 15 minutes, but only 20 minutes for TPN mixtures as opposed to 50 minutes, making the assay much cheaper and desirable for this purpose. It is much more sensitive and precise for TPN fluids with the exception of Vamin 9 preparations.

This assay has been shown to be more accurate and reproducible at levels below the reported sensitivity limits of other HPLC assays utilising UV detection. It is also quicker for the assay of pyridoxine concentration than the published HPLC assays involving the separation of the B_6 vitamers. When compared to

Gas Chromatographic methods, again it is quicker, both in terms of sample preparation and chromatography. Pyridoxine being a non-volatile molecule, it requires derivatisation before Gas Chromatography.

Although microbiological methods give very good precision and accuracy at low concentrations of pyridoxine, these are very time consuming and tedious. In addition one usually has to wait 24 hrs or more to get results.

Despite being developed for infusion fluids, this assay is also applicable to other pharmaceuticals such as multivitamin tablets, capsules, liquids etc, the chromatography being free from interference and being quick to perform, very little sample preparation is required due to the high water solubility of pyridoxine, eg in the extraction from tablets.

CHAPTER FOUR

THE STABILITY OF PYRIDOXINE IN INFUSION FLUIDS AND PARENTERAL NUTRITION FLUIDS.

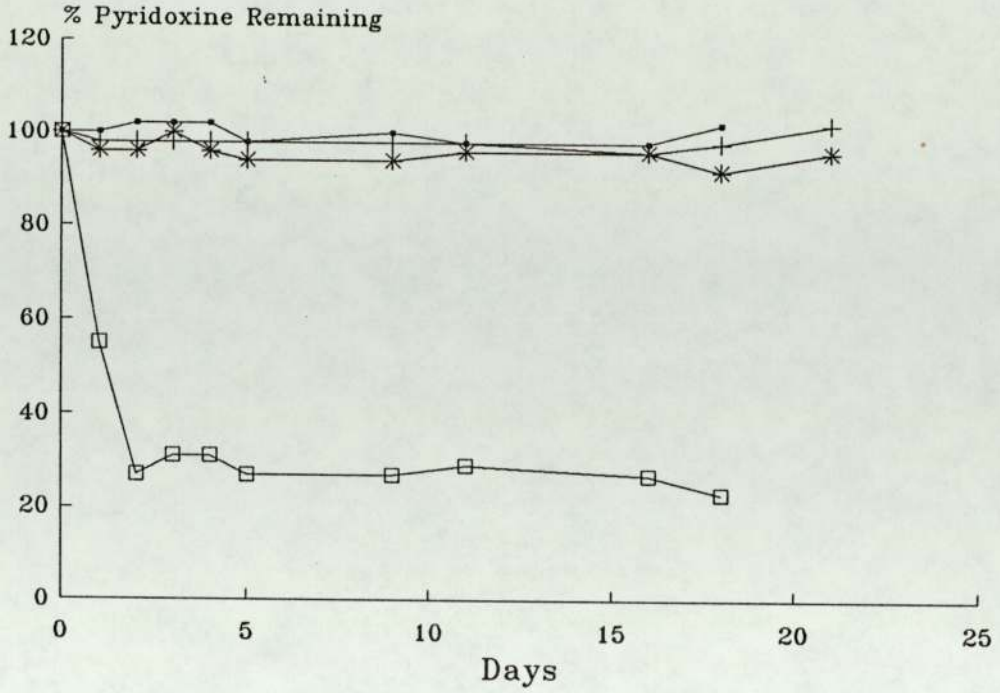
Results

Pyridoxine did not significantly degrade over an 18 day period of study in the absence of other water soluble vitamins (as in Solivito an injectable multivitamin preparation), or in the dark, in dextrose 10% and Saline 0.9% infusion fluids (see figures 4.1,4.2). However, in the light, pyridoxine in Solivito, was rapidly degraded - less than 30% of the original concentration remained after 48 hours, in both dextrose 10% and saline 0.9% infusion fluids, (see figures 4.1,4.2). During the rapid degradation of pyridoxine, in Solivito, in the light, the characteristic yellow intense colour of Solivito was diminished. This did not occur with Solivito in the dark.

Pyridoxine, in Solivito, degraded rapidly in both dextrose 10% and saline 0.9% infusion fluids such that after 48hrs there was 27-28% of the initial pyridoxine concentration (figures 4.1,4.2). However, in saline 0.9% solution, the pyridoxine concentration curve approached zero with increasing time, whereas in dextrose 10% infusion solution, the residual concentration of pyridoxine stabilised at around 27% of the initial concentration at 48 hrs, and thereafter. This suggests that the degradation of pyridoxine in the saline 0.9% goes to completion whereas there is a residual 27% of pyridoxine remaining in dextrose 10% after an 18 day period in the light.

Addamel, an injectable trace element preparation containing calcium, magnesium, iron (III), zinc and copper (II) ions significantly stabilised pyridoxine, in Solivito, to the effect of light. After 48hrs, pyridoxine, in Solivito, in saline 0.9% infusion fluid in the light, degraded to 27% of the initial concentration without Addamel, but with Addamel, at a concentration of 10ml Addamel per litre of infusion fluid, 90% of the initial concentration remained after 48 hrs. Thus the rate of degradation was decreased seven-fold on addition of Addamel. A similar rate of

Figure 4.1 The Stability of Pyridoxine in Dextrose 10% Solution



Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution in the dark.

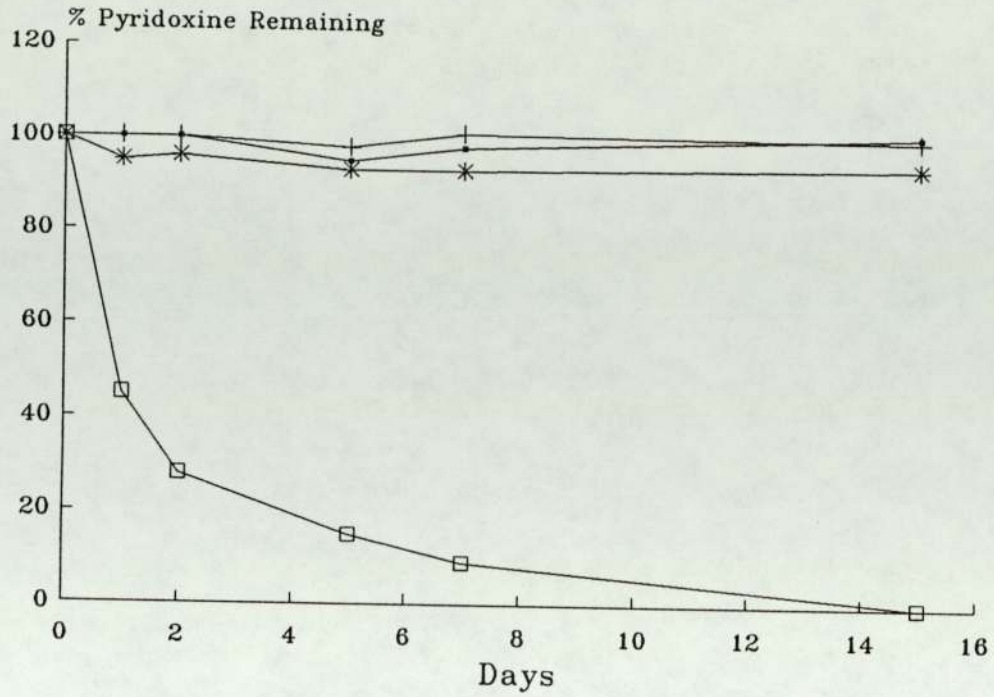
Pyridoxine 5.0mg in 1L dextrose 10% solution in the light.

Pyridoxine 5.0mg in 1L dextrose 10% solution in the dark.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution in the light.

Pyridoxine in dextrose solution was stable in both the light and the dark. Pyridoxine, in Solivito, in dextrose solution was similarly stable in the dark. In the light, pyridoxine, in Solivito, was statistically significantly degraded compared to pyridoxine in the above solutions after 24 hours by student's t-test ($P<0.001$). There was no further degradation after 48 hours.

Figure 4.2 The Stability of Pyridoxine in Saline 0.9% Solution



Pyridoxine 5.0mg in 1L saline 0.9% solution in the light

Pyridoxine 5.0mg in 1L saline 0.9% solution in the dark

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution in the dark.

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution in the light.

Pyridoxine in Saline 0.9% was stable in both the light and the dark. Pyridoxine, in Solivito, in saline 0.9% was similarly stable in the dark. In the light, pyridoxine, in Solivito was statistically significantly degraded compared to pyridoxine in the above solutions after 24 hours by student's t-test ($P<0.001$). Degradation was complete after 14 days.

degradation was observed in dextrose 10% infusion solution, but pyridoxine, in Solivito, was significantly more stable in dextrose infusion fluid than in saline 0.9% solution (see Figs 4.3 and 4.4)

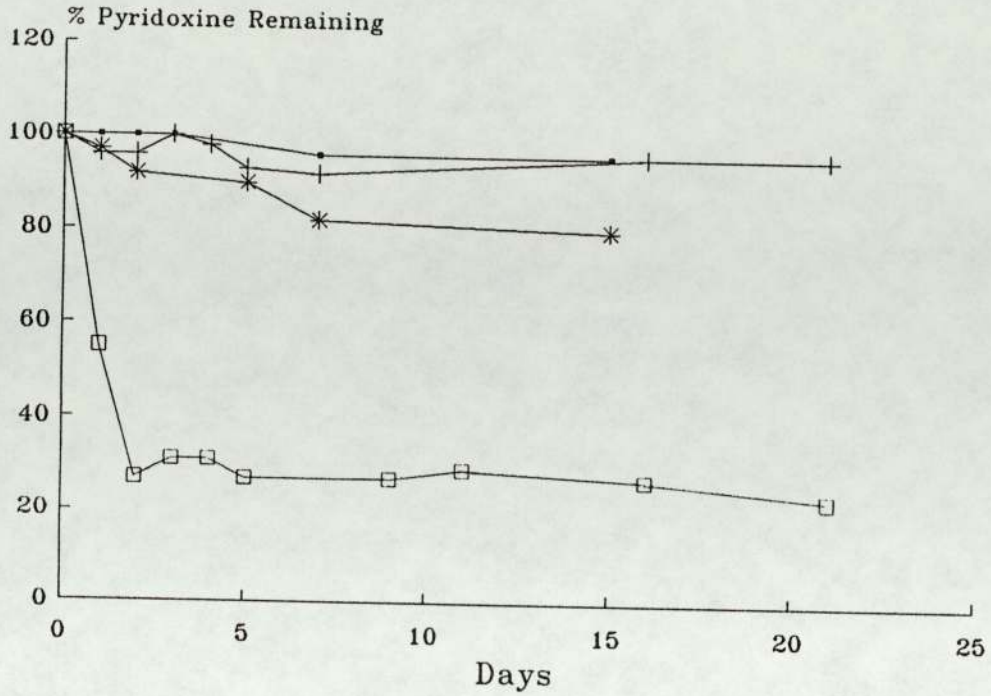
The stability of pyridoxine, in Solivito, increased with increasing Addamel concentration (see figures 4.5,4.6). In saline 0.9% solution, as the concentration was increased from 10ml/L to 30ml/L there was a statistically significant improvement in the stability of pyridoxine ($P<0.001$) (see fig 4.5). A similar pattern of pyridoxine stability was observed in dextrose 10% solution, however, changes were not statistically significant (figure 4.6).

The stability of pyridoxine, in the absence of other water soluble vitamins, was not affected by the addition of Addamel over a 15 day period, in both the light and the dark (see figures 4.7,4.8).

Pyridoxine was resistant to degradation in most amino acid solutions (see figures 4.9-4.13). There was no significant degradation in Aminoplex 24 solution diluted with either saline 0.9% or dextrose 10% solution (see figure 4.9). In Vamin 18 solution, pyridoxine on its own showed a similar pattern of stability. Pyridoxine, in Solivito, however, showed some degradation after a 15 day period. This was not statistically significant compared to pyridoxine, in the absence of other water soluble vitamins (see figure 4.10).

In Vamin 14 solution, pyridoxine significantly degraded, both on its own, and in Solivito, though there was no significant change in degradation due to the other vitamins in Solivito (see figure 4.11).

Figure 4.3 The Stability of Pyridoxine (In Solivito) in Dextrose Solution Containing Addamel



Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution with 10ml/L Addamel in the dark.

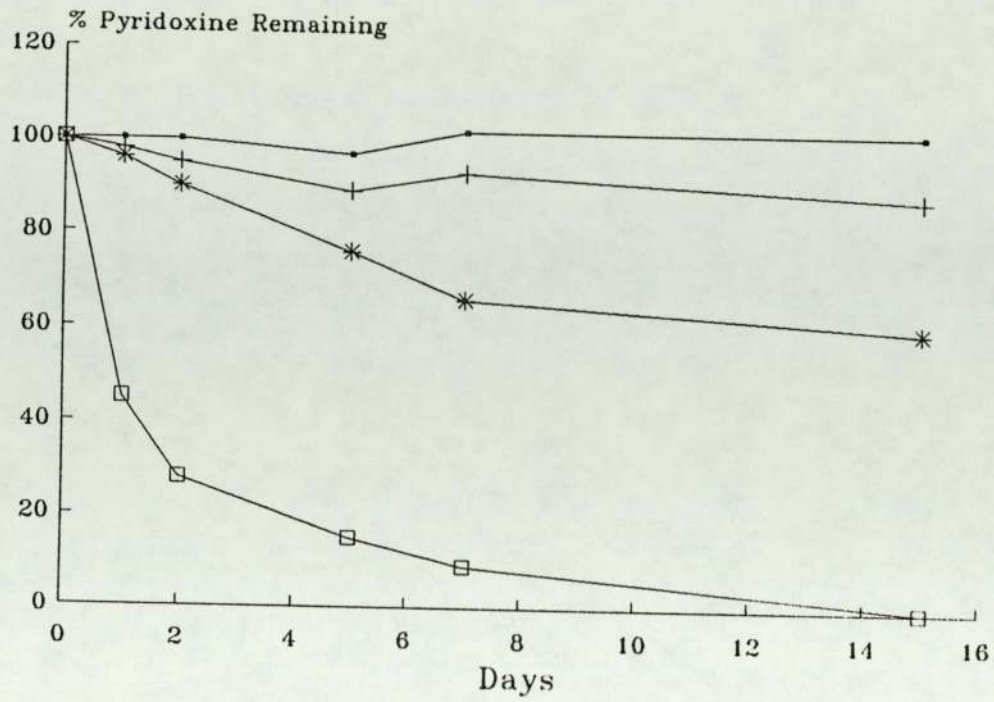
Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution in the dark.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution with 10ml/L Addamel in the light.

Pyridoxine, in Solivito, 1 vial in 1L in dextrose 10% solution in the light

Pyridoxine, in Solivito, with Addamel in dextrose 10% solution was statistically significantly more stable than an identical solution not containing Addamel by students t-test ($P<0.001$). In the dark, Addamel had no effect on the stability of pyridoxine.

Figure 4.4 The Stability of Pyridoxine (In Solivito) in Saline 0.9% Solution Containing Addamel



Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution in the dark.

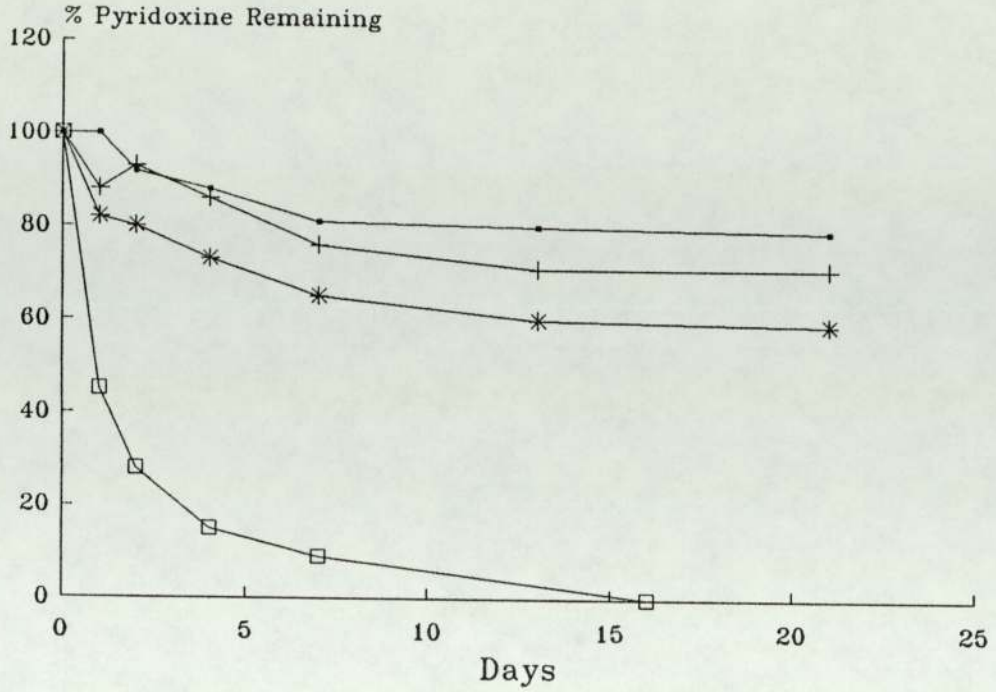
Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution with 10 ml/L Addamel in the dark

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution with 10ml/L Addamel in the light.

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution in the light.

In the light, pyridoxine, in Solivito, with Addamel in saline 0.9% solution was statistically significantly more stable than in an identical solution not containing Addamel by the student's t-test ($P<0.001$). In the dark pyridoxine, in Solivito, with Addamel in saline 0.9% solution was statistically significantly less stable than in an identical solution not containing Addamel, after 7 days by student's t-test ($P<0.05$).

Figure 4.5 The Stability of Pyridoxine in Saline 0.9% Solution Containing Varying Concentrations of Addamel in the Light.



Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution with 30ml/L Addamel.

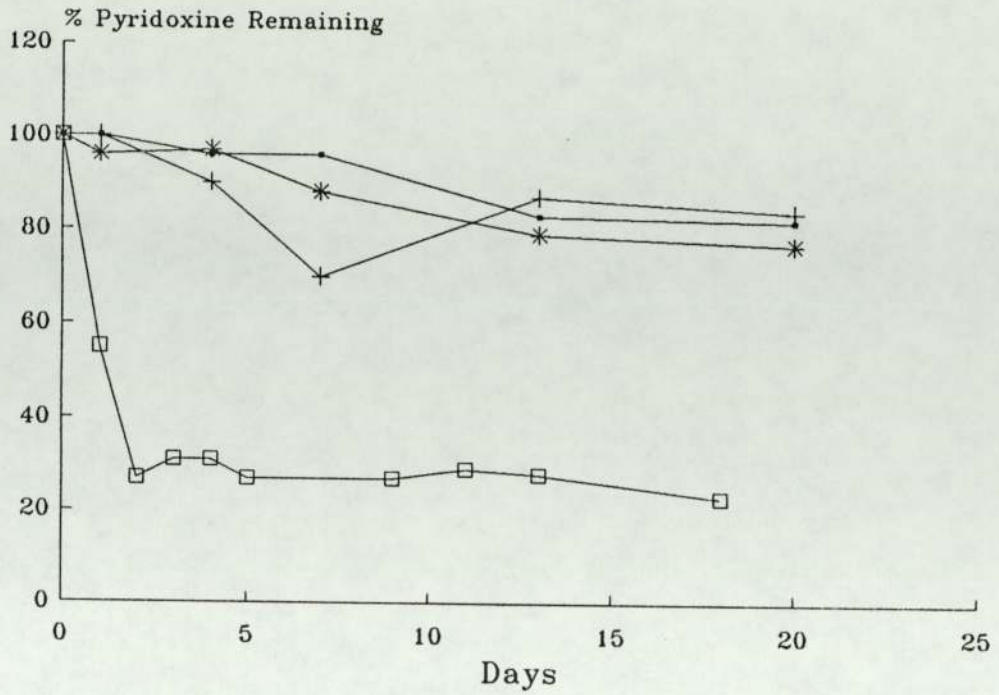
Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution with 20ml/L Addamel.

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution with 10ml/L Addamel.

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution.

As the concentration of Addamel increased, the more stable pyridoxine became, in the presence of water soluble vitamins. The addition of 30 ml/L Addamel statistically significantly increased the stability of pyridoxine compared to the addition of 10ml/L Addamel after 24 hours, by student's t-test ($P<0.001$).

Figure 4.6 The Stability of Pyridoxine in Dextrose 10% Infusion Fluid Containing Varying Concentrations of Addamel in the Light.



Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution with 30ml/L Addamel.

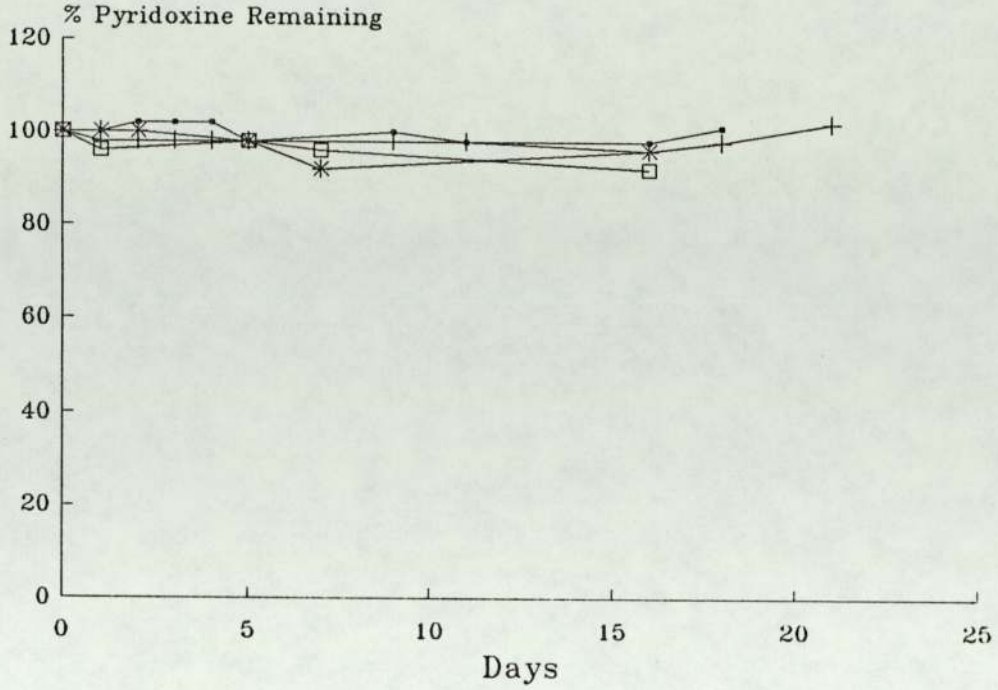
Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution with 20ml/L Addamel.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution with 10ml/L Addamel.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution.

As the concentration of Addamel was increased, then the stability was only slightly increased. No statistically significant difference was shown between the stability curves by student's t-test.

Figure 4.7 The Stability Of Pyridoxine In Dextrose 10% Solution Containing Addamel



Pyridoxine 5.0mg in 1L saline 0.9% solution in the light.

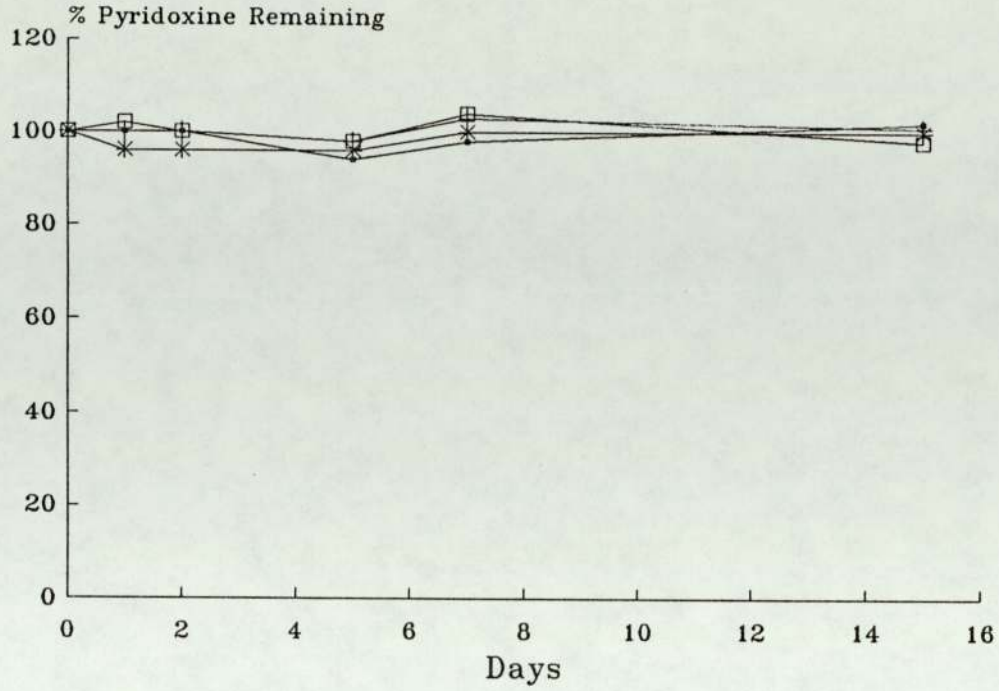
Pyridoxine 5.0mg in 1L saline 0.9% solution in the dark.

Pyridoxine 5.0mg + 10ml Addamel in 1L saline 0.9% solution in the light.

Pyridoxine 5.0mg + 10ml Addamel in 1L saline 0.9% solution in the dark.

Addamel had no effect on the stability of pyridoxine in dextrose 10% solution in either the light or the dark. No statistically significant difference was shown between the stabilities of either solution in the light or dark by student's t-test.

Figure 4.8 The Stability of Pyridoxine in Saline 0.9% Infusion Solution Containing Addamel



Pyridoxine 5.0mg in 1L saline 0.9% solution in the light.

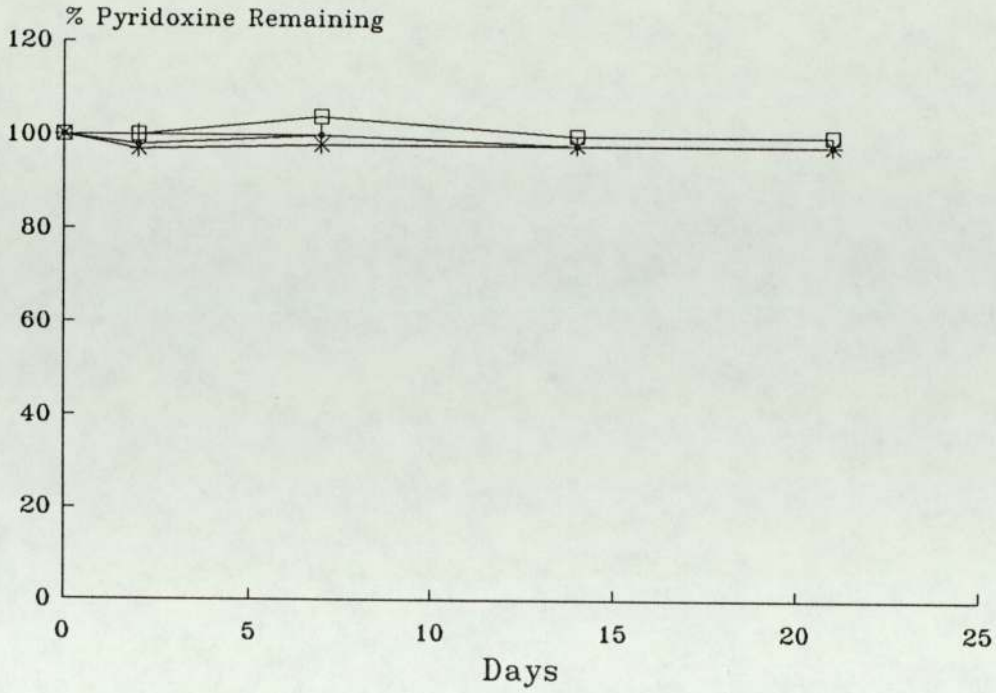
Pyridoxine 5.0mg + 10ml Addamel in 1L saline 0.9% solution in the light.

Pyridoxine 5.0mg + 10ml Addamel in 1L saline 0.9% solution in the dark.

Pyridoxine 5.0mg in 1L saline 0.9% solution in the dark.

Addamel had no effect on the stability of pyridoxine in dextrose 10% solution in either the light or the dark. No statistically significant difference was shown between the stability of either solution in the light or dark by student's t-test.

Figure 4.9 The Stability of Pyridoxine in Aminoplex 24 Diluted With Dextrose 10% Solution or Saline 0.9% solution in the Light



Pyridoxine 5.0mg in 500ml 30% Aminoplex 24 in dextrose 10% solution

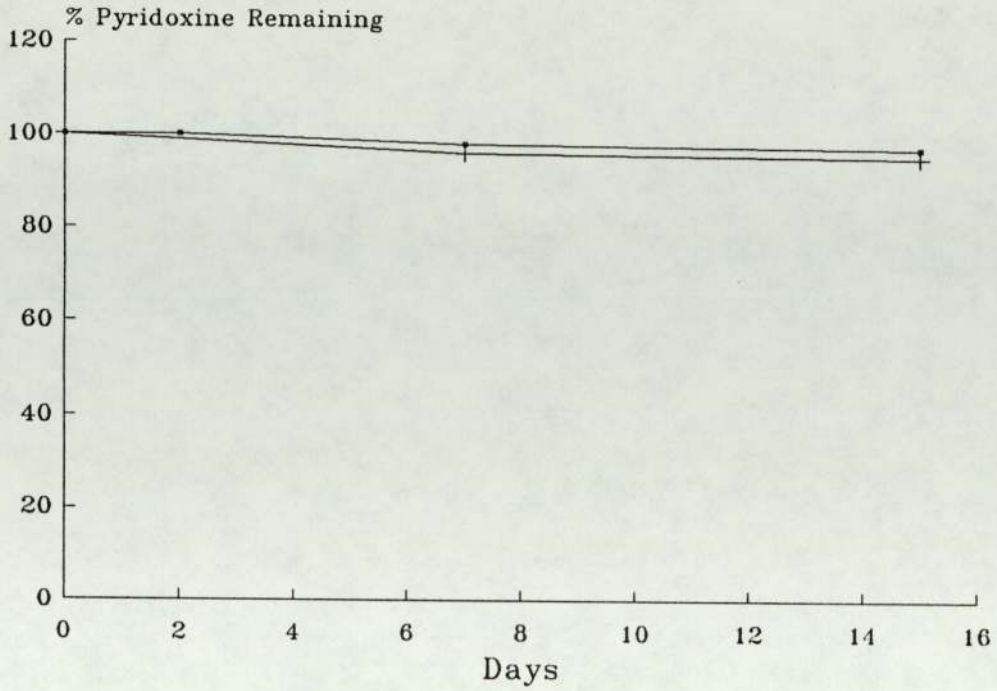
Pyridoxine, in Solivito, 1 vial in 500ml 30% Aminoplex 24 in saline 0.9% solution

Pyridoxine,in Solivito, 1 vial in 500ml 30% Aminoplex 24 in saline 0.9% solution

Pyridoxine 5.0mg in 500ml 30% Aminoplex 24 in saline 0.9% solution

Pyridoxine showed no statistically significant degradation in Aminoplex 24 solutions diluted with either saline 0.9% or dextrose 10% solutions (by student's t-test)

Figure 4.10 The Stability Of Pyridoxine in Vamin 18 Amino Acid Solution

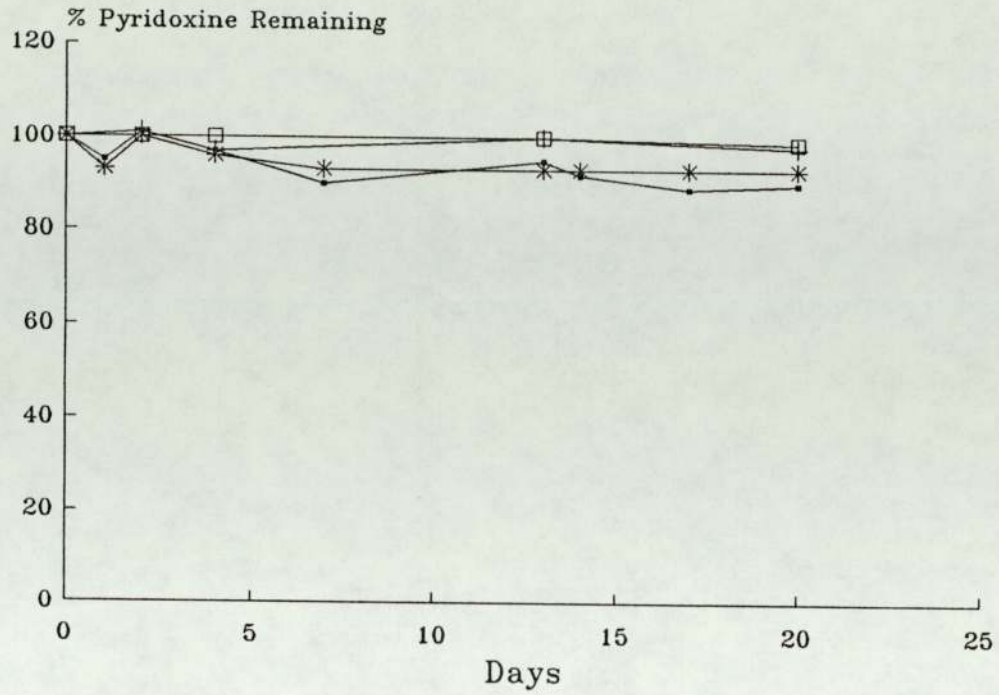


Pyridoxine, 10mg in 1L 30% Vamin 18 in saline 0.9% solution, in the light.

Pyridoxine, in Solivito, 1 vial in 1L 30% Vamin 18 in saline 0.9% solution, in the light.

Pyridoxine in dilute Vamin 18 solution showed no significant degradation after 15 days. Pyridoxine, in Solivito, in Vamin 18 solution showed 6% degradation after 15 days. This was not statistically significantly greater compared to pyridoxine, on its own, by student's t-test, after 15 days.

Figure 4.11 The Stability of Pyridoxine in Vamin 14 Amino Acid Solution



Pyridoxine, 10mg in 1L 30% Vamin 14 in saline 0.9% in the dark.

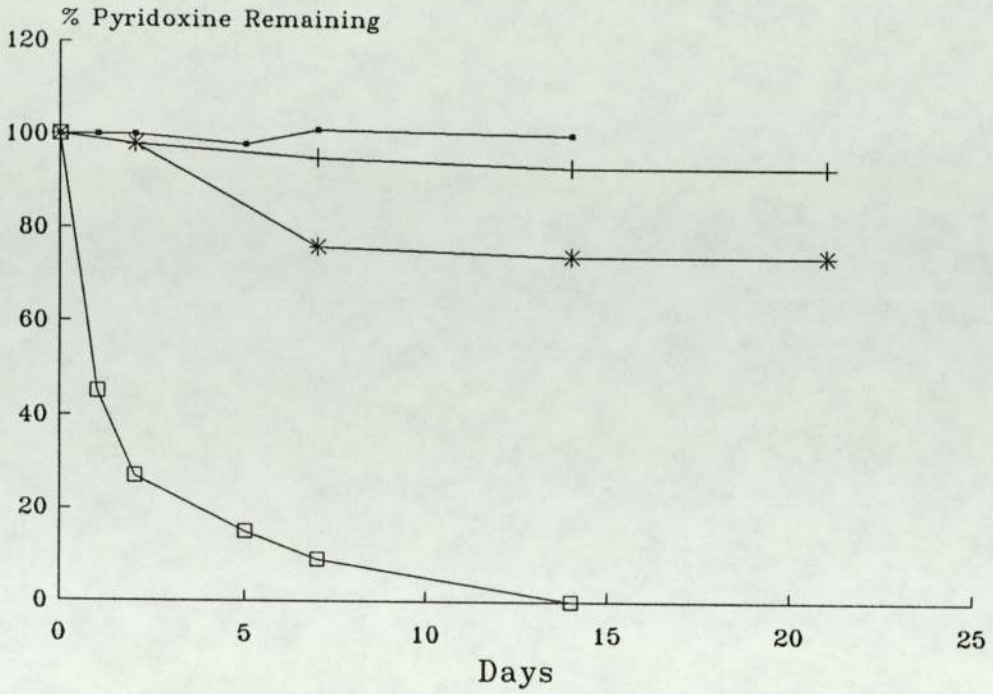
Pyridoxine, in Solivito, 1 vial in 1L 30% Vamin 14 in saline 0.9% in the dark.

Pyridoxine, 10mg in 1L 30% Vamin 14 in saline 0.9% in the light.

Pyridoxine, in Solivito, 1 vial in 1L 30% Vamin 14 in saline 0.9% in the light.

Pyridoxine was stable in the dark in Vamin 14 solution. In the light, pyridoxine showed some degradation, which was shown to be statistically significant compared to the degradation of pyridoxine in Vamin 14 in the dark, by student's t-test ($P<0.001$), after 21 days.

Figure 4.12 The Stability of Pyridoxine in Aminoplex 12 Amino Acid Solution



Pyridoxine, 5mg in 1L saline 0.9% in the light.

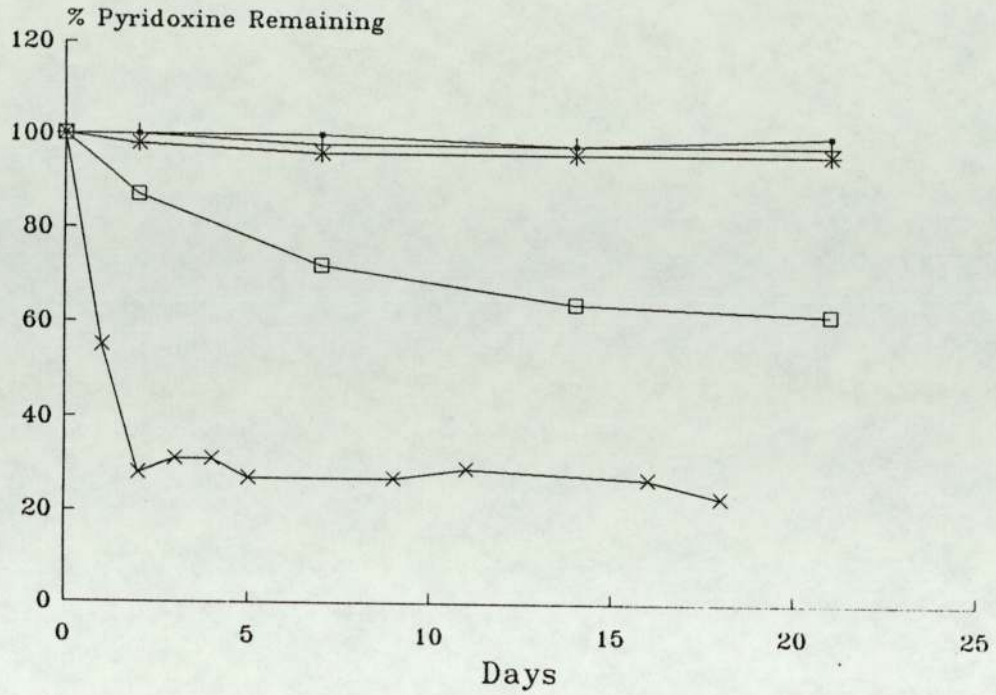
Pyridoxine, 10mg in 1L 30% Aminoplex 12 in saline 0.9% in the light.

Pyridoxine, in Solivito, 1 vial in 1L 30% Aminoplex 12 in saline 0.9% in the light.

Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% in the light.

Pyridoxine, in Aminoplex 12 solution was statistically significantly less stable than pyridoxine in saline 0.9% by student's t-test ($P<0.001$) after 14 days. Pyridoxine, in Solivito, in Aminoplex 12 solution was statistically significantly more stable than pyridoxine, in Solivito, in saline 0.9% by student's t-test ($P<0.001$) but was statistically significantly less stable than pyridoxine, on its own, in Aminoplex 12 by student's t-test ($P<0.001$) after 18 days. It was statistically significantly less stable than pyridoxine, in Solivito, in Aminoplex 24 or Vamin 14 by student's t-test ($P<0.001$).

Figure 4.13 The Stability of Pyridoxine in Vamin 9 Glucose Amino Acid Solution



Pyridoxine, in Solivito, 1 vial in 1L 30% Vamin 9 glucose in dextrose 10% solution in the dark.

Pyridoxine, 10mg in 1L 30% Vamin 9 glucose in dextrose 10% solution in the dark.

Pyridoxine, 10mg in 1L 30% Vamin 9 glucose in dextrose 10% solution in the light.

Pyridoxine, in Solivito, 1 vial in 1L 30% Vamin 9 glucose in dextrose 10% solution in the light.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution in the light.

Pyridoxine, in Solivito, in Vamin 9 Glucose solution in the light was statistically significantly more stable than pyridoxine, in Solivito, in dextrose 10% solution in the light by student's t-test ($P<0.001$) after 2 days. Pyridoxine, on its own, in Vamin 9 Glucose solution in the light was shown to be stable, no statistically significant degradation occurring after 21 days compared to pyridoxine in Vamin 9 Glucose solution in the dark or pyridoxine, in Solivito, in dextrose 10% solution, in the dark.

Significant degradation of pyridoxine occurred in Aminoplex 12 (see fig 4.12). After 7 days, 76% of the initial pyridoxine concentration remained with Solivito, but 95% remained in the absence of other water soluble vitamins.

Pyridoxine in Solivito was significantly less stable in Vamin 9 glucose compared to the other amino acid solutions, 71% remaining after 7 days (see figure 4.13).

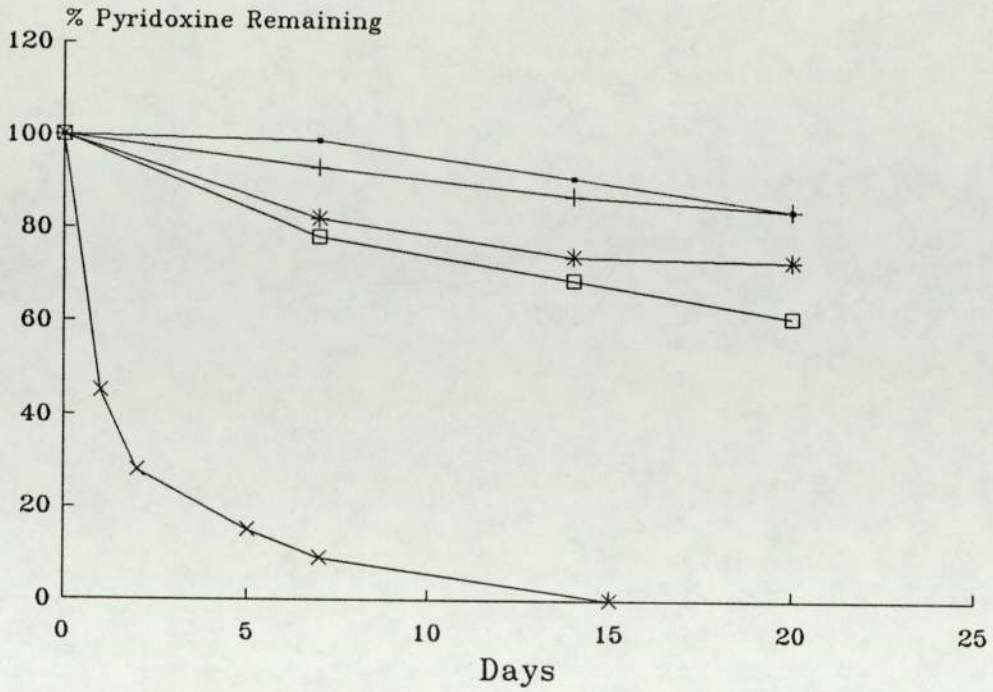
Intralipid was shown to stabilise pyridoxine to the effect of light (see figures 4.14-4.18). After 7 days in the light (fluorescent light) in undiluted Intralipid 10%, 93% of the initial concentration of pyridoxine in Solivito remained, compared to just 9% in saline 0.9% in the light. Degradation of pyridoxine in the dark, in undiluted Intralipid 10%, was similar to that in the light (see figure 4.14).

In dilute Intralipid 10% (Intralipid 10% diluted to 15%v/v with saline 0.9%) there was a significant increase in degradation, 82% of the pyridoxine remaining after 7 days compared to 93% in undiluted Intralipid 10% in the light. When an identical solution of Solivito in dilute Intralipid 10% was stored by a window receiving sunlight, the rate of degradation was faster than in the fluorescent light (see figure 4.14)

Vitlipid had little effect on the stability of pyridoxine, in Solivito, in fluorescent light or in diffused sunlight (see figure 4.15).

Pyridoxine, on its own, was shown to be more unstable in Intralipid 10% than in dextrose 10% or saline 0.9% infusion solutions, in fluorescent light (see figure 4.16). In saline 0.9%, 98% of the initial concentration of pyridoxine remained compared to 94% in undiluted Intralipid 10%. This increased rate of degradation in Intralipid 10% was statistically significantly higher than in saline 0.9% after 7 days

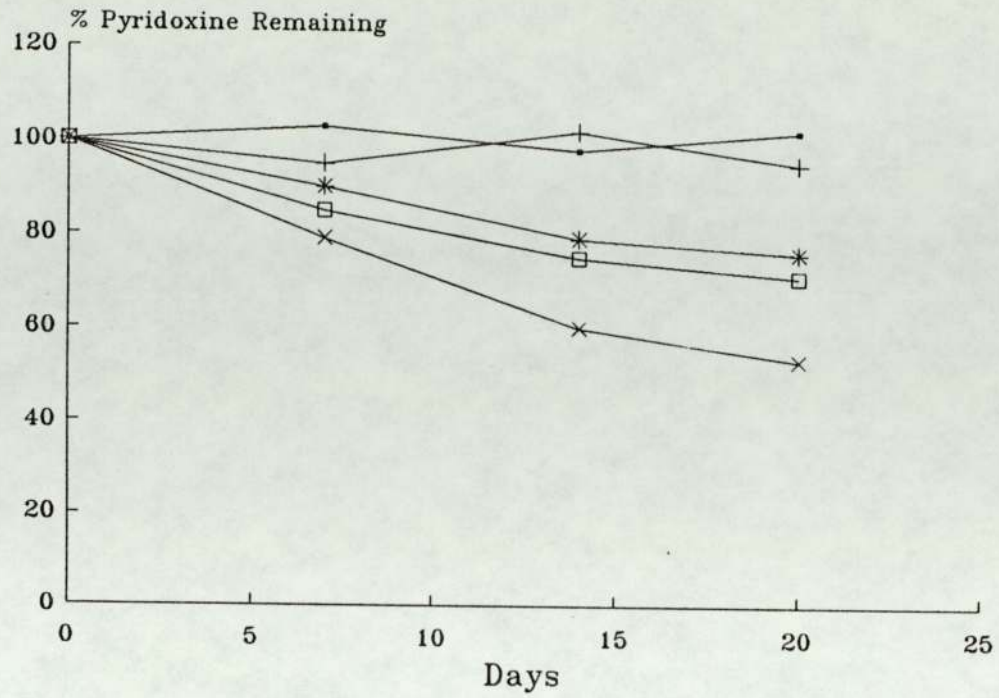
Figure 4.14 The Stability of Solivito in Intralipid 10%



- Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% in the dark.
- Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% in the light.
- Pyridoxine, in Solivito, 1 vial in 500ml 15% v/v Intralipid 10% in saline 0.9% in the light.
- Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% in diffused sunlight.
- Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% in the light.

Pyridoxine, in Solivito, in Intralipid 10% was statistically significantly more stable compared to pyridoxine, in Solivito, in saline 0.9% by student's t-test ($P<0.001$). No statistically significant difference in the stability of pyridoxine, in Solivito,in Intralipid 10% was shown in either the light or the dark. Pyridoxine, in Solivito, in diluted Intralipid 10% was statistically significantly less stable than pyridoxine, in Solivito, in Intralipid 10% by student's t-test ($P<0.001$) after 7 days. In diffused sunlight, pyridoxine, in Solivito, in Intralipid 10% was statistically significantly less stable compared to pyridoxine, in Solivito,in Intralipid 10% under all experimental conditions by student's t-test ($P<0.001$) after 7 days. Pyridoxine, in Solivito, in Intralipid 10% was statistically significantly more stable than pyridoxine, in Solivito, in saline 0.9%, by student's t-test ($P<0.001$).

Figure 4.15 The Stability Of Solivito in Intralipid 10% With Vitlipid



Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% with 10ml Vitlipid in the dark.

Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% with 10ml Vitlipid in the light.

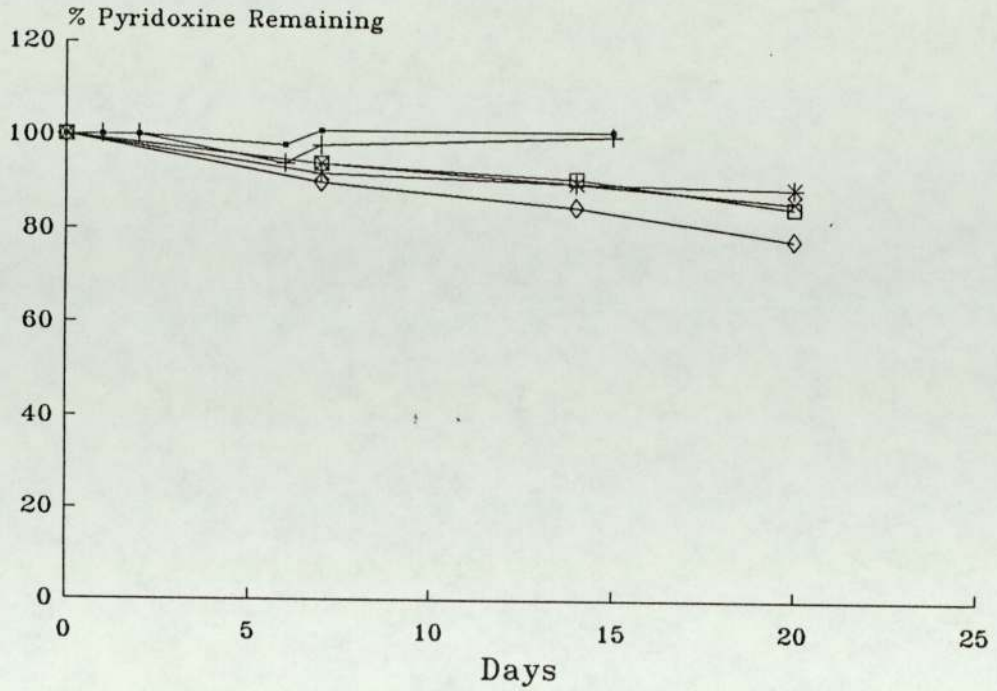
Pyridoxine,in Solivito, 1 vial in 500ml 15%v/v Intralipid 10% in saline .9% with 10ml Vitlipid,10ml Addamel in diffused sunlight

Pyridoxine, in Solivito, 1 vial in 500ml 15%v/v Intralipid 10% in saline 0.9% with 10ml Vitlipid in the light.

Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 10% with 10ml Vitlipid in diffused sunlight.

Pyridoxine, in Solivito, in Intralipid 10% with Vitlipid in the light slowly degraded at a rate which was statistically significantly greater than the degradation of pyridoxine in an identical solution in the dark after 20 days by student's t-test ($P<0.005$). Pyridoxine, in Solivito, in dilute Intralipid with Vitlipid in the light was statistically significantly less stable than in Intralipid with Vitlipid in the light after 7 days by student's t-test ($P<0.001$). In diffused sunlight, pyridoxine, in Solivito, in Intralipid with Vitlipid was statistically significantly less stable than in either Intralipid or diluted Intralipid in the light after 7 and 14 days respectively by student's t-test ($P<0.001$). The addition of Addamel to this solution statistically significantly increased the stability of pyridoxine by student's t-test after 7 days ($P<0.001$).

Figure 4.16 The Stability of Pyridoxine in Intralipid 10% Containing Vitlipid



Pyridoxine 5.0mg in 1L saline 0.9% in the dark.

Pyridoxine 5.0mg in 1L saline 0.9% in the light.

Pyridoxine 5.0mg in 500ml Intralipid 10% with 10ml Vitlipid in the light.

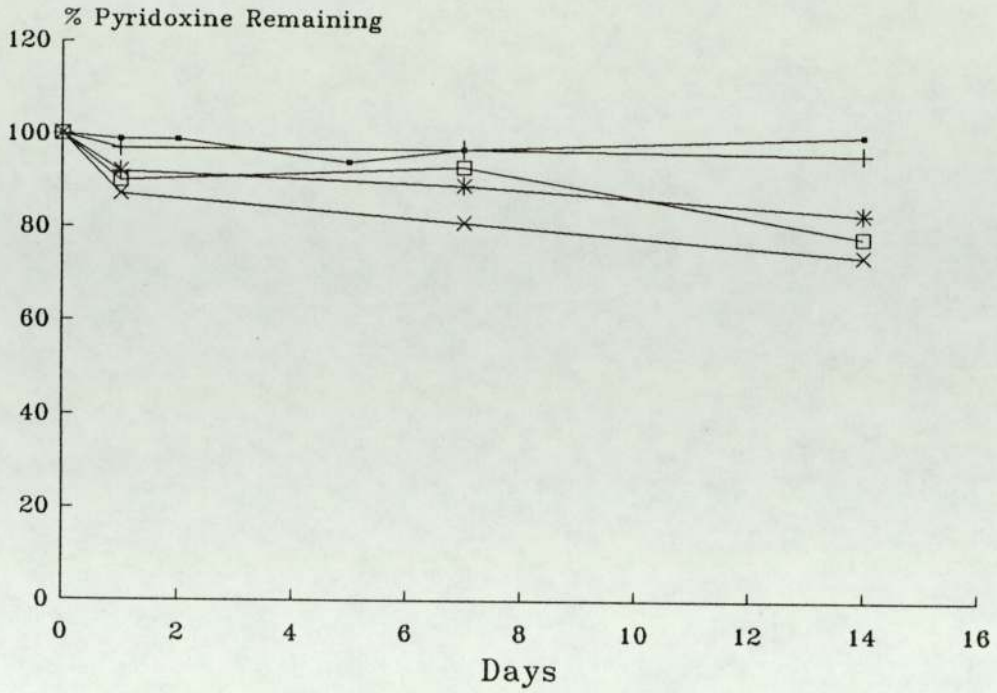
Pyridoxine 5.0mg in 500ml 15%v/v Intralipid 10% in saline 0.9% with 10ml Vitlipid and 10ml Addamel in diffused sunlight.

Pyridoxine 5.0mg in 500ml Intralipid 10% with 10ml Vitlipid in the dark.

Pyridoxine 5.0mg in 500ml 15%v/v Intralipid 10% in saline 0.9% with 10ml Vitlipid in diffused sunlight.

Pyridoxine in Intralipid 10% with Vitlipid showed a similar slow degradation in both the light and the dark. This degradation was statistically significantly greater than in saline 0.9% in the light or dark after 14 days by student's t-test ($P<0.001$). Pyridoxine in diluted Intralipid 10% with Vitlipid in diffused sunlight was statistically significantly less stable than in Intralipid 10% in the light after 20 days by student's t-test. The addition of Addamel to this solution statistically significantly increased the stability of pyridoxine compared to the stability of pyridoxine in an identical solution without Addamel by student's t-test ($P<0.001$).

Figure 4.17 The Stability of Pyridoxine in Intralipid 20% Containing Vitlipid



Pyridoxine 5.0mg in 1L saline 0.9% solution in the light.

Pyridoxine 5.0mg in 500ml Intralipid 20% with 10ml Vitlipid in the light.

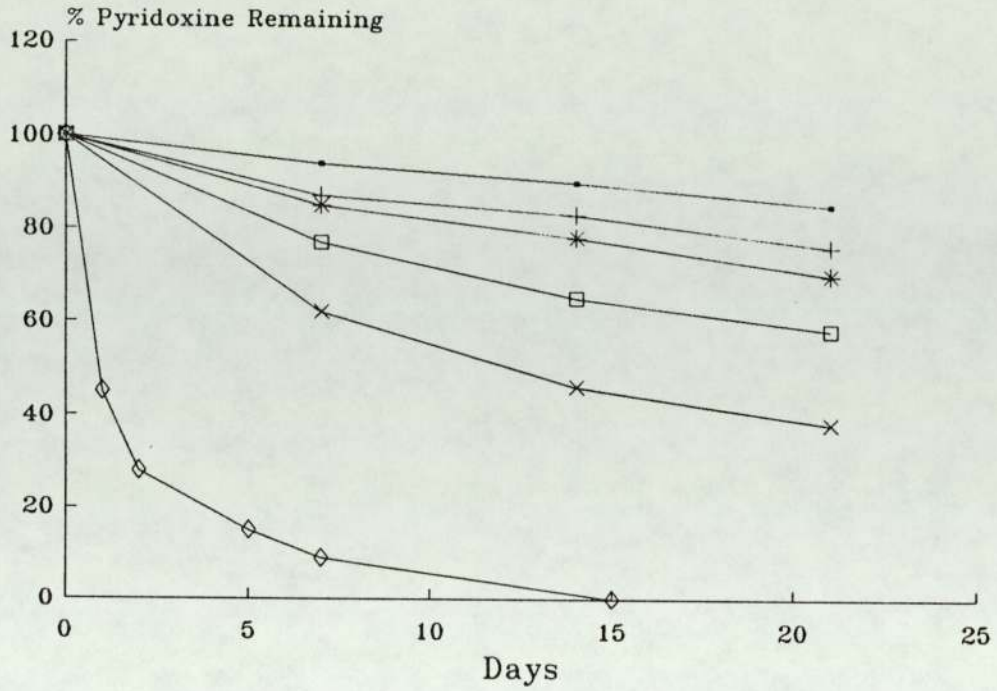
Pyridoxine 5.0mg in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid in the light.

Pyridoxine 5.0mg in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid and 10ml Addamel in diffused light.

Pyridoxine 5.0mg in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid in diffused sunlight.

Pyridoxine, in Intralipid 20% with 10ml Vitlipid showed a slow degradation in the light which was not statistically significant compared to the degradation of pyridoxine in saline 0.9% in the light, by student's t-test. Pyridoxine in diluted Intralipid 20% with Vitlipid was statistically significantly less stable than pyridoxine in undiluted Intralipid 20% after 14 days by student's t-test ($P<0.001$). In diffused sunlight, pyridoxine in diluted Intralipid 20% with Vitlipid was statistically significantly less stable than pyridoxine in diluted Intralipid 20% with Vitlipid in the light after 14 days by student's t-test. The addition of Addamel did not significantly alter the stability of pyridoxine in diluted Intralipid 20% with Vitlipid in diffused sunlight.

Figure 4.18 The Stability of Solivito in Intralipid 20% containing Vitlipid



- Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 20% with 10ml Vitlipid in the light.
- Pyridoxine, in Solivito, 1 vial in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid in the light
- Pyridoxine, in Solivito, 1 vial in 500ml Intralipid 20% with 10ml Vitlipid in diffused sunlight.
- Pyridoxine, in Solivito, 1 vial in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid and 10ml Addamel in diffused sunlight
- Pyridoxine, in Solivito, 1 vial in 500ml 15%v/v Intralipid 20% in saline 0.9% with 10ml Vitlipid in diffused sunlight
- Pyridoxine, in Solivito, 1 vial in 1L saline 0.9% solution in the light.

Pyridoxine, in Solivito, in Intralipid 20% with Vitlipid showed a slow degradation in the light. This was statistically significantly less than pyridoxine, in Solivito, in saline 0.9% in the light by student's t-test ($P<0.001$). Pyridoxine, in Solivito in diluted Intralipid 20% with Vitlipid in the light was statistically significantly less stable than in undiluted Intralipid 20% with Vitlipid in the light after 14 days by student's t-test ($P<0.001$). Pyridoxine, in Solivito, in diluted Intralipid 20% in diffused sunlight was statistically significantly less stable than in an identical solution in the light after 7 days by student's t-test ($P<0.001$). The addition of Addamel statistically significantly increased the stability of pyridoxine, in Solivito, in diluted Intralipid 20% in diffused sunlight compared to the stability of pyridoxine in an identical solution without Addamel after 7 days by student's t-test ($P<0.001$).

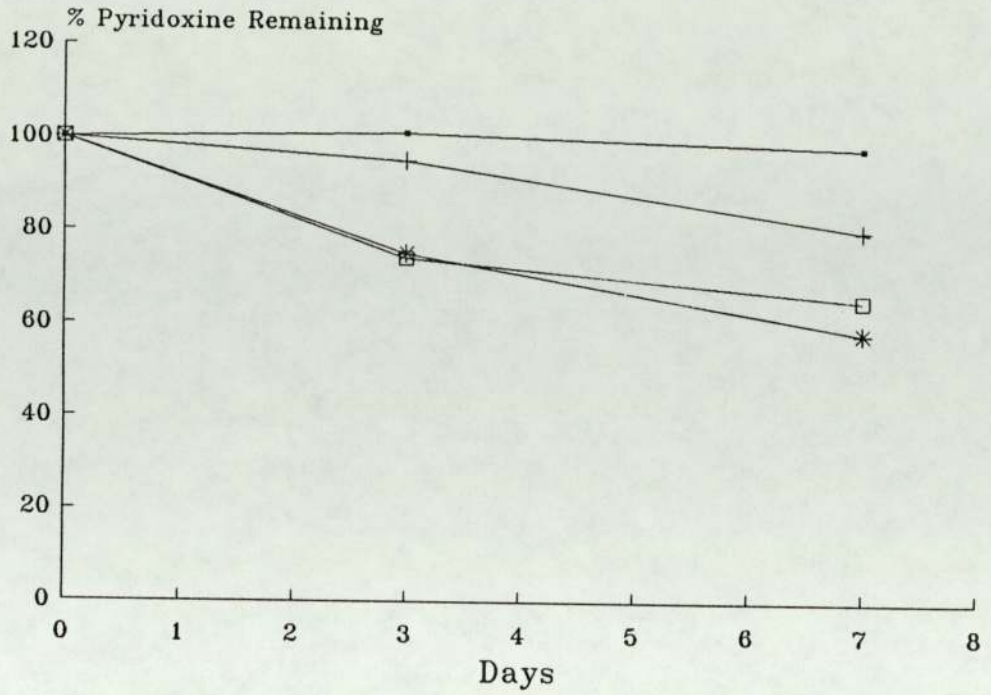
(P < 0.001). In dilute Intralipid 10%, in diffused sunlight, the rate of degradation of pyridoxine was only slightly increased compared to that in undiluted Intralipid. After 14 days 84% of the initial concentration of pyridoxine remained in dilute Intralipid in diffused sunlight 10%, but 89% remained in undiluted Intralipid 10% in fluorescent light. Thus the dilution of Intralipid and the increased light intensity increased the rate of degradation to a small extent. However, this increased rate of degradation was statistically significant (P <0.001).

The stability of pyridoxine, on its own, in Intralipid 20% was similar to that in Intralipid 10% (see figs 4.16,4.17). Similarly the stability of pyridoxine, in Solivito, in Intralipid 20% was similar to that in Intralipid 10% in fluorescent light (see fig 4.18). In diffused light, however, degradation of pyridoxine, in Solivito, was greater in undiluted Intralipid 20%. In Intralipid 10%, after 7 days, 78% of the initial concentration of pyridoxine remained compared to 85% in Intralipid 20%. This increase in rate was shown to be statistically significant (P < 0.001).

Diffused sunlight had a much greater effect on the stability of pyridoxine than dilution. In Intralipid 10%, dilution increased degradation after 7 days from 7% in undiluted Intralipid 10% to 17% in dilute Intralipid 10%, in fluorescent light, whereas in undiluted Intralipid 10% in diffused light, the rate of degradation was 22% in 7 days (see fig 4.17). This difference was statistically significant (P < 0.001).

Addition of Addamel to Intralipid stabilised pyridoxine. In dilute Intralipid 20%, after 7 days, in diffused sunlight, 77% of the initial concentration of pyridoxine, in Solivito, remained with Addamel present, but only 61% remained without Addamel (See fig 4.18).

Figure 4.19 The Stability of Pyridoxine in TPN Solutions Containing Intralipid



Pyridoxine, 2.0mg in 2.5L of a TPN solution containing Vamin 14 in the light.

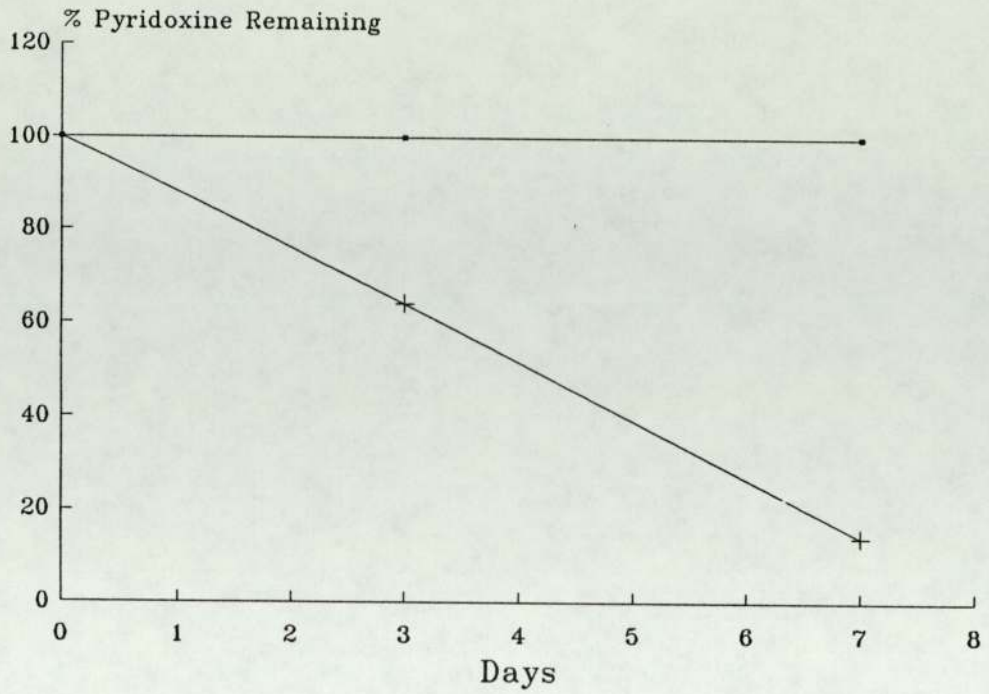
Pyridoxine, 2.0mg in 2.5L of a TPN solution Aminoplex 12 in the light.

Pyridoxine, 2.0mg in 2.5L of a TPN solution containing Vamin 14 in diffused sunlight.

Pyridoxine, 2.0mg in 2.5L of a TPN solution Aminoplex 12 in diffused sunlight.

Pyridoxine was stable in a TPN solution containing Vamin 14 in the light over a 7 day period. Pyridoxine in a similar TPN solution containing Aminoplex 12 in the light was statistically significantly less stable than pyridoxine in the TPN solution containing Vamin 14 in the light after 14 days by students t-test ($P<0.001$). In diffused sunlight, the stability of pyridoxine in the TPN solutions containing Vamin 14 or Aminoplex 12 was similar but statistically significantly less stable than in identical solutions in the light after 3 days by student's t-test ($P<0.001$).

Figure 4.20 The Stability of Pyridoxine in a TPN Solution Containing Vamin 14 And No Intralipid



Pyridoxine, 2.0mg in 2.5L of a TPN solution containing Vamin 14 protected from diffused sunlight by a black plastic liner.

Pyridoxine, 2.0mg in 2.5L of a TPN solution containing Vamin 14 with no protection from diffused sunlight.

Pyridoxine was stable in the TPN solution protected from diffused sunlight over a 7 day period. With no protection from light, pyridoxine rapidly degraded in the TPN solution in diffused sunlight.

The stability of pyridoxine was followed in sample TPN solutions. In a TPN solution containing Vamin 14, there was no significant change in pyridoxine concentration over a 7 day period (See fig 4.19). However in Aminoplex 12, significant degradation occurred over a 7 day period, only 80% of the initial concentration remaining.

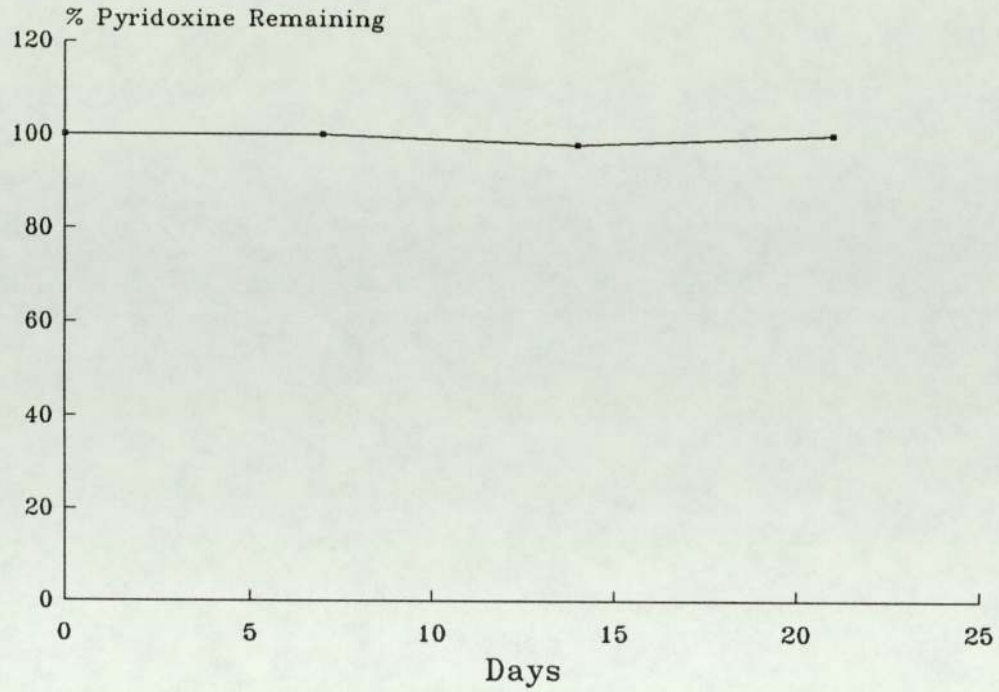
When identical solutions were left in diffused sunlight the rate of degradation was significantly increased compared to that in flourescent light. After 7 days, 58% of the original concentration of pyridoxine remained in the TPN solution containing Aminoplex 12 (see figure 4.19).

If the Intralipid in the TPN solution is omitted and replaced with dextrose 10% solution and then exposed to diffused sunlight, rapid degradation ensued, 14% of the original pyridoxine concentration remaining after 7 days. The omission of Intralipid significantly increased the rate of degradation (Compare figure 4.19 and 4.20). If an identical TPN solution was protected from light by a black plastic liner, no significant degradation occurred over a 7 day period.

In the refrigerator, stored between 4 and 8 °C, pyridoxine showed no degradation in a TPN solution containing Vamin 14 after a 21 day period (see figure 4.21).

A brief experiment was carried out to determine the effect of riboflavin on pyridoxine stability. There was a significant increase in the rate of degradation of pyridoxine between solutions containing 1.0mg/L and 5.0mg/L of riboflavin, However the change in rate of degradation was small compared to the degradation of pyridoxine in a solution containing 10 mg/L riboflavin, where no pyridoxine was detectable after 24 hours (see figure 4.22).

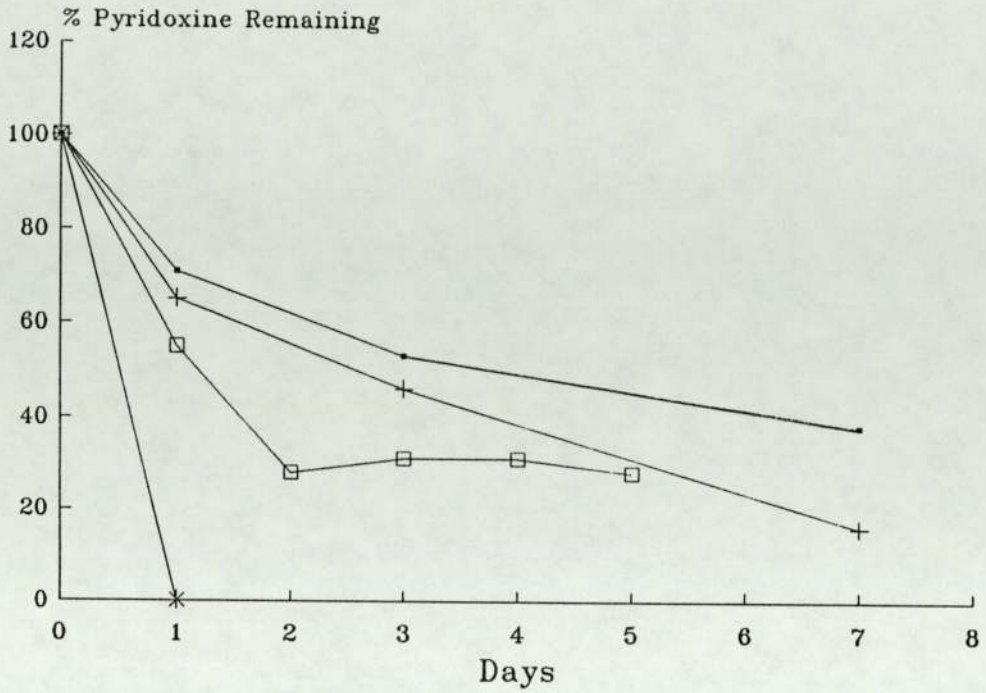
Figure 4.21 The Stability of Pyridoxine in a TPN Solution Containing Vamin 14 Stored in a Refrigerator



Pyridoxine, 2.0mg in 2.5L of a TPN solution containing Vamin 14 and Intralipid 20%

Pyridoxine was stable in a TPN solution stored in the refrigerator at 4-8°C over a 21 day period.

Figure 4.22 The Effect Of Riboflavin Concentration On The Stability Of Pyridoxine



Pyridoxine, 5.0mg in 1L dextrose 10% solution containing 1.0mg riboflavin.

Pyridoxine, 5.0mg in 1L dextrose 10% solution containing 5.0mg riboflavin.

Pyridoxine, in Solivito, 1 vial in 1L dextrose 10% solution.

Pyridoxine, 10.0mg in 1L dextrose 10% solution containing 10.0mg riboflavin.

Pyridoxine degraded in dextrose 10% solution containing riboflavin, in the light. The rate of degradation of pyridoxine increased with increasing riboflavin concentration. There was only a small increase in degradation of pyridoxine at low concentrations of riboflavin, but above 5.0mg/L riboflavin, there was rapid increase such that at 10mg/L riboflavin, no pyridoxine remained after 24 hours. In a solution of Solivito, in dextrose 10% solution, the degradation of pyridoxine was statistically significantly increased compared to the degradation of pyridoxine in dextrose solution containing riboflavin 5.0mg/L after 24 hours by student's t-test ($P < 0.001$).

Pyridoxine has been known to be unstable in the light since it was discovered, particularly in alkaline solution. There is little published evidence concerning the stability in various solutions for intravenous use. Mizuno et al (52) described the effects of individual amino acids and riboflavin on the stability of pyridoxine. Riboflavin was found to increase the degradation of pyridoxine, though amino acids had varying effects on pyridoxine stability. Chen et al (53) concluded that pyridoxine in a TPN solution not containing fat emulsion was stable in fluorescent light and diffused sunlight but not direct sunlight.

Pyridoxine was stable to the effect of light, in the absence of other water soluble vitamins in all infusion fluids tested except Intralipid. In Intralipid infusion fluid, there was shown to be a slow degradation that appeared to be independent of light. This can be compared to the results of Mizuno et al (52) who concluded that photodecomposition should be considered in solutions of pyridoxine below 0.5mM or 100mg/L. The solutions tested in this study were all in the range 1 to 10 mg/L or one tenth of this concentration.

In the presence of water soluble vitamins, pyridoxine degraded rapidly within the first 48 hours, in the light. This degradation proceeded to completion in saline 0.9% solution but stabilised at residual 27% of the initial concentration in dextrose 10% solution. The reason for this difference is unclear. The dextrose solution is slightly more acidic than saline 0.9% and this may stabilise pyridoxine towards degradation. However the rate of degradation of pyridoxine during the initial 48 hours was similar in saline 0.9% and dextrose 10% solution.

In both dextrose 10% and saline 0.9% infusion fluids, the estimated T_{90} or the time taken for the initial concentration to decline by 10% was four hours for pyridoxine, in Solivito. Degradation in saline 0.9% solution was shown to be consistent with a second order model. A plot of reciprocal concentration versus time yielding a good linear regression (see figure 4.23).

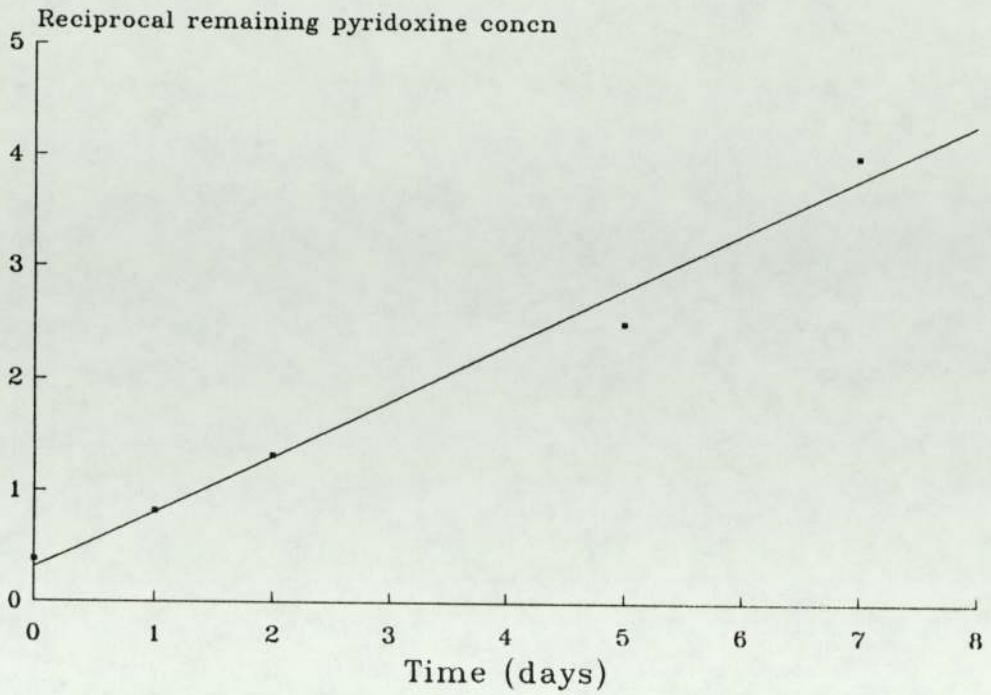
Solutions of Solivito in both dextrose 10% and saline 0.9% were an intense yellow colour when first reconstituted. This is mainly due to riboflavin present in the multivitamin injection. This yellow colour rapidly decreased in intensity, paralleling degradation of pyridoxine. Mizuno et al[52] found that riboflavin increased the rate of degradation of pyridoxine. It is likely that riboflavin is the major 'photosensitiser' of pyridoxine to the effect of light. Other vitamins may also photosensitise pyridoxine as indicated in figure 4.22.

In no experiment was a chromatogram peak corresponding to pyridoxal observed for samples taken from infusion fluids. This suggests a photo-induced dimerisation rather than a photo-oxidation of pyridoxine to pyridoxal and pyridoxic acid.

The trace metal ions in Addamel, Fe^{2+}, Cu^{2+}, Zn^{2+}, stabilise pyridoxine to the effect of multivitamin induced photodecomposition. Mizuno et al showed that pyridoxine was stabilised by some trace metal ions, eg, Cu^{2+}, Zn^{2+}. This stabilisation was not observed in the presence of sodium edetate. Solivito contains sodium edetate, thus, it would be expected that trace elements, when added to a solution containing Solivito, would not affect the stability of pyridoxine.

Comparison of the association constants for copper pyridoxine and copper edetate shows them to be 2.8 (55,56) and 18.8 (57) respectively. Thus the edetate ion shows a much greater affinity for copper(II) ions than does pyridoxine.

Figure 4.23 Second Order Plot Of The Degradation Of Pyridoxine In Saline 0.9% Solution



The reciprocal of the remaining concentration of pyridoxine versus time yielded a straight line plot with a correlation coefficient of 0.99.

This would support the results reported by Mizuno et al. Without edetate present, copper(II) ions and pyridoxine combine forming a complex. This complex must be less susceptible to effect of light, or the effects of multivitamin induced photosensitisation. When edetate ions are present, these bind the copper, leaving pyridoxine susceptible to the effect of light. This would occur at the equimolar concentrations used in the work of Mizuno et al.

Solivito contains 1.5 micromoles of edetate ion, whereas there are 5 micromoles of copper(II) ions in 10ml of Addamel. There would be, therefore at least 3.5 micromoles of copper(II) ions available to complex with pyridoxine. Other metal ions may also complex with pyridoxine. These complexes may well be less susceptible to degradation by light.

Amino acids inhibited the degradation of pyridoxine. The stability of pyridoxine increased with increasing amino acid concentration. The pH of the amino acid solution significantly affected the degradation of pyridoxine. Pyridoxine was statistically significantly less stable in diluted Aminoplex 12 solution (pH 7.4) than in diluted Vamin 14 solution (pH 5.6) ($p < 0.001$). This difference is greater than expected due to the slight difference in concentration of amino acids, in each solution. Pyridoxine would be less stable to photodegradation at higher pH. Mizuno et al showed that some amino acids stabilised pyridoxine to the effect of light, e.g., valine, leucine, whilst others increased degradation to a small extent, e.g., histidine, arginine.

In amino acid solutions for Parenteral Nutrition, which contain all amino acids, there must be an overall stabilising effect. The mechanism of any physical or chemical interaction is not known.

Intralipid retarded the photodegradation of pyridoxine. The T_{90} for pyridoxine in Intralipid was in the range of 7-14 days in fluorescent light, compared to 4 hours in saline 0.9% and dextrose 10% solutions. Thus pyridoxine, in Solivito, is stable in Intralipid when Parenteral Nutrition is infused from constituent bottles over a 24 hour period.

Pyridoxine showed some degradation in the dark in Intralipid. There must, therefore, be an interaction between pyridoxine and the components of Intralipid itself. This may not mean, however, that pyridoxine would not be bioavailable. The extraction step of the assay of pyridoxine in Intralipid preparations did not involve destruction of the emulsion, only aggregation. The oily phase would not be expected to take up pyridoxine molecules, but it is possible, pyridoxine being a polar molecule, that the oil/water interface takes up pyridoxine. As the emulsion is not destroyed, the pyridoxine at this interface would not be recovered by the extraction procedure. If this does occur, it must be a slow process, occurring over several days rather than hours.

Dilution of Intralipid produced a significant increase in degradation, when other water soluble vitamins were present. This indicates that dilution enhances the penetration of light into the emulsion. Photodegradation can only occur in a very narrow layer around the emulsion. Dilution must significantly increase this 'penetration layer', exposing more pyridoxine to the effect of light.

Diffused sunlight also significantly increased degradation in Intralipid compared to that in fluorescent light. This is due to the increased intensity of light. The estimated T_{90} of pyridoxine, in Solivito, in Intralipid is 3-5 days. Thus even if exposed to sunlight, near a window, on a ward, pyridoxine would still be stable if Parenteral Nutrition was infused from constituent bottles over 24 hours.

The addition of Addamel to Intralipid preparations, again stabilised pyridoxine to the effect of light. A brown aggregate was observed within a few hours of adding Addamel to Intralipid. This indicated that a chemical and/or complexation reaction occurred between the trace metal ions and constituent of the emulsion. This would remove metal ions from interacting with pyridoxine. This did not affect the stabilisation of pyridoxine by added trace elements to Intralipid.

Pyridoxine was shown to be stable in TPN solutions containing Intralipid in fluorescent light. The estimated T_{90} in a TPN mixture containing 1L of Aminoplex 12 was 4 days. Pyridoxine is therefore stable in a TPN solution infused over 24 hours. The estimated T_{90} for pyridoxine in a TPN mixture containing Vamin 14 could not be determined as there was no significant degradation over the 7 day period during which pyridoxine stability was monitored.

If identical solutions were placed in diffused sunlight, then the estimated T_{90} for pyridoxine in TPN solutions containing Intralipid and Aminoplex 12 or Vamin 14 was about 28 hours. However, during the experiments in diffused sunlight, TPN solutions would only be subject to continuous sunlight for a few hours in the day. Thus, the estimated T_{90} may only be a few hours when subject to diffuse sunlight. This may have implications at ward level if a TPN mixture being infused into a patient was near a window and not protected from sunlight, then significant amounts of pyridoxine will be destroyed. Some vitamins such as vitamin C, riboflavin, vitamin A, are destroyed more quickly than pyridoxine, especially in sunlight, thus a patient may only receive a fraction of the vitamin content of the infusion.

When Intralipid is omitted from TPN solutions, the rate of degradation, in diffused sunlight was similar to that when Intralipid is present in the infusion. Degradation, however, had almost proceeded to completion after 7 days. Covering the

solution with a black plastic bag inhibited degradation. Thus protection of TPN mixtures from light should still be recommended, especially as some other vitamins are more susceptible to photodegradation than pyridoxine.

Pyridoxine showed no degradation under refrigerated conditions at 4-8 °C, in a TPN mixture. This would be expected as the mixture is not exposed to light. The slow decrease in pyridoxine concentration seen in the studies with Intralipid was not seen in this experiment.

A recently published study of the stability of vitamins in TPN mixtures (58), showed most vitamins, including pyridoxine to be stable both in the dark and in normal room illumination. This is similar to results presented in this study. However, the effect of diffused sunlight, and prolonged storage were not investigated.

Pyridoxine has thus been shown to be stable in the dark, and in the absence of other water soluble vitamins. In the light, with other water soluble vitamins present, pyridoxine degraded rapidly. Photosensitisation of pyridoxine by riboflavin was probably the major mechanism of degradation of pyridoxine in multivitamin infusion fluids. In an experiment investigating the effects of riboflavin on the degradation of pyridoxine, in the concentration range 1-5 mg/L riboflavin, the rate of degradation was approximately 75% that in an infusion of Solivito in dextrose 10% (where the riboflavin concentration is approximately 2mg/L, other vitamins may photosensitise pyridoxine since riboflavin did not independently induce a decrease in pyridoxine concentration as rapidly as that induced by multivitamin solution.

Riboflavin photosensitises pyridoxine probably via a free radical mechanism. A possible mechanism is shown in mechanism 1 in figure 4.24

Figure 4.24 Mechanisms By Which Riboflavin May Produce Free Radicals

Mechanism 1

Pyridoxine + Riboflavin ----------> Oxidised pyridoxine free radical + Reduced riboflavin free radical

Mechanism 2

EDTA + riboflavin ----------> Oxidised EDTA Free radical + Reduced riboflavin free radical.

Riboflavin photosensitises pyridoxine by oxidising pyridoxine and simultaneously producing free radicals. The riboflavin free radical then reduces oxygen to form superoxide and riboflavin (54). The oxidised pyridoxine free radical may then react with itself to form a dimer or with other substrates. Superoxide may also oxidise substrates in the mixture such as pyridoxine, though if this occurs it is probable that 4-pyridoxic acid would be formed which could not be detected by the assay. Superoxide may also spontaneously revert to hydrogen peroxide and oxygen.

Metal ions were found to stabilise pyridoxine. As well as possibly forming complexes with pyridoxine, metal ions may increase the spontaneous dismutation of superoxide ions to oxygen and hydrogen peroxide. Disodium edetate is also capable of photosensitisation forming edetate radicals by riboflavin (61) shown in mechanism 2 in figure 4.24. EDTA free radicals could then react with pyridoxine, forming pyridoxine free radicals. The presence of trace elements which complex with EDTA may reduce this reaction. Thus, there may be more than one mechanism by which metal ions reduce the riboflavin induced photosensitisation of pyridoxine.

If the photosensitisation of pyridoxine does occur by the above mechanism, then repeating these experiments with added superoxide dismutase and catalase would produce oxygen and reduce the amount of superoxide present. Pyridoxine degradation would then be reduced, similar to the reduction by the addition of Addamel.

In Intralipid, pyridoxine showed a slow loss in both the light and the dark from the aqueous phase. Further investigations are needed to show that this is true. This may be done by repeating the experiments, assaying the emulsion concentrate after centrifugation for pyridoxine.

CHAPTER FIVE

THE INVESTIGATION OF PYRIDOXINE REQUIREMENTS IN PATIENTS REFERRED FOR TOTAL PARENTERAL NUTRITION

Results

The Direct Assay For Pyridoxal-5-Phosphate

Singkamani et al (32) established optimal conditions for the enzyme reaction. They reported that if the pH of assay solutions was kept between pH 4.4 and pH 7.0, no significant change in the ΔOD/min of the solution occurred. In their neutralisation step of the extraction procedure, the perchlorate extract was neutralised by a fixed amount of 1 nmol/L KCl solution such that the pH of the solution was between 2.0 and 3.0. My observations were that the final pH of the extract had to be monitored carefully such that it varied only between pH 6.0 and 7.0. Although Sinkamani et al added constant volumes in the neutralising step, in this experiment the volume added had to be titrated to the pH. Approximately 150 microlitres of 2nmol/L KCl solution were added, the variation being 140 to 160 microlitres.

Linearity

Calibration curves were obtained for aqueous Pyridoxal-5-Phosphate (PLP) standard solutions and for the serum spiked with PLP. Curves were found to be linear up to the 40 pmoles PLP per sample corresponding to 200nmol/L PLP. Correlation coefficients were 0.95 in aqueous tris-buffer solution (See fig 5.1) and 0.88 in serum (see figure 5.2).

Precision and Accuracy

Inter-batch precision was assessed by the analysis of 15 samples of the same serum sample containing 17.3 nmol/L of PLP. The coefficient of variation obtained was 14.8%. Intra-batch precision was found to be 16.2%.

Figure 5.1 ΔOD/Min versus Amount Pyridoxal-5-Phosphate Standard Added To 545 mmol/L Tris Buffer

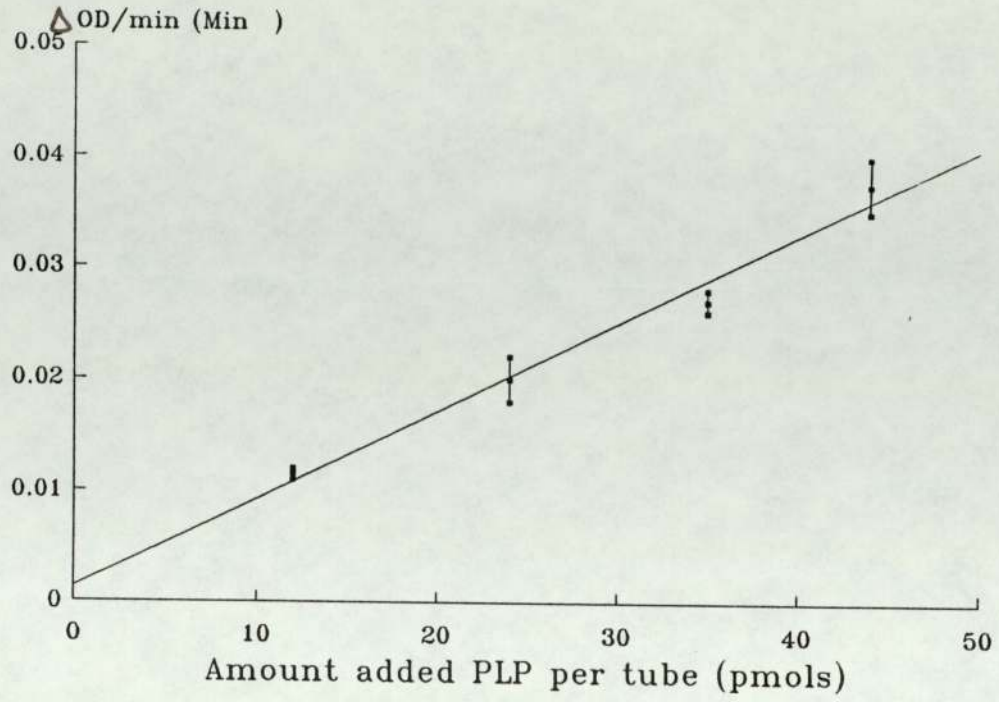
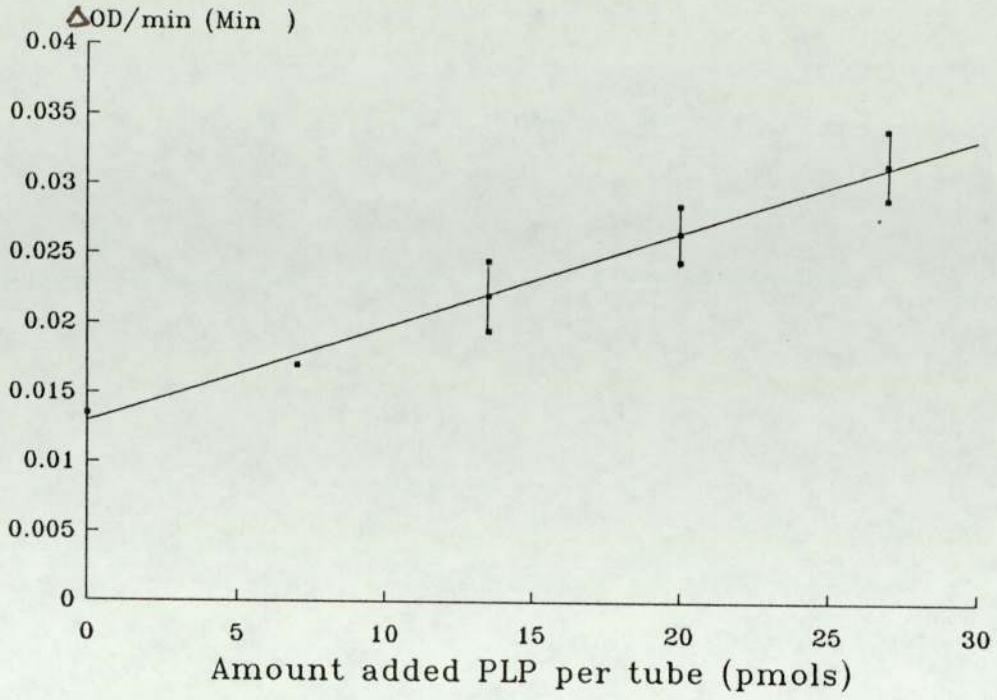


Figure 5.2 ΔOD/Min versus Amount Pyridoxal-5-Phosphate Standard Added To Serum



Samples were stable for several months at -20°C. Once thawed they were assayed on the same day to avoid any decomposition. Samples were not refrozen.

The Investigation Of Serum Pyridoxal-5-Phosphate Concentration In Patients Receiving TPN

Nine out of the eleven patients investigated had serum PLP concentrations below the stated reference range of 20-80 nmol/L prior to receiving Parenteral Nutrition (see table 5.3, figure 5.5). After seven days, serum PLP concentrations had risen in nine out of the eleven patients. In six of these patients, the rise in serum PLP concentration was shown to be statistically significant ($P<0.05$) by the student's t-test.

After seven days the serum PLP concentration of seven patients were within the reference range (see table 5.3, figure 5.5). Of the other four patients, one patient (JR) had a serum concentration which had been above the reference range throughout the study. The other three patients (VP, ED, SM) still had serum concentrations below the stated reference range.

In two patients (SD, ED) studied, the serum PLP concentration fell, though this was not shown to be significant by the students t-test. Both patients were seriously ill with complications (see table 5.4) and died soon after their investigation was completed.

No correlations were found between the rise in serum PLP concentration and the amount of nitrogen (amino acids) received daily, or the patients weight (see tables 5.3, 5.4).

Table 5.3

Change In Serum Concentration of PLP in Patients Receiving Total Parenteral Nutrition for More than One Week

Patient and sex	Day 0 (nmol/L ± SEM*)	Day 7 (nmol/L ± SEM*)
CS (F)*	8.2 ± 0.5	25.5 ± 0.9
CS (F)**	12.3 ± 0.9	39.2 ± 1.4
AT (F)	14.5 ± 1.4	30.1 ± 1.4
TB (M)	14.1 ± 1.9	20.5 ± 2.5
JR (F)	78.4 ± 5.0	84.5 ± 6.4
AVS (F)	29.2 ± 3.6	43.8 ± 5.5
HW (F)	17.3 ± 0.5	50.2 ± 3.6
WA (M)	14.6 ± 0.9	38.8 ± 1.8
VP (F)	8.2 ± 0.9	15.5 ± 1.4
ED (M)	14.1 ± 2.3	10.5 ± 0.9
SM (F)	7.8 ± 0.9	5.9 ± 0.9

* All SEM's quoted for four values

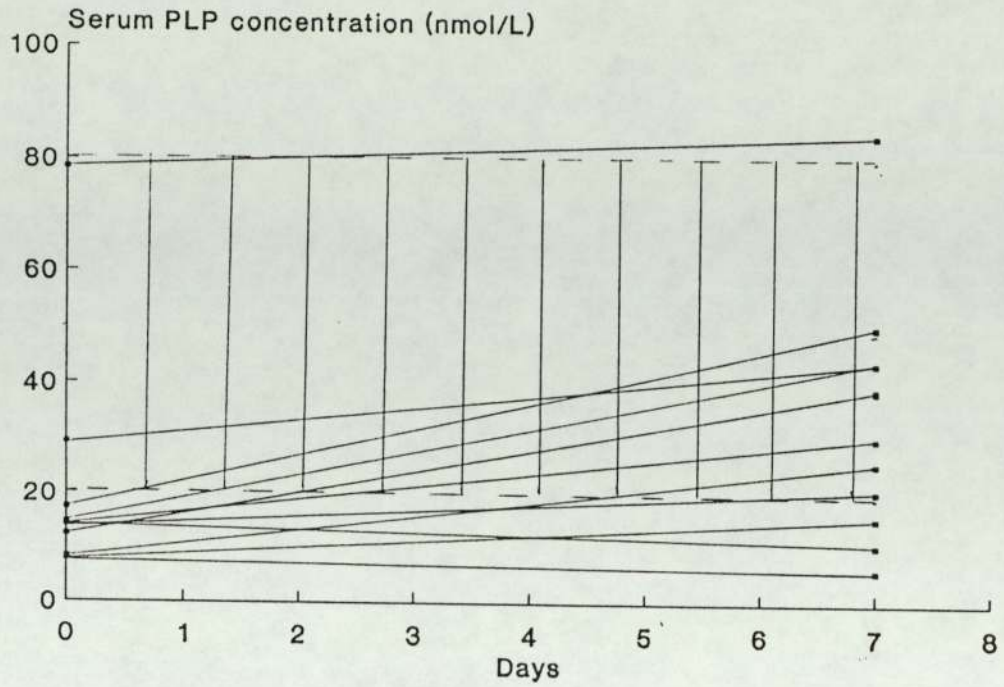
** Patent CS received two courses of TPN more than one month apart.

Table 5.4

Primary Disease States of Patients Investigated

Patient	Age (yrs)	Weight (Kg)	Primary Disease	Average amount of N_2 recieved per day (g)
CS	31	59	Neuroleptic induced malignant hyperthermia	9
AT	53	65	Cervical cancer	9
TB	64	59	Septic shock	14
JR	42	40	Chronic cancer	9
AV-S	68	60	Gastric fistula	9
HW	33	40	Perforated peptic ulcer	9
WA	69	59	Oesophageal cancer	18
VP	76	70	Intestinal surgery	14
ED	83	60	Oesophageal cancer	14
SM	46	120	Necrotising cellulitis systemic fungal infection	14

Figure 5.5 The Change In Serum Pyridoxal-5-Phosphate Concentration in Patients Receiving Total Parenteral Nutrition For One Week Or More



Serum pyridoxal-5-phosphate concentration at day 7 was statistically significantly raised in seven out of eleven of the patients compared to day 0 by student's t-test ($P<0.05$). All patients received 2.0mg pyridoxine per day via their TPN regimen.

| | | | The stated reference range

Total Parenteral Nutrition requires constant monitoring to ensure adequate nutritional status. It is both cost effective and easy to monitor the nutritional macronutrients such as water, carbohydrates and nitrogen. Although many assays have been developed for micronutrients - vitamins and trace elements, it still remains expensive to monitor the status of these nutrients. However it is still essential to give adequate amounts of these micronutrients to prevent and treat nutritional deficiencies.

The precision of the assay of pyridoxine stated by Sinkamani et al (32) could not be matched in these experiments. Following the method in their paper exactly, no result could be obtained, probably due to the very acidic conditions. A number of modifications were therefore made.

Sinkamani et al added 1pmol PLP as an internal standard to 0.2ml of sample or standard. Following extraction with perchloric acid the acidic extract was neutralised with 0.3ml of 1N KCl solution to give a final extract pH of 2.0 - 3.0. In this investigation, the internal standard was increased from 1 pmol to 5 pmol of PLP. This was added to 0.5ml of sample. This provided a greater amount of PLP for assay and thus decreasing the percentage error.

After extraction, an exact volume of sample was taken for neutralisation, 0.40ml was neutralised with approximately 0.15ml of 2N KCl. This exact volume was taken as the total volume varied between 0.39 and 0.43 microlitres, which after neutralisation caused marked variation in sample volumes, with associated variation in the PLP concentration for the sample. As already stated, the samples were

neutralised to pH 6.0-7.0 as variations in the ΔOD/min were noted outside this range. Furthermore, if the pH was below 5.0, the reaction in the assay was found to be negligible.

Results from the investigation of pyridoxine requirements in TPN show that prior to initiation of Parenteral Nutrition, most patients were biochemically deficient in vitamin B_6. However none showed any clinical deficiency of the vitamin.

There could be several reasons for the deficiency. Firstly, patients who recieve Parenteral Nutrition have often undergone a period of partial or total starvation which would lower the amount of PLP stored in the skeletal muscle and thus plasma PLP concentration. Secondly, most of these patients have undergone some stress or trauma with associated catabolism. They may still be suffering severe stress during Parenteral Nutrition. The associated increase in protein metabolism will increase the need for PLP which will be drawn out of the muscle store during starvation.

A daily input of 2mg of pyridoxine increased the plasma PLP concentration in all but two patients. After seven days however, plasma PLP concentrations in all patients but one of the patients (JR) were below 50nmol/L, the middle of the stated normal range (20-80 nmol/L). It may be argued therefore that patients would benefit from an initial injection of vitamin B_6 at the start of Parenteral Nutrition. This would be followed by the daily infusion of pyridoxine via the TPN regimen. It is common practice for 'stat' doses of some vitamins eg. folic acid, hydroxycobalamin, to be given by intramuscular injection prior to the initiation of TPN.

The two patients whose plasma PLP concentrations fell during TPN both died shortly after the study. Patient ED was suffering from oesophageal cancer and had been malnourished for some time with considerable muscle wastage. This would explain

his low levels due to reduced storage capacity combined with malnutrition. Patient SM had suffered from several severe infections, and was thus under severe stress. Administration of 2mg was not sufficient to increase plasma PLP concentrations in these patients. However it may be that these moribund patients were not able to assimilate nutrients including vitamins.

When blood samples were taken on day 0, patients were receiving little or no nutrition. This was followed by seven days continuous nutrition including continuous pyridoxine input. This would be expected to increase plasma PLP concentration to some extent. The rate of input of pyridoxine was approximately 80 micrograms per hour. This would be converted to PLP very quickly by the liver and then taken up by the muscle tissue. The plasma half-life of PLP is 2-3 hours (5). The continous administration of pyridoxine in this dosage is, therefore, unlikely to significantly contribute to the increase in plasma PLP concentration, this being due to the increase in amount of PLP stored in muscle tissue.

The administration of 2mg of pyridoxine daily in a TPN system maintained and improved the vitamin B_6 status, as determined by the direct measurement of plasma PLP concentration, in most of patients in this study. However the plasma PLP concentration after 7 days was still in the lower half of the stated normal range. Suggested methods of improving this are to give an initial 'stat' dose of pyridoxine at the begining of TPN. A suggested dose would be 10-20mg pyridoxine hydrochloride. This should be followed by giving increased daily amounts of pyridoxine during TPN.

Since the completion of this study, Solivito, the multivitamin injectable preparation employed in the TPN regimens at Dudley Road Hospital has been reformulated, with increases in all the vitamins, and is now called Solivito N. The pyridoxine content has been increased from 2mg to 4mg. This would seem a more

appropriate dose of pyridoxine for patients on TPN and is also in line with the recommendations made by other authors (22,24). A further study is to be carried out using Solivito N to confirm this hypothesis.

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APPENDIX 1

THE CONTENT OF SOLUTIONS FOR TOTAL PARENTERAL NUTRITION USED IN THIS STUDY

1. Addamel

Addamel contains:

Calcium Chloride.$2H_2O$		73.5	mg
Magnesium Chloride		30.4	mg
Ferric Chloride.$6H_2O$		1.35	mg
Zinc Chloride		0.27	mg
Manganese Chloride.$4H_2O$		0.79	mg
Copper Chloride.$2H_2O$		85	mcg
Sodium Fluoride		210	mcg
Potassium Iodide		17	mg
Sorbitol		0.3	g
Water	to	1	ml

The solution has a pH of 2.5 and 10ml corresponds to :

Ca^{2+}	5	mmol
Mg^{2+}	1.5	mmol
Fe^{3+}	50	micromol
Zn^{2+}	20	micromol
Mn^{2+}	40	micromol
Cu^{2+}	5	micromol
F^-	50	micromol
I^-	1	micromol
Cl^-	13.3	micromol

2. Aminoplex 12

One litre solution contains :

Amino acids

L - Isoleucine	3.8	g
L - Leucine	5.8	g
L - Lysine.HCl	6.8	g
L - Methionine	4.8	g
L - Phenylalanine	6.9	g
L - Threonine	3.2	g
L - Tryptophan	1.4	g
L - Valine	4.5	g
L - Arginine	9.2	g
L - Histidine	2.2	g
L - Alanine	10.0	g
L - Glutamic acid	2.0	g
Glycine	4.4	g
L - Proline Aspartate	12.0	g
L - Ornithine	2.0	g
L - Serine	2.4	g

Electrolytes :

Sodium	35	mmol
Potassium	30	mmol
Magnesium	2.5	mmol

Chloride	67	mmol
Acetate	5	mmol

Additional nutrient :

L - Malic acid	46	g

1 L provides 12.4g of utilisable nitrogen and has a pH of 7.4

3. Aminoplex 24

One litre solution contains :

Amino acids

L - Isoleucine	7.6	g
L - Leucine	11.6	g
L - Lysine.HCl	6.8	g
L - Lysine - L - Malate	10.4	g
L - Methionine	9.6	g
L - Phenylalanine	13.8	g
L - Threonine	6.4	g
L - Tryptophan	2.8	g
L - Valine	9.0	g
L - Arginine	18.4	g
L - Histidine	4.4	g
L - Alanine	20.0	g
L - Glutamic acid	4.0	g

Glycine	8.8	g
L - Proline	24.0	g
L - Ornithine - L - Aspartate	4.0	g
L - Serine	4.8	g

Electrolytes :

Sodium	35	mmol
Potassium	30	mmol
Magnesium	2.5	mmol
Chloride	67	mmol
Acetate	5	mmol

Additional nutrient :

L - Malic acid	45	g

1 L provides 24.9g of utilisable nitrogen and has a pH of 7.4

4. Intralipid

A white oil in water emulsion containing :

	Intralipid 10%	Intralipid 20%
Fractionated Soya-bean oil	50 g	100 g
Fractionated egg phospholipid	6 g	6 g
	120	

Glycerol	11 g	11 g
Water for injections	to	500 ml

It has a pH of 7 and provides 1100 kcal (Intralipid 10%) or 2000 kcal (Intralipid 20%)

5. Solivito

1 vial of Solivito corresponds to the following formula

Thiamine Mononitrate (B_1)	1.24	mg
Sodium Riboflavine Phosphate (B_2)	2.47	mg
Nicotinamide	10	mg
Pyridoxine Hydrochloride (B_6)	2.43	mg
Sodium Pantothenate	11	mg
Biotin	0.3	mg
Folic acid	0.2	mg
Cyanocobalamin (B_{12})	2	mcg
Sodium Ascorbate (C)	34	mg
Glycine	100	mg
Sodium edetate	0.5	mg
Methylhydroxybenzoate	0.5	mg

It contains the following quantities of water soluble vitamins :

Thiamine	1.2	mg
Riboflavine	1.8	mg

Nicotinamide	10	mg
Pyridoxine	2	mg
Pantothenic acid	10	mg
Biotin	0.3	mg
Folic acid	0.2	mg
Ascorbic acid	30	mg
Vitamin B_{12}	2	mcg

6. Vamin 9

One litre contains :

Amino acids

L - Arginine	3.3	g
L - Alanine	3.0	g
L - Aspartic acid	4.1	g
L - Cysteine	1.4	g
L - Glutamic acid	9.0	g
Glycine	2.1	g
L - Histidine	2.4	g
L - Isoleucine	3.9	g
L - Leucine	5.3	g
L - Lysine	3.9	g
L - Methionine	1.9	g
L - Phenylalanine	5.5	g
L - Proline	8.1	g
L - Serine	7.5	g
L - Threonine	3.0	g

L - Tryptophan	1.0	g
L - Tyrosine	0.5	g
L - Valine	4.3	g

Electrolytes :

Sodium	50	mmol
Potassium	20	mmol
Calcium	2.5	mmol
Magnesium	1.5	mmol
Chloride	55	mmol

It has a pH of 5.2 and contains 9.4g of utilisable nitrogen per litre.

7. Vamin 9 Glucose

This is identical to Vamin 9 but with an added 100g of anhydrous glucose.

8. Vamin 14

One litre contains :

Amino acids

L - Alanine	12.0	g
L - Arginine	8.4	g
L - Aspartic acid	2.5	g

L - Cysteine	420	mg
L - Glutamic acid	4.2	g
Glycine	5.9	g
L - Histidine	5.1	g
L - Isoleucine	4.2	g
L - Leucine	5.9	g
L - Lysine	6.8	g
L - Methionine	4.2	g
L - Phenylalanine	5.9	g
L - Proline	5.1	g
L - Serine	3.4	g
L - Threonine	4.2	g
L - Tryptophan	1.4	g
L - Tyrosine	170	mg
L - Valine	5.5	g

Electrolytes :

Sodium	100	mmol
Potassium	50	mmol
Calcium	5	mmol
Magnesium	8	mmol
Chloride	100	mmol
Sulphate	8	mmol
Acetate	135	mmol

It has a pH of 5.6 and contains 13.5g of utilisable nitrogen per litre.

9. Vamin 18 electrolyte free

One litre contains :

Amino acids

L - Alanine	16.0	g
L - Arginine	11.3	g
L - Aspartic acid	3.4	g
L - Cysteine	560	mg
L - Glutamic acid	5.6	g
Glycine	7.9	g
L - Histidine	6.8	g
L - Isoleucine	5.6	g
L - Leucine	7.9	g
L - Lysine	9.0	g
L - Methionine	5.6	g
L - Phenylalanine	7.9	g
L - Proline	6.8	g
L - Serine	4.5	g
L - Threonine	5.6	g
L - Tryptophan	1.9	g
L - Tyrosine	230	mg
L - Valine	7.3	g

It contains no electrolytes and has a pH of 5.6 and contains 18.0g nitrogen per litre.

10. Vitlipid Adult

One ampoule of Vitlipid Adult contains

Retinol Palmitate		750	mcg
Calciferol		3	mcg
Phytomenadione		150	mcg
Fractionated Soya-bean oil		1	g
Glycerol		225	mg
Water for injections	to	10	ml