FOLATE AND ZINC STATUS OF CHRONIC HEMODIALYSIS PATIENTS

By

DEBORAH JANE REID

B.H.Ec., University of British Columbia, 1982

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Division of Human Nutrition,
School of Family and Nutrition Sciences)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

September 1990

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Human Nutrition

The University of British Columbia
Vancouver, Canada

Date Sept. 20/90

DE-6 (2/88)

ABSTRACT

Folate supplementation at a level of 15 to 35 mg per week is routinely prescribed for many chronic hemodialysis patients in B.C. In recent studies involving these levels of folate supplementation, RBC folate concentrations ranged from near the upper limit of normal to 1.5 times this upper limit. Initially there was research suggesting that high dose folate supplementation impaired zinc absorption but more recent studies refute this hypothesis. A beneficial effect of high dose folate supplementation is lowering of plasma homocysteine levels. This may be desirable since the homocysteinemia observed in chronic renal failure patients may be a factor in their commonly occurring premature vascular disease.

The present study addressed folate needs on a nutritional basis but did not investigate folate's effect on homocysteine levels. The study involved chronic hemodialysis patients and was designed to:

1. assess whether patients consuming the Recommended Nutrient Intake for folate, require a folate supplement to maintain normal folate stores;
2. assess whether patients receiving a supplement of 5 mg of folate per day will have RBC folate levels exceeding the upper limit of the normal range;
3. compare serum zinc concentrations (and in some cases hair zinc levels as well) of patients receiving no zinc supplement or a 22.5 mg per day zinc supplement, to each other and to normal values;

4. assess whether a supplement of 5 mg of folate per day is associated with impaired zinc status;
5. in the event that a 5 mg per day folate supplement is associated with impaired zinc status, assess whether a supplement of 22.5 mg of zinc per day is associated with an improvement in zinc status; and
6. determine average daily energy, protein, folate and zinc intakes of patients.

A 2x2 factorial quasiexperimental design was employed. The study included 21 clinically stable chronic hemodialysis patients between the ages of 25 and 69, who were receiving folate and/or zinc supplements at certain specific levels. Subjects were entered into treatment groups based on the following folate/zinc supplementation levels: no folate, no zinc; no folate, 22.5 mg zinc/day; 5 mg folate/day, no zinc; 5 mg folate/day, 22.5 mg zinc/day. Folate status was assessed using RBC folate concentration. Serum zinc concentration was measured in all subjects. Hair zinc level was determined in 6 of the zinc-supplemented subjects. A food frequency questionnaire was developed to determine dietary folate and zinc intakes. Subjects kept 3 day food records so average daily energy and protein intakes could be determined.

Study results indicated no significant difference in protein intake (g/kg b.w.) or energy intake (expressed as a percent of requirement) among the four treatment groups. Differences in dietary folate intakes among the four treatment groups as well as between zinc-supplemented and non zinc-supplemented subjects, were not significant ($p \leq 0.05$). Mean dietary

folate intake for all study subjects was 4.2 ug/kg b.w. RBC folate concentration was normal in both treatment groups receiving no supplemental folate. In contrast, the RBC folate concentration for both folate-supplemented groups was approximately 6.5 to 7 times the upper limit of the normal range. The difference between RBC folate concentration for folate supplemented and unsupplemented groups was highly significant ($p<0.00001$) and remained so when analysis of covariance was done with number of months of folate supplementation as the covariate. RBC folate levels did not differ significantly between zinc-supplemented and unsupplemented groups ($p\leq 0.05$). Differences in dietary zinc intakes among the four treatment groups as well as between zinc-supplemented and non zinc-supplemented subjects were not significant ($p\leq 0.05$). Mean dietary zinc intake for all study subjects was 9.39 mg/day. Serum zinc levels were below normal in both treatment groups receiving no supplemental zinc. The 22.5 mg zinc, no folate group had a serum zinc concentration near the lower limit of the lower range while that in the 22.5 mg zinc, 5 mg folate group was slightly below normal. When all zinc supplemented subjects were combined, serum zinc concentration was just within the normal range. Hair zinc analysis was conducted in a subgroup of 6 zinc-supplemented subjects and a group of non zinc-supplemented healthy controls. Hair zinc level was significantly higher in the zinc-supplemented subjects than in the controls ($p\leq 0.01$).

In conclusion, folate supplementation does not appear to be required on a nutritional basis in clinically stable chronic hemodialysis patients

not receiving medications known to affect folate status, who are consuming a diet providing a minimum of 1 g of protein per kg b.w. and 4.6 ug of folate per kg b.w. The low serum zinc concentrations observed in both zinc-supplemented and non zinc-supplemented patients may have been due to a shift of zinc from serum to other "zinc pools" in the body as reported in the literature.

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

TABLE OF CONTENTS CONTINUED

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDICES CONTINUED

ACKNOWLEDGMENTS

I would like to thank the following people for their help with my thesis project.

My advisor, Dr. Leichter and committee members, Dr. Susan Barr, Dr. John Price, and Dr. Melvin Lee for their advice throughout the course of this study.

Dr. Jim White and Dr. Daniel Perlman for advice and assistance with statistical analysis of data.

Staff at Willow Hemodialysis Unit and the hemodialysis units at Vancouver General Hospital, St. Paul's Hospital and the Royal Columbian Hospital for accommodating the study so willingly.

Vancouver General Hospital Nutrition Services Department for the Ethel Pipes Bursary and for the use of their nutrient analysis program.

Colleagues and family, in particular my husband and my friend Beverlee Kell for their ongoing encouragement.

Most importantly, participating patients for their cooperation with the study's demands.

This study was supported by a grant from the Kidney Foundation of Canada.

Financial support from a University Teaching Assistantship (1987/88) is also gratefully acknowledged.

CHAPTER ONE

INTRODUCTION

A deficiency of water-soluble vitamins, especially ascorbic acid, pyridoxine and folic acid has been associated with regular hemodialysis (Sullivan and Eisenstein 1972, Kopple et al. 1981, Lasker et al. 1963). Major factors implicated are: (1) increased losses of water-soluble vitamins into the dialysate and (2) decreased intake of these vitamins due to protein and potassium restrictions (Feldman and Singer 1974). Accordingly, most chronic hemodialysis patients have routinely been supplemented with the water-soluble vitamins, often including 7-35 mg of folate per week. Recently, the practice of routine folate supplementation at such a high level has been questioned. This is because research (DeBari et al. 1984, Ramirez et al. 1986a, Ramirez et al. 1986b, Sharman et al. 1982) has shown serum and red cell folate levels to be well above the normal range in folate supplemented hemodialysis patients.

Whether such high dose folate supplementation is beneficial or harmful is controversial (Wicken et al. 1988, Brattstrom et al. 1988). Homocysteinemia is thought to be damaging to vascular endothelium (Harker et al. 1974). High dose folate supplementation has been found to lower plasma homocysteine levels (Brattstrom et al. 1988, Wilcken et al. 1988). Wilcken et al. (1988) have suggested that the homocysteinemia observed in chronic renal failure (CRF) patients may be a factor in their commonly occurring premature vascular disease. Based on the foregoing, high dose folate supplementation may be of benefit to CRF patients. However,

researchers (Anonymous 1985, Prakash and Petrie 1982, Hunter and Barnes 1970, Leung et al. 1985) have documented anecdotal information suggesting that high dose folate supplementation has adverse side effects. Those side effects discussed included: slow fetal heart rate and maternal infection at delivery, behavioral changes in psychiatric patients, gastrointestinal disturbances including bitter taste and nausea, altered sleep patterns including vivid dreams and insomnia, and finally irritability.

In addition to these side effects there has been concern that administering such large amounts of folic acid may decrease zinc absorption. When zinc was administered after a 14 day period of 350 ug folate per day, the 4 hour plasma zinc curve was reduced to 79% of that observed prior to folate therapy (Simmer et al. 1987). Folic acid supplementation (400 ug every second day for 5-6 months) in males with a mild zinc deficiency affected zinc balance (Milne et al. 1984). Specifically, fecal zinc increased significantly on both control and low zinc diets but not on a high zinc diet. The mechanism whereby folate may impair zinc absorption has not been established. Animal and in vitro investigations (Ghishan et al. 1986) suggested that the increase in fecal zinc following folate supplementation was not due to formation of insoluble zinc-folate complexes in the intestine.

More recent studies (Butterworth Jr. et al. 1988, Keating et al. 1987) do not support the contention that high level folate supplementation impairs zinc absorption. Women with cervical dysplasia who were treated for 4 months with 10 mg folate per day showed no significant change in plasma or red cell zinc concentrations (Butterworth Jr. et al. 1988). In another study involving humans (Keating et al. 1987) 10 mg folate had no

effect on serum zinc response curves after ingestion of 25 mg zinc. Finally, rat studies (Keating et al. 1987) showed no effect of folate on zinc uptake by the kidney, liver or bone. In summary, there is no conclusive evidence of harmful effects associated with high dose folate supplementation.

Abnormally high RBC folate levels have been observed in chronic hemodialysis patients on high dose folate supplements. However, whether such patients can maintain normal RBC folate levels without supplemental folate has not been clearly established. The effect withdrawing hemodialysis patients' folate supplements had on RBC folate concentration, was investigated by Ramirez et al. (1986a), Ramirez et al. (1986b), and Swainson and Winney (1983). Twelve months after discontinuing a 1 mg per day folic acid supplement, most but not all patients maintained a normal RBC folate level (Ramirez et al. 1986a, Ramirez et al. 1986b). In another study (Swainson and Winney 1983), all patients had RBC folate levels in the normal range 12 months after a 5 mg per day folic acid supplement was withdrawn. In the foregoing studies (Ramirez et al. 1986a, Ramirez et al. 1986b, Swainson and Winney 1983), subjects started out with high normal, or higher than normal folate stores making it difficult to firmly conclude that normal folate stores are maintained without folate supplements. Furthermore, none of the previous studies assessed dietary folate intake.

The present study involved chronic hemodialysis patients who were clinically stable (i.e., symptoms associated with their chronic renal failure were relatively stable under present treatment regimes and they were not suffering from any acute disease processes). It was designed to:

1. assess whether patients consuming the Recommended Nutrient Intake (RNI) for folate require a folate supplement to maintain normal folate

stores (reflected by normal RBC folate levels). The RNI for a nutrient is the level of that nutrient estimated to be sufficiently high to meet the requirements of almost all individuals in a group with specified characteristics (age, sex, body size, physical activity, type of diet). Estimates of requirements are not appropriate for persons recovering from previous undernutrition or those taking drugs which interact with nutrients. They may be inappropriate for those with diseases that interfere with the absorption, utilization or excretion of nutrients (Department of National Health and Welfare 1983);

2. assess whether patients receiving a supplement of 5 mg of folate per day will have RBC folate levels exceeding the upper limit of the normal range;
3. compare serum zinc concentrations (and in some cases hair zinc levels as well) of patients receiving no zinc supplement, or a 22.5 mg per day zinc supplement, to each other and to normal values;
4. assess whether a supplement of 5 mg of folate per day is associated with impaired zinc status;
5. assess whether in the event that a 5 mg per day folate supplement is associated with impaired zinc status, providing a supplement of 22.5 mg of zinc per day is associated with an improvement in zinc status; and
6. determine average daily energy, protein, folate and zinc intakes of patients.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The purposes of this section of the thesis are to:

1. give background information to enhance the reader's understanding of this thesis; and
2. present literature which instigated the present study.

First, an overview on folate and zinc content of the diet of chronic hemodialysis patients in the 1960s/70s and in the 1980s is provided to help the reader understand later discussions examining folate and zinc supplementation practices in these patients.

Secondly, assessment of folate status, whether folate supplementation is indicated, and whether high dose folate supplementation has any harmful or beneficial effects are discussed. Next, assessment of zinc status and the zinc status of chronic renal failure patients are reviewed.

Finally, validity of the three day food record is examined since this tool is used in the present study. The literature review concludes with discussions on: (i) current folate and zinc supplementation practices in chronic hemodialysis patients in British Columbia; and (ii) how these practices as well as recent research on folate and zinc supplementation and utilization in these patients led to formulation of objectives for the present study.

2.2 The 1960s/70s and 1980s Hemodialysis Diets; Impact on Folate and Zinc Intake

2.2.1 The 1960s/70s Hemodialysis Diet: Impact on Folate and Zinc Intake

Studies conducted in the 1960s (Whitehead et al. 1968, Hampers et al. 1967, MacKenzie et al. 1968, Siddiqui et al. 1970) on folate status of chronic hemodialysis patients are somewhat vague in describing the diet patients consumed. Protein was limited with restrictions ranging from 50-70 g protein per day (Hampers et al. 1967, MacKenzie et al. 1968, Siddiqui et al. 1970, Royal Victoria Hospital Dept. of Dietetics 1970). This meant high protein foods such as meat, seafood, poultry, eggs and cheese were typically limited to a total of 3-5 ounces per day with milk restricted to 6-8 ounces per day (Royal Victoria Hospital Dept. of Dietetics 1970). This restriction of animal flesh, eggs and dairy products also served to limit phosphorous intake to some extent. Further phosphorous restriction was achieved by disallowing legumes, nuts and whole grain products (Royal Victoria Hospital Dept. of Dietetics 1970). Some of the richest sources of zinc are red meat, liver, egg yolks, shellfish, poultry, legumes, nuts, milk, cheese and whole grains (Dept. of National Health and Welfare 1983, Pennington and Church 1980, Freeland et al. 1980, Murphy et al. 1975) so this diet was also restricted in zinc. Based on zinc values from food composition tables (Nutricom Nutrient Analysis Software, Pennington and Church 1980, Freeland et al. 1980, Murphy et al. 1975), a typical 1960s hemodialysis diet (60 g protein) (Royal Victoria Hospital Dept. of Dietetics, 1970) provided approximately 4.8-10.5 mg zinc per day depending on the type of animal flesh chosen.

As well as being low in zinc, the diet was probably also low in folate. Lentils, dairy products, whole grains and animal flesh, especially liver contribute some of the folate in an individual's diet. Accordingly, the previously discussed protein and phosphorous restrictions limited folate intake to some degree. Potassium restrictions ranging from 1000-2000 mg potassium per day were also sometimes imposed (MacKenzie et al. 1968, Siddiqui et al. 1970) and would have further restricted folate intake. Specifically, two of the major dietary sources of potassium, fruits and vegetables, which are also good sources of folate (Hoppner et al. 1972, Perloff and Butrum 1977), were limited. For example, a 1968 hemodialysis diet providing 1400 mg potassium, included only 1 cup of cooked vegetables (other than potatoes which are very rich in potassium) and 2 small servings of fruit (Rodwell-Williams 1973). To decrease the potassium content of vegetables through leaching, patients were sometimes asked to cut their vegetables into small pieces and cook them in a large volume of water (Siddiqui et al. 1970, Robinson 1972). This process would also have decreased the folate content of these vegetables. Considering all of the foregoing dietary restrictions, findings of 50-72 ug of folate in a 1960s hemodialysis diet (Whitehead et al. 1968, MacKenzie et al. 1968) are not surprising.

2.2.2 The 1980s Hemodialysis Diet: Impact on Folate and Zinc Intake

The 1980s hemodialysis diet is more liberal than the 1960s/70s hemodialysis diet especially with respect to protein and potassium. A protein intake of 1-1.2 g per kg body weight is advised (B.C. Dietitians' and Nutritionists' Assoc. 1984, Gillet et al. 1987). Accordingly, a 60 kg patient would be allowed 72 g protein per day. In this diet high protein

foods such as meat, seafood, poultry and eggs would typically be limited to 6 ounces per day with milk restricted to 8 ounces per day. One ounce of cheese or 15 mLs of peanut butter would be allowed in place of one ounce of animal flesh three times per week (B.C. Dietitians' and Nutritionists' Assoc. 1984). As discussed previously, this restriction of animal flesh, eggs and dairy products limits phosphorous intake to some extent. To further restrict phosphorous, legumes and nuts are disallowed as in the 1960s diet. Whole grain cereals other than bran cereals and bran muffins are now permitted (B.C. Dietitians' and Nutritionists' Assoc. 1984). Allowing whole grains and liberalizing protein intakes has increased dietary zinc modestly over that found in the 1960s hemodialysis diet. Based on zinc values from food composition tables (Nutricom Nutrient Analysis Software, Pennington and Church 1980, Freeland et al. 1980, Murphy et al. 1975), a 1980s hemodialysis diet providing 72 g protein (B.C. Dietitians' and Nutritionists' Assoc. 1984) would provide approximately 6.9-14.3 mg zinc, again depending on the type of animal flesh chosen.

In addition to being more liberal in zinc, the 1980s hemodialysis diet is more liberal in folate. As discussed previously, restrictions on animal flesh are less severe. This in conjunction with the inclusion of whole grains increases dietary folate somewhat. Of major significance are increases in dietary potassium from 1960s levels of 1000-2000 mg per day (MacKenzie et al. 1968, Siddiqui et al. 1970) to 1980s levels of 2350-3150 mg per day (B.C. Dietitians' and Nutritionists' Assoc. 1984). Typically, these diets include 1 small potato, 1 to 1.5 cups of vegetables and 3-4 small servings of fruit. Furthermore, except in the case of potatoes, processing vegetables to leach potassium is no longer advised (B.C.

Dietitians' and Nutritionists' Assoc. 1984). Accordingly, the folate losses associated with this are avoided. Based on folate values from food composition tables (Nutricom Nutrient Analysis Software, Hoppner et al. 1972, Perloff and Butrum 1977), a typical 72 g protein, 2730 mg potassium diet would provide approximately 187-323 ug folate per day depending on which vegetables were chosen.

2.3 Assessing Folate Status in CRF Patients

To assess folate status accurately, a parameter which reflects tissue folate levels needs to be chosen. Serum folate concentration falls after only three weeks of an inadequate folate intake but it takes at least sixteen weeks before tissue folate depletion is reflected as a decline in RBC folate concentration (Herbert 1983, Nutrition Foundation 1980). Megaloblastic anemia occurs soon after RBC folate level falls below normal (Herbert 1983, Nutrition Foundation 1980).

Accordingly, while serum folate may be useful in detecting an impending folate deficiency, RBC folate must be measured to assess tissue folate stores. One factor which can affect serum and RBC folate levels is the vitamin B_{12} status of an individual: vitamin B_{12} deficiency leads to an increase in serum folate and a decrease in red cell folate in many cases (Schneider and Stroinski 1987). However, vitamin B_{12} deficiency is not very common. It occurs in strict vegans (vegetarians who avoid animal flesh, eggs and dairy products), individuals with inadequate intrinsic factor production (e.g., Pernicious Anemia, gastric atrophy, gastrectomy) and in those with a diseased or resected terminal ileum. Thus, if these conditions have been ruled out, RBC folate alone can be used with reasonable confidence to assess folate status.

RBC folate can be determined using a microbiological assay or a radioimmunoassay. Since antibiotics and some other drugs interfere with the microbiological assay, most laboratories use the radioimmunoassay today. Based on nonuremic populations, the normal range for RBC folate concentration is about 150-770 ng/ml (Shrier 1983). This varies slightly between laboratories due to differences in radioassay kits and populations.

Whether RBC folate concentration is altered by uremia is not known. In normal individuals who are not vitamin B_{12} deficient, megaloblastic anemia indicates advanced folate deficiency. However, mean corpuscular volume is often not elevated in similar subjects with CRF because concurrent microcytic and/or normocytic anemia tend to suppress formation of megaloblasts. Accordingly, RBC folate concentration appears to be the best indicator of folate status in CRF at present.

2.4 Do Chronic Hemodialysis Patients Need Folate Supplements?

In the 1960s several researchers (Hampers et al. 1967, Whitehead et al. 1968, MacKenzie et al. 1968, Siddiqui et al. 1970) looked at folate status in chronic hemodialysis patients. At this time patients were dialyzing 24-34 hours per week on Kiil dialyzers and in some cases (MacKenzie et al. 1968, Siddiqui et al. 1970) were reported to be consuming only 50-72 ug folate per day because of extensive soaking and cooking of food to remove potassium.

In two studies (Whitehead et al. 1968, Siddiqui et al. 1970), normal RBC folate levels were found in twelve of the fourteen patients with no megaloblastic changes observed in any of the patients. However, Whitehead et al. (1968) noted occasional giant metamyelocytes in the smears of two

of eight patients studied. MacKenzie et al. (1968) observed giant metamyelocytes in all six study subjects. Metamyelocytes are precursors to polymorphonuclear leukocytes (Quesenberry 1987). Giant metamyelocytes indicate megaloblastic anemia (Jandl 1987).

Other researchers (Hampers et al. 1967, Siddiqui et al. 1970) found fairly convincing evidence suggestive of folate deficiency in chronic hemodialysis patients. Hampers et al. (1967) did not measure red cell folate but observed a normal serum folate in only one of ten patients. Bone marrow aspirates indicated megaloblastic changes ranging from minimal to definite in all ten patients. Two patients with definite megaloblastic changes showed distinct improvements in their bone marrow smears after treatment with 15 mg folic acid per day for 6-8 weeks.

Siddiqui et al. (1970) became interested in hypersegmentation of polymorphonuclear leukocytes (nuclei containing more than the usual five or less lobes [Colonotero and Wheby 1987]) since this frequently occurs in uremics. They wondered if it was a consequence of folic acid deficiency (previously documented) or a manifestation of the uremic state. Ten hemodialysis patients underwent polymorphonuclear cell lobe counts and serum folate measurements before and after 12 weeks of folate supplementation at 5 mg per day. Serum folate levels were at the low end of the normal range initially, and increased thirtyfold through folate supplementation. Lobe counts were abnormally high in nine patients and fell to a normal level after folate supplementation, suggesting that they had folate deficiencies prior to supplementation.

These often quoted early studies are probably responsible for the practice of routinely supplementing chronic hemodialysis patients with folate. Recognizing that the hemodialysis diet today provides more folate

and that dialysis treatment duration has been shortened, recent studies have reinvestigated the need for routine folate supplementation in chronic hemodialysis patients.

DeBari et al. (1984) determined the plasma and red blood cell folate levels of twelve hemodialysis patients who had received 1 mg folate daily for an unspecified period of time. Red cell folate levels were compared to those of healthy controls both before and after a one month treatment with 1 mg folate per day. Plasma folate was much higher in hemodialysis patients than in controls (61±23 versus 13.2±2.5 ng/ml). Red cell folate was also greatly increased in dialysis patients in comparison to both supplemented and unsupplemented controls (965±529, 242±73 and 186±67 ng/ml respectively). According to Henderson et al. (1984), the normal range for RBC folate is 106-815 ng/ml.

Ramirez et al. (1986a, 1986b) monitored plasma and red blood cell folate levels for a 12 month period in 15 hemodialysis patients after their 1 mg per day folate supplement had been withdrawn. Prior to discontinuation of folate supplements, RBC folate was elevated at 1035±265 ng/ml (normal:100-700). At 6 months red blood cell folate was normal in all subjects but at 12 months it was low in a few subjects. Approximate means were 270 and 390 ng/mL at 6 and 12 months respectively. It is questionable that RBC folate rose during the second six months when patients received no folate supplements. One possible explanation is that some subjects received blood transfusions near their twelve month RBC folate determinations. This could have falsely elevated red blood cell folate levels. The authors never mentioned withholding transfusions. In fact, they mentioned intra dialytic transfusions as a possible factor in increases observed in plasma vitamin levels over the course of dialysis.

Laboratory error may also have been a factor causing the twelve month red blood cell folate value to be higher than the six month value. In conclusion, the reader has to be somewhat skeptical about these results.

Sharman et al. (1982) evaluated the effect of a six month period of a 5 mg folate supplement daily. Although actual red cell folate levels were not reported, they were noted to be significantly elevated as would be expected.

Swainson and Winney (1983) followed folate status in six hemodialysis patients for twelve months after discontinuation of a 5 mg per day folate supplement they had been receiving for at least one year. Serum folate decreased from more than 18 to 7.1±1.5 ng/ml (normal: 6-20) over the twelve month period. Red blood cell folate levels were not reported but they were noted to be in the normal range at the end of twelve months.

In the foregoing studies (DeBari et al. 1984, Ramirez et al. 1986a, Ramirez et al. 1986b, Sharman et al. 1982, Swainson and Winney 1983), RBC folate levels were always measured in hemodialysis patients who had been receiving high dose folate supplementation. In four studies (DeBari et al. 1984, Ramirez et al. 1986a, Ramirez et al. 1986b, Swainson and Winney 1983), RBC folate levels were remeasured six to twelve months after folate withdrawal. Red blood cell folate levels prior to discontinuation of supplements were not always reported, but when they were, they were high normal or above normal. Accordingly, these studies were assessing how long it takes to deplete such folate stores rather than whether normal folate stores can be maintained without folate supplements in chronic hemodialysis patients.

Another factor which weakened some of these studies (DeBari et al. 1984, Ramirez et al. 1986a, Ramirez et al. 1986b) was the fact that blood transfusions were not withheld. This could have falsely elevated red blood cell folate levels. Finally, none of these studies assessed dietary folate intakes of subjects.

Despite these design limitations, two studies (Sharman et al. 1982, Swainson and Winney 1983) concluded that folate supplements are not indicated in chronic hemodialysis patients who are eating well. The other studies did not make this statement recognizing a need for further research. The present study was designed to overcome some of the previously discussed limitations. Two groups of chronic hemodialysis patients were compared, a group who had never received folate supplements and a group who had received 5 mg folate per day since starting hemodialysis. Subjects had been on hemodialysis for 11.7-94.7 months. Blood transfusions were held for at least four weeks prior to RBC folate determination to avoid falsely elevating it. Dietary intakes of folate, protein and energy were determined.

2.5 Does High Dose Folate Supplementation Have Any Beneficial or Harmful Effects?

2.5.1 Decreasing Plasma Homocysteine May Be Beneficial

The fact that chronic renal failure patients are at high risk for premature vascular disease is well documented (Lowrie et al. 1974, Ibels et al. 1977, Green et al. 1983). The reason for this is not well established. It is thought that hypertension is relevant in some patients and abnormal lipid metabolism a contributor in others, especially those receiving hemodialysis. However, it is difficult to account for this

accelerated atherogenesis on the basis of the plasma lipid levels observed in these patients (Somer et al. 1980). Another factor in CRF patients which may increase the risk of vascular disease is mild homocysteinemia.

Patients with chronic renal insufficiency have been found to have an elevated plasma homocysteine level with the concentration increasing as renal function declines (Wilcken and Gupta 1979, Wilcken and Gupta 1980, Wilcken et al. 1981). A marked increase in plasma homocysteine and methionine is observed in untreated homocystinuria patients and is known to be associated with premature vascular disease (Mudd et al. 1985). Such premature vascular disease also occurs in enzyme disorders which limit remethylation of homocysteine to methionine (Mudd and Levy 1983). In these patients plasma methionine is low with homocysteine elevated. This suggests that the vascular disease may be homocysteine induced (Wilcken et al. 1988).

A detailed mechanism for homocysteine's role in vascular damage has not been elucidated. However, Harker et al. (1974) found patchy desquamation of vascular endothelium and arterial thrombosis in baboons with induced homocysteinemia but not in control animals. Furthermore, in four homocysteinuric patients, they observed threefold increases in platelet turnover and twenty percent increases in fibrinogen and plasminogen turnover. The turnover rates of these blood clotting factors were essentially normalized in three of the patients whose plasma homocysteine was reduced through pyridoxine administration. The foregoing observations suggest that an elevated plasma homocysteine concentration enhances thrombogenic activity and raises interest in homocysteinemia as a factor in the precocious vascular disease observed in chronic renal failure.

Recent research (Wilcken et al. 1988, Brattstrom et al. 1988) has focused on folic acid therapy as a means of lowering plasma homocysteine levels. High dose folate supplementation is felt to enhance remethylation of homocysteine to methionine. Brattstrom et al. (1988) administered 5 mg of folic acid per day for a fourteen day period to forty-two subjects free of vascular and other diseases. Twelve hour fasting total plasma homocysteine and methionine levels were measured before and after folate treatment. Plasma homocysteine decreased in all but the two subjects with the lowest initial concentrations. The mean reduction of homocysteine by 52% was highly significant ($p<0.01$). Plasma methionine concentration was not affected.

Other research in this area (Wilcken et al. 1988) has focused specifically on individuals with chronic renal insufficiency not yet receiving dialysis (serum creatinine: 560 ± 240 umol/L). Folic acid (5 mg/day) was administered for 7-32 days (mean: 15 ± 6 days). Ten hour fasting plasma free homocysteine and methionine levels determined before and after folate treatment were compared to values of healthy controls not receiving folate. Prefolate plasma homocysteine levels were above normal in twenty of twenty-one patients studied. Folate therapy caused a decrease in plasma homocysteine in all patients. Although the group's decrease was highly significant ($p<0.0001$), the mean value attained was still above that of the controls. Whether or not administration of folate for a longer period of time would result in greater benefits is not established.

Whether such decreases in the plasma homocysteine of CRF patients are significant in terms of decreasing risk of vascular disease is not known. Specifically, the impact of lowering plasma homocysteine levels on

premature vascular disease in CRF patients has not been examined. However, epidemiologic studies in the general population (Wilcken and Wilcken 1976, Brattstrom et al. 1984, Freedman et al. 1982, Kang et al. 1986, Boers et al. 1985, Murphy-Chutorian et al. 1985, Wilcken et al. 1983) have found higher plasma homocysteine levels in people with cerebral, coronary or peripheral arterial disease than in healthy controls. This suggests that elevated plasma homocysteine levels might constitute a risk factor for the development of premature vascular disease.

2.5.2 Can Folate Be Toxic?

Many of the effects attributed to folate oversupplementation are based on anecdotal information. Nevertheless, they warrant discussion considering the gross elevations in serum and red cell folate which have been observed in dialysis patients.

A brief report (Anonymous 1985) on pregnant women who received 1 mg folate daily, attributed several complications to high serum folates. Specifically, the quartile with the highest serum folate levels developed complications at delivery including abnormally slow fetal heart rate and maternal infection. Actual serum folate levels were not given. Furthermore, the authors also mentioned having observed low plasma levels of zinc and albumin in some of the women with obstetric complications. Accordingly, the observed fetal/maternal complications were certainly not clearly linked to folate toxicity.

Prakash and Petrie (1982) have related behavioral changes in psychiatric patients to elevated tissue folate levels. A patient receiving diphenylhydantoin was started on 1 mg folic acid daily when his

serum folate was measured at 3.2 ng/ml (normal:5-20). After 5 months his serum folate was 23.7 ng/ml with considerable change in his quiet, blunt affect. He had become excitable and quarrelsome, and frequently giggled without reason. Unfortunately, serum diphenylhydantoin levels were not reported. Folate supplementation decreases serum levels of this drug, sometimes to an ineffective level.

A second patient on lithium carbonate was started on 3 mg folic acid per day when a red blood cell folate of 142 ng/ml was discovered (normal: 225-600). Two weeks later, his RBC folate was 611 ng/ml. Uncharacteristic verbosity, irritability and hostility were reported.

In both patients, excitable behavior was felt to improve after folic acid supplements were tapered.

Hunter and Barnes (1970) studied the effects of 15 mg of folic acid daily in fourteen healthy volunteers. After one month of supplementation, serum folate had reached levels ranging from 65 to more than 180 ng/ml with 4 to 24 ng/ml the normal range. Folate supplementation was stopped at this point because of side effects in the majority of subjects. Specifically, a variety of symptoms including gastrointestinal disturbances, a constant bitter taste and significantly altered sleep patterns were reported. Within three weeks of discontinuing folate, all symptoms were reportedly alleviated.

In a double blind study (Leung et al. 1985) with six month treatments of 5 mg folate daily or placebo in continuous ambulatory peritoneal dialysis (CAPD) patients, nausea, headache, vivid dreams and irritability were slightly more common during folate supplementation. However, occurrence of symptoms was not significantly different between treatments.

In a group of these patients with serum and red blood cell folates seven and fourteen times the upper limits of normal, respectively, there were many complaints of insomnia (Leung et al. 1985). The insomnia resolved on stopping folate supplements.

The mechanisms through which folate produces such side effects are not clearly understood but the previously discussed work in humans suggests that the CNS is involved. High dose folate administration has also been shown to affect the CNS of rats causing neuroexcitability and decreased learning capacity (Bachevalier and Botez 1978).

2.5.3 Zinc-Folate Interactions

Researchers first became interested in the effect of folate supplementation on zinc absorption because of the practice of routine folate supplementation in pregnancy. Meadows et al. (1983) studied the availability of zinc in ten healthy subjects prior to and after supplementation with iron and folic acid. Plasma zinc concentration was followed for six hours after fasted subjects ingested 50 mg zinc. After fourteen days of 100 mg iron and 350 ug folic acid per day, zinc bioavailability was reassessed twenty-four hours after taking the last tablet.

The plasma zinc-time curve appeared to be affected by the iron and folic acid supplement. Specifically, area under the curve and peak zinc concentration were reduced significantly. The authors attributed this decrease in zinc absorption to competition between iron and zinc, failing to discuss a possible role of folate.

Simmer et al. (1987) furthered this research, first studying the effect of iron/folate supplements on intestinal zinc absorption in ten pregnant women. Initially, subjects were not receiving iron or folate supplements. After a twelve hour fast, they were given 25 mg zinc orally. To prevent nausea, all women were given a cup of tea and a small bun both before and after zinc ingestion. Plasma zinc concentration was followed for four hours after zinc administration. Following this, the women received 100 mg iron and 350 ug folic acid daily for fourteen days. Twenty-four hours after taking the last iron/folate supplement, zinc absorption was reassessed, using the protocol previously followed.

Oral iron/folate supplementation appeared to decrease zinc absorption. Specifically, after supplementation the area under the plasma zinc concentration-time curve was reduced to 49% of the baseline value, and peak zinc concentration was significantly decreased ($p<0.02$).

To separate the effects of iron and folate, Simmer et al. (1987) also investigated the effect of folate alone on zinc absorption in healthy nonpregnant females. After an overnight fast subjects were given 50 mg zinc. Plasma zinc levels were followed for four hours. Subjects were then supplemented with 350 ug folate daily for fourteen days. Twenty-four hours after the final dose of folate, zinc absorption was reassessed as previously. After the period of folate supplementation, the area under the plasma zinc-time curve was reduced significantly to 79% of the baseline value, compared with 49% after the period of iron plus folate supplementation. Superficial inspection of these results would suggest that folate alone affected zinc bioavailability less than iron and folate given in combination. However, many factors need to be considered in comparing these two experiments (Simmer et al. 1987).

In the experiment on pregnant women 25 mg zinc was given, while in the later experiment on nonpregnant women 50 mg zinc was given. The pregnant condition may have affected zinc absorption. Furthermore, with the pregnant women, a bun and tea were consumed prior to and immediately after the zinc dose. Valberg et al. (1985) found that giving solids with a dose of zinc significantly decreased area under the plasma zinc time curve in the five hour post zinc administration. Accordingly, the snacks these pregnant women received may have decreased absorption of the dose of zinc they were administered. In summary, the only conclusion that can be drawn from these two experiments (Simmer et al. 1987) is that both folate and iron appear to decrease zinc absorption.

Whether changes in plasma zinc concentration are a good indicator of zinc absorption must also be considered. Valberg et al. (1985) have investigated this using radioactive zinc. Subjects received ^{65}Zn added to a liquid or a turkey patty with plasma zinc concentration followed for five hours. In both cases, zinc absorption (assessed through ^{65}Zn stool counts) was strongly correlated with AUC_5 (area under the plasma zinc time curve). Although slopes of the two regression lines were identical, y intercepts were significantly different indicating a lower plasma zinc response with the turkey meat for an equivalent level of ^{65}Zn absorption. Accordingly, caution should be exercised in using increases in plasma zinc concentration as an index of zinc absorption unless conditions are standardized with the relationship between plasma zinc concentration and zinc absorption defined for specific test meals.

Other researchers interested in folate/zinc interactions have assessed zinc absorption by monitoring fecal and urinary zinc. Specifically, Milne et al. (1984) examined the effects of a much lower level of folic acid

supplementation (400 ug every second day for 5-6 months) on fecal and urinary zinc excretion in men in whom zinc deficiency was induced. Fecal zinc increased significantly during folate supplementation on both control and low zinc diets but not on a high zinc diet. Urinary zinc decreased significantly on all three diets.

Although plasma zinc appeared to be related to dietary zinc, this relationship was not statistically significant. Perhaps this was because the study included such a small number of subjects. The decline in plasma zinc concentration while on the low zinc diet was more pronounced in the folate-supplemented subjects. Conversely, increases in plasma zinc concentration during the high zinc diet, were more pronounced in subjects without folate supplementation. The authors suggested that supplemental folate may have decreased zinc absorption through formation of an insoluble chelate.

Interested in these findings, Ghishan et al. (1986) used rats to determine whether insoluble zinc-folate complexes actually occur. First, zinc absorption was studied in vivo in the presence and absence of folate. These studies showed a significant decrease in zinc absorption in the presence of folate and a significant decrease in folate absorption in the presence of zinc. Charcoal-binding studies were performed to determine whether these findings were the result of folate-zinc complexes. In these studies, folate and zinc chloride solutions were combined with a charcoal medium. After centrifugation, folate which complexed with zinc remained in the supernatant while uncomplexed folate bound to the charcoal. At pH 2 (gastric pH) zinc-folate complexes formed but at pH 6 (duodenal pH) these complexes dissolved. The authors concluded that under normal physiological conditions, zinc and folate do

not form complexes. Some of the foregoing research suggests but certainly does not prove that high dose folate supplementation impairs zinc absorption. More recent research (Butterworth Jr. et al. 1988) refutes this hypothesis. Fifty women with mild and moderate cervical dysplasia were randomly assigned to receive 10 mg/day of either folic acid or Vitamin C (placebo). Plasma and erythrocyte zinc levels were measured after two months (all women) and four months (21 women) on the supplements. The level of zinc in plasma and red blood cells showed no significant change in either the folate-treated or placebo group at 2 or 4 months. Keating et al. (1987) have also challenged the supposition that folate interferes with zinc absorption. Using human subjects they found no difference in serum zinc response curves when 25 mg zinc was given with or without 10 mg of folic acid.

In summary, initial research suggested that high dose folate supplementation impairs zinc absorption. However, more recent research does not support this hypothesis.

2.6 Assessing Zinc Status

2.6.1 Introduction

Zinc is found in most tissues in the human body. Levels are highest in the eyes and prostate, intermediate in bone, liver, muscle and other soft tissues and lowest in adipose tissue (Prasad 1979, Shils and Young 1988). Finding a diagnostic test to assess zinc status is very difficult because in both mild and severe zinc deficiency, there is almost no reduction in the zinc concentrations of most tissues (Golden 1989). Other nutrients such as protein behave similarly to zinc. Both zinc and protein are locked into tissues and are fundamental to the integrity and day to

day functioning of tissues. However, small amounts of zinc may be released during certain metabolic readjustments in the body such as during a period of decreased protein synthesis. Golden (1989) suggests that a negative zinc balance is as likely to be due to a dietary protein deficiency or a metabolic readjustment as to a zinc deficiency. Keeping the foregoing in mind, a discussion of the numerous tests which have been used to assess zinc status is in order.

2.6.2 Plasma or Serum Zinc

Plasma or serum zinc is commonly used as an indicator of zinc status because of its ease of measurement. Solomons (1979) notes that plasma and serum zinc do not reflect body zinc stores but Golden (1989) has criticized this statement on the basis that total body zinc probably bears little relationship to the likelihood of an individual suffering from zinc deficiency. Solomons feels that serum zinc is an unreliable indicator of zinc status because it reflects both serum albumin concentration and the affinity of albumin for zinc. Golden has criticized the argument that plasma zinc may be low secondary to hypoalbuminemia noting that even when plasma albumin is at the lower limit of normal, only about one of every fifty albumin molecules is associated with a zinc atom. However, both authors (Golden 1989, Solomons 1979) agree that circulating zinc is not a very specific measure of zinc deficiency since it is subject to metabolic alterations as well as nutritional limitation. Fox example, low plasma zinc levels are seen in conditions of infection, inflammation, carcinoma and acute stress such as myocardial infarction (Golden 1989, Solomons 1979, Patrick and Dervish 1984) because of redistribution of zinc that exists in the free pool. However, studies involving subjects with induced

zinc deficiencies and TPN patients receiving no trace minerals, found predictable falls in circulating zinc suggesting that serum/plasma zinc can reflect zinc status (Solomons 1979).

2.6.3 Zinc in Erythrocytes, Leukocytes and Neutrophils

Erythrocyte zinc has been proposed as an indicator of zinc status based on reductions in RBC zinc observed in human volunteers fed zinc deficient diets (Buerk et al. 1973) and in patients with Sickle Cell Anemia, a disorder felt to be associated with zinc deficiency (Prasad and Cossack 1982). However, other researchers failed to observe a decrease in RBC zinc in subjects with a marginal zinc deficiency. Since RBC zinc is more difficult to measure than serum or plasma zinc, it has not been looked at as frequently (Solomons 1979).

Leukocyte zinc is even more tedious to measure than erythrocyte zinc. Zinc's role in the leukocyte is not confirmed but its involvement with zinc metalloenzymes is generally accepted (Patrick and Dervish 1984). It may also be involved in membrane stabilization or modulation of membrane function. Dietary deprivation of zinc has been shown to reduce leukocyte zinc but reductions have also been observed in patients with serious metabolic disturbances (Patrick and Dervish 1984). In these individuals leukocyte zinc level did not rise with zinc supplementation but returned to normal when the metabolic defect was corrected. Accordingly, as with plasma zinc, leukocyte zinc is not a useful measure in those with metabolic disturbances. However, it may be useful in monitoring effectiveness of zinc therapy in metabolically stable individuals (Patrick and Dervish 1984).

Neutrophil zinc concentration has also been proposed as an indicator of zinc status with some evidence to support this proposal (Prasad and Cossack 1982). Neutrophils are rich in zinc and have a rapid turnover rate. Subjects who had a negative zinc balance induced over a three month period showed a highly significant decrease in neutrophil zinc concentration. Similarly, in Sickle Cell Anemia, neutrophil zinc concentration was significantly lower than in controls (Prasad and Cossack 1982).

2.6.4 Zinc Metalloenzymes

Zinc is an integral part of a number of metalloenzymes and is a cofactor for a number of zinc-dependent enzymes (Roth and Kirchgenssner 1980). Whether the activity of a particular enzyme decreases in response to a zinc deficiency depends upon how tightly the zinc cation is bound to the protein. For this reason only a few of the known metalloenzymes respond quickly and sensitively to a low zinc intake and then only in certain tissues (Roth and Kirchgenssner 1980). In terms of assessing zinc status in humans, the list becomes even smaller since only a limited number of tissues are readily accessible. Zinc metallo and dependent enzymes which have been used to assess zinc status in man include alkaline phosphatase, lactic dehydrogenase, ribonuclease and carbonic anhydrase (Solomons 1979, Roth and Kirchgenssner 1980, Prasad et al. 1978).

Serum alkaline phosphatase activity has been measured in volunteers in whom a slight zinc deficiency was induced. Activity slowly decreased following zinc restriction and doubled within eight weeks of supplementing the diet with zinc (Prasad et al. 1978). In patients with Acrodermatitis Enteropathica, a disorder thought to be due to decreased zinc absorption,

serum alkaline phosphatase activity has been found to be reduced (Neldner and Hambidge 1975). After two weeks of treatment with 44-88 mg zinc per day, alkaline phosphatase activity returned to normal and skin lesions, diarrhea, decreased appetite and depression resolved. These and other examples which have been cited, suggest that determination of serum alkaline phosphatase activity before and after zinc supplementation is a useful approach to the diagnosis of zinc deficiency in man (Roth and Kirchgenssner 1980).

Lactate dehydrogenase activity may also be an indicator of zinc status. In the previously discussed study (Prasad et al. 1978) where a mild zinc deficiency was induced, serum lactate dehydrogenase activity decreased during zinc depletion and rose significantly during repletion.

The ribonuclease enzyme, in contrast to alkaline phosphatase and lactate dehydrogenase, shows increased activity in zinc deficient tissues. There is evidence in humans that its activity may be a measure of zinc status. In the previous study (Prasad et al. 1978) serum ribonuclease activity increased during zinc depletion and decreased during the repletion phase. Sickle Cell Anemia has been found to result in a reduced plasma zinc concentration and an elevated ribonuclease activity (Roth and Kirchgenssner 1980). With zinc therapy plasma zinc increased while ribonuclease activity decreased. Similar results were observed in a child with Acrodermatitis Enteropathica (Roth and Kirchgenssner 1982).

Finally, there is some evidence that carbonic anhydrase activity may be an indicator of zinc status. When measured in the erythrocytes of Sickle Cell patients and controls (Prasad et al. 1976, Prasad 1976) carbonic anhydrase activity correlated ($r=0.9$) with red cell zinc concentration and was reduced in patients with Sickle Cell Disease.

2.6.5 Hair Zinc Concentration

In reviewing the hair zinc literature, Dorea and Paine (1988) found both evidence supporting and questioning hair zinc concentration as an indicator of zinc status.

They investigated whether hair zinc content indicates if zinc content of other body tissues is within normal limits. Most studies found no association between hair zinc content and zinc levels in other organs, body fluids and secretions. This is not surprising, because as previously discussed, the zinc in body tissues is not readily exchangeable. Whether severe zinc deficiency characterized by stunted growth is associated with a decrease in hair zinc is controversial with evidence for (Dorea and Paine 1988, Marginal Comments 1976) and against (Dorea and Paine 1988).

A second area investigated was whether an association exists between zinc intake and hair zinc content. In all but one study Dorea and Paine (1988) presented, there was no relationship between dietary zinc and hair zinc content. However, this was not always the finding in studies where zinc intake had been experimentally increased. Hair zinc concentration has been found to increase with zinc supplementation in healthy individuals, in some individuals with Acrodermatitis Enteropathica, in adults with suspected zinc deficiency and in children recovering from zinc deficiency. In other studies involving healthy individuals, uremics and patients with Acrodermatitis Enteropathica, zinc supplementation did not result in increases in hair zinc concentration. The effect of a decreased zinc intake on hair zinc concentration has only been looked at in animals and decreases were observed.

Clearly, a consensus has not been reached regarding hair zinc as an indicator of zinc status. Even if one was convinced that hair zinc concentration is a valuable measurement, there are many variables affecting it which are difficult to control. These include hair colour, age, sex, exposure to zinc in the environment and season (Taylor 1986, Hambidge 1980).

2.6.6 Taste Acuity and Salivary Zinc

Researchers have shown interest in taste acuity testing as a functional parameter of zinc status analagous to dark adaptation testing for subclinical Vitamin A deficiency (Solomons 1979). But reports are conflicting on whether zinc supplements improve hypoguesia. According to Solomons (1979), few conclusions can be drawn about the relationship between zinc deficiency and taste acuity.

Closely related to taste acuity measurement is salivary zinc determination. It has been suggested that a deficiency of gustin, a zinc-containing salivary protein, mediates impairment of taste in zinc-deficient subjects (Solomons 1979). In support of salivary zinc concentration as an indicator of zinc status is the observation that it was decreased fivefold in patients with idiopathic hypogeusia (Solomons 1979). Radioactive zinc administered intravenously, was found to concentrate in these patients' saliva. Others (Solomons 1979) who administered a zinc-deficient diet to subjects for 21 days, found decreases in the zinc concentration of solid components of saliva reflecting the progression of zinc depletion. Further investigation of this possible relationship between saliva zinc concentration and hypogeusia is warranted.

2.7 Zinc Status of Chronic Renal Failure Patients

Certain metabolic alterations associated with chronic renal failure may invalidate some of the previously discussed measurements of zinc status. Plasma and red blood cell zinc concentration are examples of measurements which may be invalid. Whether a low plasma zinc in renal failure represents a true zinc deficiency or merely a shift from plasma into other body pools such as red blood cells has been debated. The fact that elevated RBC zinc levels (Mahajan et al. 1979a, Rose and Willden 1972, Mahajan et al. 1978) and decreased plasma zinc levels (Mahajan et al. 1979a, Condon and Freeman 1970, Halsted and Smith 1970, Burge et al. 1984, Mahajan et al. 1980) have often been observed in CRF patients, lends support to the foregoing hypothesis. Unfortunately, plasma and RBC zinc have seldom been measured concurrently and there are reports of normal RBC zinc concentrations (Mansouri et al. 1970) and normal to high serum zinc concentrations (Mansouri et al. 1970, Rose and Willden 1972, Mahler et al. 1971) in uremics.

The fact that the plasma concentrations of some zinc-dependent enzymes are elevated in chronic renal failure patients makes it difficult to use them as indicators of zinc status. When laboratory animals are being studied, enzymes can be extracted from tissues that have been removed making it possible to determine specific activities (units of activity/mg of enzyme) of these enzymes. In contrast, when living humans are being studied, the only readily accessible tissue is blood. When zinc-dependent enzyme activity has been used to assess zinc status, enzyme activity per millilitre of plasma (rather than specific activity) has usually been used (Neldner and Hambidge 1975, Prasad et al. 1975, Prasad et al. 1978,

Mahajan et al. 1979a). This is probably because the extraction of enzymes from plasma is very difficult. Expressing enzyme activity as units of activity per millilitre of plasma may be valid for normal individuals. However, with disorders which cause plasma concentrations of given zinc-dependent enzymes to increase, there is a false impression of increased enzyme activity.

Zinc-dependent enzymes which may be elevated in chronic renal failure include serum alkaline phosphatase and serum ribonuclease. Serum alkaline phosphatase may be elevated in CRF due to the commonly associated metabolic bone disease (David 1977). Serum ribonuclease, an extremely stable enzyme, is also greatly elevated in renal failure presumably because it is normally excreted in the urine (Reddi 1978). One might also expect erythrocyte carbonic anhydrase activity to be affected by uremia. Specifically, if RBC zinc concentration is elevated then erythrocyte carbonic anhydrase activity might also be elevated.

Finally, whether the diminished taste acuity reported in chronic renal failure is a function of zinc deficiency or some other aspect of the uremic syndrome remains controversial. Despite these limitations, the literature on zinc status of CRF patients is worth reviewing.

Taste acuity and plasma zinc levels have frequently been used to assess zinc status of uremic individuals. Burge et al. (1984) examined dietary zinc intakes, serum zinc, and recognition thresholds for sour and sweet in subjects with mild, moderate and severe renal failure (creatinine clearances of 41-75, 15-40 and 5-14 ml/min respectively). Mean zinc intake, based on two day food diaries, showed no significant differences among the three groups. Serum zinc decreased as renal function declined. There was a significant inverse relationship between creatinine clearance

and threshold for sweet and sour. As blood urea nitrogen increased, recognition threshold for sour but not sweet increased. Serum zinc was inversely correlated to recognition threshold for sour only.

Another study (Mahajan et al. 1979b) investigated the effect of zinc supplementation on the hypogeusia of uremia. Eleven stable hemodialysis patients received either 25 mg elemental zinc or a placebo once a day. The study also included 20 control subjects with normal renal function. After 6-12 weeks, plasma zinc concentration had increased significantly in the treatment group reaching a level comparable to that of controls. At entry to the study, mean detection and recognition thresholds for NaCl, sucrose, HCl, and urea were significantly higher in uremics than in controls. After 6-12 weeks there were significant decreases in detection and recognition thresholds for NaCl, sucrose, and urea but not HCl, in the zinc treated group.

Finally, Mahajan et al. (1979b) looked at plasma, RBC, WBC and hair zinc concentrations in predialysis, hemodialysis and peritoneal dialysis patients. Plasma, hair and leukocyte zinc levels were significantly decreased in all uremic patients in comparison to controls. Conversely, RBC zinc was significantly increased in the three uremic groups.

In conclusion, the literature does not reveal a reliable means of assessing zinc status in CRF patients. Metabolic changes associated with chronic renal failure may alter plasma and RBC zinc levels as well as plasma levels of some zinc-dependent enzymes. One recommendation is to measure several parameters including serum, hair and neutrophil zinc levels (Gibson 1988).

2.8 Is a 3 Day Food Record a Valid Tool For Determining Average Daily Energy, Protein, Folate, and Zinc Intakes?

2.8.1 Introduction

Validating the 3 day food record as a tool for determining individuals' average daily energy, protein, folate, and zinc intakes requires demonstrating that it indeed determines these average daily intakes. Such validation requires that the truth be known about dietary intakes. "When the method purports to measure usual intake over an extended period of time, revealing the true intake either presents overwhelming practical difficulties (direct observation of many individuals over a long period) or is actually impossible (if the definition of "usual" is not limited in time)" (Block 1982). Since "the truth" is not available to investigators, approaches have included "validating" one method against another method which has wider acceptance, or demonstrating that a method elicits a usual intake by showing that it produces similar results on two different occasions (Block 1982). Both of these approaches have been used by researchers (Chalmers et al. 1952, Marr and Heady 1986, Jackson et al. 1986, Tremblay et al. 1983, Stuff et al. 1983) trying to validate the 3 day food record as a tool for determining average daily intakes of energy, protein and several vitamins and minerals. One final consideration in the validation of a 3 day food record, is deciding what degree of precision or repeatability is acceptable. For example, one researcher may be satisfied if nutrient intakes determined on two different occasions vary by up to 20% while another researcher may accept no more than a 10% variation.

2.8.2 Validity of a 3 Day Food Record in Determining Average Daily Energy Intake

Some researchers (Bastios et al. 1987) have found that 3 days of recording is not adequate to assess an individual's "average daily energy intake." However, others (Marr and Heady 1986, Jackson et al. 1986, Tremblay et al. 1983) have cited evidence suggesting that 3 days of recording is adequate. Marr and Heady (1986) determined energy intakes of 151 males who had kept 7 day weighed food records. Based on energy intake, subjects were classified into a bottom, middle or top tertile. The authors then developed a table which compared shorter food records (a portion of the 7 day records) to 7 day food records in ability to correctly classify subjects into the previously outlined tertiles. Subjects were considered grossly misclassified if the shorter food record placed them in the top tertile when the 7 day food record placed them in the bottom tertile. The converse was also considered gross misclassification. The researchers essentially ignored subjects who fell into the top or bottom tertile with one method and into the middle tertile with the other method. Using a 3 day food record, 70% of subjects were correctly classified with 4% grossly misclassified.

Jackson et al. (1986) used a different approach to determine the minimum number of days food intake should be recorded to assess an individual's average daily energy intake. Eighteen subjects (11 male, 7 female; ages 25-69) recorded food intakes for 14 days. Next, a computer generated the 12 sets of records for 3 consecutive days in the 14 days. Each subject's mean energy intakes based on their 3 day food records, were compared to mean energy intake based on their 14 day food record. The authors determined whether all energy intakes (based on 12 individual 3

day food records) differed by 5% or less from mean energy intakes based on respective 14 day food records. They had arbitrarily decided that meeting this criterion would deem the 3 day food record reliable in monitoring energy intake of an individual. In their study, 96% of the 3 day food record mean energy intakes came within 5% of their respective 14 day mean energy intakes. Finding that 96% of the 3 day records met this demanding "5% criterion" indicates that a 3 day food record is reliable in characterizing an individual's energy intake.

Tremblay et al. (1983) also assessed the reliability of a 3 day food record in determining average daily energy intake. From a group of 1539 subjects (ages 9-59), 26 cooperative adults kept two 3 day food records (approximately 1 week between records). Paired t-tests and intraclass correlation coefficients were used to compare energy intakes estimated from subjects' two food records. There was a difference of 8% in energy intake between the two records (2319$\pm$646 Kcal versus 2124$\pm$473 Kcal) and this difference was not statistically significant. A significant ($p \leq 0.01$) intraclass correlation coefficient of 0.72 was felt by the authors to reflect acceptable reproducibility of energy intake results.

This study (Tremblay et al. 1983) differs from the previous studies (Marr and Heady 1986, Jackson et al. 1986) in its approach to validation of the 3 day food record. Validation involved examining reproducibility of results rather than comparing 3 day record results to those from a method with greater acceptance. Block (1982) has criticized the "reproduction of results" method. Specifically, since it is not known whether what is being measured remains unchanged, one does not know whether disimilar results on two occasions indicate an unreliable method or a reliable method which is measuring a change in intake.

Tremblay et al. (1983) found energy intake to be similar on both occasions it was measured but there was only a one week period between measurements. Considering this plus the validation method used, these results are weaker than those of previously discussed studies (Marr and Heady 1986, Jackson et al. 1986). Nevertheless, collective examination of all 3 studies offers considerable support for the 3 day record as a tool for determining average daily energy intake.

2.8.3 Validity of a 3 Day Food Record in Determining Average Daily Protein Intake

There is research for and against the 3 day food record as a means of assessing average daily protein intake (Bastios et al. 1987, Stuff et al. 1983, Tremblay et al. 1983). Bastios et al. (1987) found that an average of 42 days of recording is needed to characterize average daily protein intake with 10% of that determined with a 1 year food record. However, other researchers (Stuff et al. 1983, Tremblay et al. 1983) with less stringent repeatability criteria showed the 3 day food record to be reasonably reliable in the determination of an individual's average daily protein intake.

Stuff et al. (1983) assessed protein intakes of 40 lactating females using both 3 day and 7 day food records. The 3 day record included 2 days randomly selected from the 5 weekdays and 1 day selected from the weekend (the authors did not specify how this was selected). Individual protein intakes based on the 3 day records were comparable to those based on the 7 day records which the authors considered representative of true intakes. Specifically, an intraclass correlation coefficient of 0.76 suggested reasonably good agreement between the two methods.

Tremblay et al. (1983) took a different approach to assessing the reliability of a 3 day food record. From a group of 1539 subjects (ages 9-59), 26 cooperative adults kept two 3 day food records (approximately 1 week between records). Paired t-tests and correlation analysis were used to compare protein intakes as estimated from subjects' two food records. Protein intake was significantly lower (16.8%) in the second record (79.4 ± 19.8 g versus 95.4 ± 29.1 g; $p<0.01$). Although this difference in protein intake was statistically significant, a precision of 16.8% would be considered reasonable by some researchers. A significant ($p<0.01$) intraclass correlation coefficient of 0.72 also suggested reasonable agreement between the 3 day and 7 day food records.

In conclusion, there is support for the 3 day food record as a tool for determining average daily protein intake. However, this is not quite as strong as the support related to average daily energy intake determination.

2.8.4 Lack of Validity of a 3 Day Food Record in Determining Average Daily Folate and Zinc Intakes

Several studies (Tremblay et al. 1983, Stuff et al. 1983, Karkeck 1987, Bastios et al. 1987) have assessed the validity of a 3 day food record as a method of determining an individual's average daily intake of many vitamins and minerals. Unfortunately, folate and zinc intakes have not been investigated in these studies probably because food composition data bases are incomplete for these nutrients. However, reviewing the foregoing studies with respect to intake of micronutrients such as iron, vitamin A and ascorbic acid is useful. Specifically, many good sources of iron are also good sources of zinc while ascorbic acid and vitamin A are

supplied largely by fruits and vegetables which are also significant sources of folate.

Tremblay et al. (1983) had 61 subjects keep two 3 day food records. After average daily iron, vitamin A and ascorbic acid intakes were calculated, intraclass correlation coefficients were determined as a measure of within individual agreement for nutrient estimates by the two methods. The correlation coefficients were 0.73, -0.06, and 0.54 for iron, vitamin A and ascorbic acid respectively. Even though those for iron and ascorbic acid were significant ($p<0.01$), only that for iron was large enough to suggest reasonable agreement in nutrient estimation between repeated 3 day food records.

In another study (Stuff et al. 1983) involving 40 lactating females, iron intakes were determined using a 3 day food record and a 7 day food record. Unfortunately vitamin intakes were not assessed. As in the previous study, an intraclass correlation coefficient was used as an indicator of within individual agreement for the two methods of determining iron intake. Furthermore, a regression equation was used to estimate the 95 percent prediction interval within which an individual's 7 day record iron would lie, based on their 3 day record value. A highly significant ($p<0.005$) correlation coefficient of 0.82 suggested good agreement between the two methods. However, the prediction interval (expressed as a percentage of Recommended Daily Allowance) was $\pm$19 percent suggesting poor agreement between the 3 day and 7 day records.

Karkeck (1987) has summarized the findings of several studies which investigated the number of recording days required to classify 80 percent of the population into tertiles of nutritional intake with 95 percent confidence. The range in days required for iron, vitamin A, and ascorbic

acid were 12 to 19, 46 to 64 and 6 to 14 respectively again pointing to the inadequacy of a 3 day food record.

Finally, Bastios et al. determined the average daily intake of several nutrients (referred to as usual intakes) for 16 females and 13 males using a year long period of recording. Confidence intervals constructed for each individual's usual intakes, were used to determine the number of days required to estimate intake within 10 percent of usual intake. Average number of days required and ranges were as follows: females: iron - 66 (28-142), vitamin A - 474 (152-1372), ascorbic acid - 222 (83-328); males: iron - 68 (18-130), vitamin A - 390 (115-1724), ascorbic acid - 249 (90-900). This final study emphasizes the point made by previously discussed studies.

Specifically, the 3 day food record is not a valid tool for determining average daily intakes of micronutrients such as iron, vitamin A, ascorbic acid, folate and zinc. The large number of recording days that would be required to determine usual or average daily folate and zinc intake would not be feasible for most studies. Accordingly, the diet history method has been recommended for such endeavours (Block 1982). The history method in this case refers to any method involving an extensive interview designed to elicit usual intake.

A form of the history method was used in the present thesis project. Specifically a food frequency questionnaire requiring a lengthy interview was developed. It was designed to elicit usual intake of all significant dietary sources of folate and zinc.

2.9 Summary

In the 1960s, chronic hemodialysis patients were often found to have folate deficiencies. Such deficiencies might be expected considering reports of folate levels as low as 5-72 ug folate per day in the "hemodialysis diet." Alleviation of folate deficiencies with a 5 mg per day folate supplement is probably what led to fairly routine folate supplementation in chronic hemodialysis patients.

It was not until the 1980s that clinicians once again became interested in studying folate status of chronic hemodialysis patients. The "hemodialysis diet" had been liberalized and could now provide the RNI of 3 ug folate/kg body weight. However, many nephrologists continued to prescribe 7-35 mg of folate per week for their chronic hemodialysis patients. When serum and RBC folate levels were examined in such patients, they were found to be high normal to well above the normal range.

Attempts were made at assessing whether these chronic hemodialysis patients required folate supplements to maintain normal folate stores. Specifically, RBC folate levels were remeasured 6-12 months after folate supplements had been withdrawn. However, such studies did not actually assess whether patients could maintain normal folate stores without folate supplementation. Instead they determined whether patients' high normal or above normal folate stores would become depleted when folate supplements were withdrawn. Furthermore, these studies failed to determine dietary folate intakes of patients.

The high RBC folate levels observed in CRF patients receiving a supplement of 7-35 mg of folate per week, has raised concern about folate toxicity. There are anecdotal reports suggesting adverse effects with

high dose folate supplementation, however, there are no controlled studies indicating folate toxicity. Preliminary studies raised the additional concern that high dose folate supplementation may impair zinc absorption. But more recent studies do not support this hypothesis.

Research on high dose folate supplementation and the possibility of an effect on zinc status, has raised the interest of renal dietitians in B.C. This is because many chronic hemodialysis patients in B.C. are on very large doses of folate in comparison to the RNI of 3 ug/kg body weight. Specifically, most of these patients have routinely been prescribed one vitamin B complex with C tablet daily and a 5 mg folate tablet either 3 or 7 times per week. Folate has been given separately because the B-complex vitamins the provincial government provides for CRF patients (Beminal with C and Z-Bec) do not contain folate. While Beminal with C only provides B vitamins and vitamin C, Z-Bec also provides 22.5 mg of zinc per tablet.

"Hemodialysis diets" prescribed today, are generally higher in zinc than those prescribed in the 1960s and early 1970s. Nevertheless, some nephrologists have routinely prescribed Z-Bec over Beminal with C because of research suggesting poor zinc status in CRF patients. However, actual dietary zinc intakes of chronic hemodialysis patients have seldom been determined. This has contributed to the controversy over whether impaired taste acuity in chronic hemodialysis patients is due to poor zinc status. Another factor in this controversy is the difficulty zinc status assessment of CRF patients poses.

In conclusion, it is not clear from the literature whether chronic hemodialysis patients can maintain normal folate status by consuming a diet providing the RNI for folate. Furthermore, although recent studies

do not support the contention that high dose folate supplementation impairs zinc absorption, this has not been investigated in chronic hemodialysis patients. Accordingly, the present study was designed to assess whether such patients, consuming a diet providing the RNI for folate, maintain normal RBC folate levels. The study also investigated whether supplementing these patients with 5 mg of folate per day was associated with a decrease in serum zinc.

CHAPTER THREE

METHODS

3.1 Subject Selection

All study subjects were recruited from the Willow Hemodialysis Unit and hemodialysis units at Vancouver General Hospital, St. Paul's Hospital, the Royal Columbian Hospital, the Royal Inland Hospital and the Royal Jubilee Hospital. The patients selected for the study met the following criteria:

1. were clinically stable (i.e., symptoms associated with their chronic renal failure were relatively stable under present treatment regimes and they were not suffering from any acute disease processes). Clinical stability was verified by the patient's nephrologist.
2. male or female; 25-69 years old.
3. presently on hemodialysis (a minimum of 8 hours per week) and on hemodialysis for a minimum of 6 consecutive months prior to the study.
4. free of liver disease, alcoholism, malabsorption, small intestine resection or any other disorder known to affect folate or zinc status and not a vegan.
5. not on medications thought to affect folate or zinc metabolism (Table 1) except for ASA, Calcium Carbonate, Colace, 1,25 vitamin D_3, Digoxin, Ducolax and Iron. The rationale for these exceptions follows Table 1.
6. receiving blood transfusions less than once a month.
7. considered reliable by the unit's dietitian to participate in dietary assessment procedures.

8. on one of the following folate/zinc supplementation regimes during at least the last 6 months of hemodialysis:

   - no folate, 22.5 mg zinc per day*
   - no folate, no zinc
   - 5 mg folate per day, 22.5 mg zinc per day
   - 5 mg folate per day, no zinc

   *After patient screening was completed, it became apparent that the "no folate, 22.5 mg zinc group" was going to be extremely small. Since there were 12 eligible patients on no folate, no zinc, the possibility of starting some of them on 1 Z-Bec per day (includes 22.5 mg zinc) was considered. Five patients were randomly selected from this group and started on 1 Z-Bec per day. The intention was to enter these patients into the study after 4 months on Z-Bec because based on the literature (Prasad et al. 1978, Myers and Hamilton 1951) it was felt that this would be sufficient time to elicit changes in serum and hair zinc levels. When these patients' records were rechecked after about 3 months had elapsed, only 3 remained on hemodialysis, 2 having received transplants. Two of these 3 patients agreed to participate in the study and were included in the "no folate, 22.5 zinc group." They were placed on 22.5 mg zinc per day; one patient took it for 3 months, 19 days stopping 19 days prior to study bloodwork (because she felt Z-Bec was causing her to be nauseated); the other took it for 3 months, 25 days (in the first 3 months, 5 days, intake varied from 5-7 tablets per week the patient claimed, because she had not been informed of the importance of taking the tablet daily even though she knew she was in a study) prior to study bloodwork.

The study was undertaken after approval was granted by UBC's Clinical Screening Committee for Research Involving Human Subjects and by the individual hospitals from which the subjects were drawn. Each prospective subject was given a recruitment letter (Appendix A) in person at their hemodialysis unit. After they had read the letter, if they were interested in participating in the study they were questioned in order that a Verification of Eligibility for the Study form (Appendix B) could be filled out. Part of this questioning was aimed at verifying medications and vitamin/mineral supplements patients were on. To aid in this process a poster displaying samples of commonly used medications and vitamin/mineral supplements was used. In cases where patients could describe but did not know the name of a given medication, this was verified using patient medication lists in the Nursing cardex. When eligibility was verified, the patient signed a consent form (Appendix C).

Twenty-one subjects were entered in the study. Treatment groups they fell into are outlined in the next section.

TABLE 1. Medication of hemodialysis patients which may affect folate or zinc metabolism (see Appendix D for list of medications reviewed)

MEDICATION	EFFECTS ON FOLATE/ZINC METABOLISM
Acetylsalicylic Acid* (ASA)	- affects folate utilization (Roe 1985) - alters transport of folate by competing for serum protein binding sites; may cause folate depletion (Grant and DeHoog 1985)
Aldomet	- increases need for folate (Powers and Moore 1983)
Alka Butazoladine	- decreases folate absorption (Powers and Moore 1983)
Calcium Carbonate*	- calcium is an inhibitor of zinc absorption (The Nutrition Foundation 1984)

TABLE 1 continued

MEDICATION	EFFECTS ON FOLATE/ZINC METABOLISM
Cloxacillin	- may decrease folate utilization (Grant and DeHoog 1985)
Colace*	- may increase absorption of minerals (Grant and DeHoog 1985)
Colchicine	- may decrease folate absorption (Powers and Moore 1983)
Digoxin*	- increases urinary zinc (Grant and DeHoog 1985)
Dilantin	- subnormal serum folate levels as well as macrocytosis have been observed in patients receiving Dilantin (Roe 1985)
Ducolax*	- decreases absorption of glucose and possibly other nutrients (Grant and DeHoog 1985)
1, 25 $(OH)_2$* Vitamin D_3	- acts as a facilitator in zinc absorption (The Nutrition Foundation 1984)
Ferrous Iron*	- ferrous iron is thought to inhibit zinc absorption; the inhibition mechanism and the level of iron which has this effect have not been established (Meadows et al. 1983, The Nutrition Foundation 1984)
Lasix	- increases urinary zinc (Roe 1985,Grant and DeHoog 1985) - increases serum zinc (Powers and Moore 1983)
Oral Contraceptives	- folic acid metabolism affected; serum folate decreased (Powers and Moore)
Phenobarbital	- is a folate antagonist (Roe 1985) - decreases serum folate (Powers and Moore 1983) - can lead to megaloblastic anemia (Canadian Pharmaceutical Assoc. 1987)
Premarin	- decreases absorption of water-soluble vitamins; decreases serum folate (Powers and Moore 1983)
Seconal	- may cause folate deficiency (Roe 1985)
Sinemet	- Sinemet is a combination of the drugs L-Dopa and Carbidopa. It is suspected but not proven that chronic L-Dopa treatment which causes excessive utilization of the de novo pathway for methyl group synthesis, may increase folate requirements (Roe 1985)

Rationale for Including Subjects on Certain Drugs* which may affect Folate/Zinc Metabolism

Colace, 1, 25 $(OH)_2$ Vitamin D_3, Calcium Carbonate, Ferrous Iron

These are standard medications for many chronic hemodialysis patients. There is insufficient evidence for an interaction between these medications and folate/zinc to justify exclusion of patients on these medications from the present study (The Nutrition Foundation Inc. 1984, Grant and DeHoog 1985).

ASA

Many hemodialysis patients are receiving a low dose of ASA (350 mg/day) as an anticoagulant to prevent graft clotting. The research suggesting that ASA competes for folate-binding proteins, has involved patients with rheumatoid arthritis taking large doses of ASA (Roe 1985). Furthermore, whether urinary folate or red cell folate levels are affected has not been investigated. On this basis, patients taking less than 350 mg ASA per day were included in the study.

Digoxin

Considering the low urine output of patients included in this study, the increases in urinary zinc with Digoxin treatment, are probably negligible. Accordingly, patients on Digoxin were not excluded from the study.

Ducolax

Decreased absorption of nutrients with Docolax treatment was mentioned in one reference only (Roe 1985) with the study quoted being unpublished. Accordingly, patients on Ducolax were not excluded from the study.

3.2 Study Design

A 2x2 factorial quasiexperimental design was chosen. In the present study, the term quasiexperimental refers to the fact that subjects were not randomly assigned to treatment groups (Polit and Hungler 1985). Each of the four treatment groups included subjects from more than one hemodialysis unit as outlined in Figure 1 below. Independent variables were folate supplementation level (no supplement or 5 mg folate per day) and zinc supplementation level (no supplement or 22.5 mg zinc per day). Dependent variables were folate status and zinc status. Red blood cell folate concentration was used as an indicator of folate status while serum zinc concentration was used as an indicator of zinc status. Hair zinc concentration was also used in subjects who did not perm, bleach or dye their hair.

Additional measurements taken to assess general nutritional status included: frame size, height and post-dialysis weights (mean of post-dialysis weights at the five dialyses prior to RBC folate blood collection). Subjects kept 3 day food records so that average daily energy and protein intakes could be determined. A food frequency questionnaire was used to determine average daily folate and zinc intakes.

FIGURE 1. Study Treatment Groups

	No zinc	22.5 mg zinc per day
no folate	n = 5 3 - VGH 2 - SPH	n = 4 1 - Willow 3 - SPH*
5 mg folate per day	n = 6 4 - VGH 1 - Willow 1 - RCH	n = 6 2 - VGH 4 - Willow

Legend

* - 2 of these subjects were on no folate/no zinc initially; they were then placed on 22.5 mg zinc/day; one patient took it for 3 months, 19 days stopping 19 days prior to study bloodwork; the other took it for 3 months, 25 days (in the first 3 months, 5 days, intake varied from 5-7 tablets/week) prior to study bloodwork.

Willow: Willow Dialysis Unit
SPH: St. Paul's Hospital
RCH: Royal Columbian Hospital
VGH: Vancouver General Hospital

Table 2 Characteristics of Subjects Receiving No Zinc and No Folate Supplements

Subject	Age* (years)	Sex	Etiology of Chronic Renal Failure	Consecutive* Months on HD	Months of Other Treatments Prior to HD	Hours of Dialysis /week
B.A.	50.54	M	Glomerulonephritis	94.0	6PD,2HD, 2.27 Tx	12
D.L.	61.83	M	Glomerulonephritis and Hypertension	8.37	nil	12
T.L.	69.10	F	Nephrosclerosis due to Hypertension	6.90	12.7 PD	12
M.Mc	72.21	F	not known	94.47	nil	8
M.B.	46.08	F	Glomerulonephritis	94.70	39 PD	12
	59.95+11.39 (mean + SD)			56.69 (mean)		11.2+1.79 (mean + SD)

Legend to Abbreviations

* - at the time RBC folate was determined
HD - hemodialysis
PD - peritoneal dialysis
TX - kidney transplant

Table 3 Characteristics of Subjects Receiving a Supplement of 22.5 mg Zinc Daily and No Folate Supplement

Subject	Age* (years)	Sex	Etiology of Chronic Renal Failure	Consecutive* Months on HD	Months of Other Treatments Prior to HD	Hours of Dialysis /week
H.L.	47.86	M	not known	51.30	nil	12
D.R.	39.17	M	Malignant Hypertension	8.63	nil	12
A.K.	66.58	F	not known	156.0	nil	9
M.Be	49.04	F	Thrombocytopenia	11.40	nil	8
	50.66+11.49 (mean + SD)			56.83 (mean)		10.25+2.06 (mean + SD)

Legend to Abbreviations

* - at the time RBC folate was determined
HD - hemodialysis

Table 4 Characteristics of Subjects Receiving a Supplement of 5 mg Folate Daily and No Zinc Supplement

Subject	Age* (years)	Sex	Etiology of Chronic Renal Failure	Consecutive* Months on HD	Months of Other Treatments Prior to HD	Hours of Dialysis /week
R.C.	60.12	F	Hypertension	86.0	nil	12
K.H.	57.69	F	Analgesic Nephropathy and Essential HT	41.60	nil	9
N.S.	57.33	F	Polycystic Kidney Disease	121.40	nil	12
Sh.S	38.53	F	Chronic Pyelonephritis	165.17	8PD	10.5
S.S.	27.05	M	Urethral Stricture	12.93	nil	12.5
G.S.	48.21	M	Polycystic Kidney Disease	20.77	nil	8
	48.16+13.09 (mean + SD)			74.75 (mean)		10.66+1.83 (mean + SD)

Legend to Abbreviations

* - at the time RBC folate was determined
HT - hypertension
HD - hemodialysis
PD - peritoneal dialysis

Table 5 Characteristics of Subjects Receiving Supplements of 5 mg Folate and 22.5 mg Zinc Daily

Subject	Age* (years)	Sex	Etiology of Chronic Renal Failure	Consecutive* Months on HD	Months of Other Treatments Prior to HD	Hours of Dialysis /week
J.B.	30.74	M	IgA Nephropathy	30.17	nil	12
D.B.	51.08	M	Polycystic Kidney Disease	11.70	nil	11.25
T.N.	25.89	M	Lupus Nephritis	46.70	3PD	12
J.S.	35.34	M	Glomerulonephritis and Hypertension	123.00	nil	13.5
W.H.	69.46	M	probably hypertension	36.47	8PD	9
W.W.	27.55	M	possibly MODY	24.90	nil	12
	40.01+17.04 (mean + SD)			45.49 (mean)		11.63+1.48 (mean + SD)

Legend to Abbreviations

* - at the time RBC folate was determined
HD - hemodialysis
PD - peritoneal dialysis
MODY - Maturity Onset Diabetes in Youth

3.3 Rationale For Lack of Randomization of Subjects into Treatment Groups

The ideal design for this study would have involved subjects recently diagnosed with endstage renal disease just starting hemodialysis who had not been receiving any vitamin or mineral supplements. Of course, only subjects meeting criteria similar to those previously outlined would be eligible for the study. They would be randomly assigned to one of the 4 treatment groups for a period of 12 months with RBC folate, serum zinc concentration, and hair zinc concentration determined at entry and every 3-4 months thereafter. Dietary intakes of folate, zinc, energy and protein would be measured at similar intervals.

This kind of approach could be taken if all hemodialysis units in Vancouver, through some integrated approach, could randomize eligible patients into these treatment groups. However, it is unlikely that the attending nephrologists would agree to such a design protocol. Even if they were in agreement, it would probably take several years to achieve sufficient subject numbers, especially considering attrition due to kidney transplants. Accordingly, the previously discussed quasiexperimental design was chosen.

Each treatment group included patients from more than one hemodialysis unit and a total of eight nephrologists were involved in the care of these patients. Accordingly, the bias which could have occurred if each treatment group had consisted of subjects from one centre under the care of one nephrologist, was avoided. Nevertheless, the chosen study design does not allow generalization of results to the entire population of chronic hemodialysis patients.

3.4 Formulation of a Food Frequency Questionnaire to Assess Average Daily Dietary Intakes of Folate and Zinc

A food frequency questionnaire was developed to assess average daily dietary intakes of folate and zinc in all study subjects. The questionnaire was intended to allow:

1. comparison of treatment groups with respect to mean intake of these nutrients.
2. examination of individual folate and zinc intakes with a reasonable degree of accuracy.

A decision was made to include foods for which a "typical serving" provided 10 ug or more total folacin and/or 0.45 mg or more zinc, each representing approximately 5% of the RNI for adults at the time formulation of the questionnaire began. This decision stemmed from the Nutrition Canada Survey's finding that individuals eat approximately 20 servings of food items per day. Based on this, consuming 20 servings of food items which each provided at least 5% of the RNI would ensure meeting the RNI. Accordingly, this "5% level" seemed reasonable. Using tables (Hoppner et al. 1972, Perloff and Butrum 1977, Pennington and Church 1980, Briggs and Calloway 1979, Freeland et al. 1980, Murphy et al. 1975) which indicated folate and zinc content of folate-rich and zinc-rich foods, initial lists of folate-rich and zinc-rich foods were compiled. Foods were grouped in the following categories: breads and cereals, meat and alternates, milk and yogurt, vegetables and soup, fruits, and desserts and supplements. A beverage group was not included since beverages other than fruit juice, milk, and nutritional supplement beverages such as Ensure^{R} were found to be insignificant sources of folate and zinc.

Next each list was expanded to:

1. be more comprehensive. For example, the zinc-rich breads and cereals list which included only whole wheat or rye bread, was expanded to include items such as white bread, pumpernickel bread, white rolls, whole wheat rolls, crumpets, muffins, Danish Pastry, etc. After checking zinc and folate content of cereals in the cereal section at Safeway, the cereal list became fairly comprehensive in terms of cereals on the Canadian market.
2. include moderate food sources of zinc/folate which are commonly consumed in quantities larger than a typical serving resulting in provision of 10 ug or more total folacin and/or 0.45 mg or more zinc per day.

After these expanded lists had been compiled with "typical servings" beside each food, their Canadian Nutrient File (CNF) values for folate and zinc were recorded using the NUTRICOM nutrient analysis software package. In cases where folate or zinc values were missing, other sources (Hoppner et al. 1972, Perloff and Butrum 1977, Pennington and Church 1980, Briggs and Calloway 1979, Freeland et al. 1980, Murphy et al. 1975, COMPUTRITION Nutrient Analysis Software) were referred to. In cases where folate or zinc content was not available, it was estimated. For example, a zinc value could not be found for croissants so a 50 g portion was estimated to have the same zinc content as a 50 g portion of white bread. All of these estimations as well as all cases where a source other than the CNF has been used, have been documented in the Detailed Folate and Zinc Food Frequency Questionnaire (Appendix E).

A different approach was taken with the cereal list. Specifically, for all fortified cereals, zinc values were recorded from cereal boxes

when available and from NUTRICOM in other cases. Folate was recorded as 0.06 mg/100 g cereal, Canada's approved level of folate supplementation for cereal. For nonfortified cereals such as Shredded Wheat, Red River and rolled oats, zinc as well as folate were taken from the CNF when available with previously discussed sources referred to as necessary.

One final procedure was used to decrease the number of questions in the food frequency questionnaire. Specifically, when two or more foods were very similar in content of the nutrient(s) they were considered to be rich in (folate, zinc, or both), an average of their folate/zinc levels were taken. For example, this was done for several vegetables where both fresh cooked and frozen cooked values were available. It was also done with cheese and cereals.

3.5 Validation of the Food Frequency Questionnaire

Validation procedures were intended to verify that the food frequency questionnaire included the majority of significant dietary sources of folate and zinc. Twelve individuals (5 female, 7 male; ages 28-68) were instructed on recording their food intake for 3 consecutive days on a specific food intake form (Appendix F).

Intake records were reviewed for completeness and ambiguities clarified with subjects. Each intake record was analyzed using the NUTRICOM nutrient analysis software package. After each food was entered, its nutrient listing on the monitor screen was checked to see if folate or zinc values were missing. Foods for which values were missing were noted. For each of these foods, folate and or zinc values were determined as discussed in the previous section, from other sources or through estimation. These procedures allowed determination of total folate and

zinc intake for each of the three days and thus average intakes over the three days. Procedures used to fill in missing folate and zinc values for foods consumed by participants in the validation study, are outlined in Appendices G and H respectively.

A food frequency questionnaire was completed for each of these individuals. Specifically, each three day intake record was reviewed to determine average daily intake of each item on the food frequency questionnaire. Next, the correlations between average daily folate and zinc intakes as determined by the two methods were established using regression analysis (STATS-2 software program by StatSoft). The correlation coefficient for average daily folate intakes was 0.95 while that for average daily zinc intakes was 0.97. Both correlation coefficients were highly significant with $p \leq 0.00005$ (see Appendices I and J for correlation plots).

To assess the food frequency's ability to determine individual's entire folate and zinc intakes, average daily folate and zinc intakes as determined by the food frequency, were expressed as a percentage of average daily intakes as determined by the three day food intake records. For the 12 subjects in the validation study, the food frequency questionnaire underestimated 3 day food record folate and zinc intakes by 9 and 13 percent respectively. It was assumed that this underestimation also occurred in the entire study population. Accordingly, average daily folate and zinc intakes were determined by multiplying subjects' food frequency folate and zinc values by 100/91 and 100/87 respectively.

3.6 Additions to the Food Frequency Questionnaire for East Indian and Chinese Study Subjects

All individuals involved in validation of the food frequency questionnaire were Caucasian. This was unfortunate considering a number of study subjects were East Indian or Chinese who included some traditional foods in their diet. This led to a concern that the food frequency questionnaire may have omitted traditional foods rich in folate and or zinc, and therefore underestimated average daily folate/zinc intake. Accordingly, East Indian and Chinese study subjects were asked about some additional foods.

In the case of the "East Indian diet" it was decided that Daal, a frequently consumed lentil soup, and Chapatis, an unleavened bread made from whole wheat flour, should be included. Since lentils were already included in the food frequency it was decided that subjects would be asked about their intake of Daal and its thickness so that the volume of lentils in a given volume of soup could be estimated. Chapatis were added to the bread section. Finally, all East Indian subjects were asked to identify any traditional vegetables they consumed. Okra and eggplant were identified. Both were found to be significant sources of folate and zinc so they were added to the vegetable section. Folate and zinc content of these traditional East Indian foods are outlined in Appendix M.

With the "Chinese diet," the generous use of Oriental vegetables, many of them leafy greens, had to be considered. A well educated middle-aged Chinese subject was asked to outline all of the traditional vegetables in the "Chinese diet" other than Bok Choy and Chinese Cabbage which were already in the food frequency. His list included: Bitter Melon, Chinese Winter Melon, Lo Bok, Bamboo Shoots, Gai Choy, Gai Lan, Wong Choy, You

Choy, and Shanghai Bok Choy. Neither Bitter Melon or Chinese Winter Melon were found in the Canadian Nutrient File. Since they have been categorized as a summer squash and a winter squash respectively (Beck 1984) a decision was made to count them in the food frequency under zucchini and winter squash respectively. Lo Bok was found to be a significant source of folate while bamboo shoots provided insignificant amounts of folate and zinc (CNF). Lo Bok was added to the vegetable list. None of the remaining vegetables were listed in the CNF or other tables previously used. Coloured photographs were available for all but Wong Choy and You Choy (Beck 1984). In general, all of these leafy green vegetables had light to medium green stalks and medium to dark green leaves as does Bok Choy. On this basis, they were all assumed to be similar in folate content. Chinese subjects were asked about their intake of Bitter Melon, Chinese Winter Melon, Lo Bok, Gai Choy, Gai Lan, Wong Choy, You Choy, and Shanghai Bok Coy. They were also asked about consumption of other Chinese vegetables since Chinese greens tend to be referred to by many different names. Folate/zinc content of the foregoing Chinese vegetables are outlined in Appendix M.

3.7 Administration and Analysis of 3 Day Food Records

Each subject was asked to record his/her food intake for 3 days: a dialysis weekday, a nondialysis weekday, and a nondialysis Saturday or Sunday. Subjects were asked to use the food intake record form (Appendix F). During a dialysis treatment, 10-15 minutes was spent with each subject reviewing written recording instructions included in their food intake record form. Additional instructions were given for foods to be recorded in volume measures. For items such as cereal or beverages which

were consistently taken in the same volume from the same bowl or glass, subjects were told that quantifying this volume once with measuring cups would suffice. They were asked to measure the amount of butter, peanut butter, salad dressing, etc. the first time these were used. With vegetables, pasta, rice and soup they were asked to measure the volume their serving spoon held the first time they served these items. Individuals were asked to specify how vegetables were prepared (e.g., boiled, baked). To ensure that recording of meat, fish, poultry and cheese was done accurately, all diagrams included in the food record form were reviewed. Subjects were asked if there were any meat/alternates they consumed which were not included in the diagrams and if this was the case, quantification of these items was discussed. Individuals were asked to specify how items were prepared (e.g., broiled, fried) and whether they removed the skin from poultry. Next, the instructions on items to be recorded by size were discussed with the included diagrams reviewed. Finally, the need for detail regarding type of food, brand name, and content of mixed dishes was discussed.

Immediately following completion of recording, each subject's intake form was reviewed for ambiguities. The individual was questioned regarding missing details with diagrams and food models used when portions were missing. Details regarding meals served at a hospital during a dialysis recording day were checked through Food Services staff at the hospital.

All three day intake records were analyzed for energy and protein content using the COMPUTRITION nutrient analysis software package in the Nutrition Services Department at Vancouver General Hospital.

3.8 Administration and Analysis of Food Frequency Questionnaires

After procedures for keeping the 3 day food record had been explained, the food frequency questionnaire was administered. Individuals were advised that they would be asked about their intake of a large number of different foods in a typical year. Specifically, they were to indicate how often they ate each food and how much they ate at a time. They were told there may be items they ate daily or weekly, items they ate only a few times a year as well as items they never consumed.

Food items were read off to subjects to elicit an intake frequency response. Subjects were given one or two breaks during questioning if they desired since questioning took 1.25-1.5 hours. For foods whose serving size was not standard, food models or actual food samples were displayed. For example, with the breads and savoury grain products groups a 50 g croissant, whole wheat crackers and 1/2 cup models of rice and macaroni were employed. Measuring cups and a bowl were available to help individuals quantitate cereal consumption. Food models, food samples and measuring cups were used to help quantitate vegetables, fruit, juice, milk and desserts. Meat, fish, poultry, cheese, legumes and nuts were quantitated with the aid of diagrams in the food intake record form as well as measuring cups.

Subjects were allowed to quote frequency of intake in any manner they desired (e.g., every second day, once every 3 months, twice a week for 4 months of the year). In recording, these frequencies were converted to average daily unit consumption. For example, if a person ate 3 slices of whole wheat bread per week with one slice considered a unit, their average daily unit consumption was 3/7.

The food frequency questionnaire was entered into a personal computer as a spread sheet using Lotus 1,2,3 release 2.01. A copy was made for each subject and their average daily unit intakes for each food were entered. Commands were used to multiply average daily unit intakes by ug folate/unit and mg zinc/unit and then to total each of these columns to determine average daily folate and zinc intake, respectively.

3.9 Blood Collection

Subjects on a folate and or zinc supplement stopped the supplement(s) for a minimum of 24 hours before having blood collected. They also fasted a minimum of 5 hours before blood collection. No subject had received a blood transfusion for a minimum of 4 weeks prior to hematocrit, serum folate or RBC folate determinations. Compliance to all of these procedures was verified before blood was drawn. All blood was collected predialysis by nurses in the hemodialysis units. For most patients all blood samples were collected on one occasion. In some cases, incorrect blood storage or errors made by the laboratory necessitated collecting blood on more than one occasion.

After cleansing the skin with alcohol, a nurse inserted a needle into an artery and one into a vein with plastic tubing attached that would eventually be hooked up to the dialyzer. The plastic tubing was connected to a plastic syringe and approximately 30 ml blood was drawn to be added to unstoppered vacutainers as outlined below.

Hematocrit

Approximately 5 ml of blood was syringed into a vacutainer containing EDTA (1.5 mg/ml). The vacutainer was promptly stoppered and agitated.

RBC Folate

Eight to 10 ml of blood was syringed into a vacutainer containing EDTA (1.5 mg/ml). The vacutainer was promptly stoppered and agitated.

Serum Folate

Five to 6 ml of blood was syringed into a heparin-free vacutainer. The vacutainer was promptly stoppered.

Serum Zinc

Eight to 9 ml of blood was syringed into a heparin-free, zinc-free vacutainer with care to avoid getting blood on the rim of the vacutainer. The vacutainer was promptly stoppered with care to avoid blood contact with the stopper which could cause zinc contamination of the specimen.

3.10 Blood and Serum Transport and Storage

Immediately after blood was drawn it was stored in a fridge or portable cooler at 4°C.

RBC Folate

Within 2-3 hours of collection, samples were transferred to 7 ml plastic vials and stored in a freezer at -17°C where they were kept until analysis. All samples were analyzed within one month.

Serum Folate

Within 2 hours of collection, samples were centrifuged at 2000 RPM for 10 minutes and serum transferred to 7 ml plastic vials. Within 3 hours of collection, serum was stored in a freezer at -17°C where it was kept until analysis.

Serum Zinc

Within 2 hours of collection, samples were centrifuged at 2000 RPM for 10 minutes and serum transferred to 25 ml glass scintillation vials with

plastic screw caps lined with linear-polyethylene. Serum samples were stored in a freezer at -17°C until analysis. The scintillation vials had been soaked in 2N reagent grade HNO_3 for 3 days then washed 3 times with quartz distilled and milli-Q demineralized water. They were air dried in a filtered air, positive pressure laminar flow hood. Serum transfer was carried out in a fumehood in a clean laboratory with a positive pressure filtered air system. Glass pasteur pipettes were used for the transfer. They had been soaked overnight in 3% (v/v) HCl, thoroughly rinsed with distilled, deionized water and oven dried in a similarly washed plastic container. Disposable surgical gloves previously rinsed with distilled, deionized water and dried with white paper towel were worn during the transfer.

3.11 Blood and Serum Analysis

Hematocrit

Except in the case of Willow Dialysis Unit whose patients had their hematocrits done at Vancouver General Hospital, patients had their hematocrits done at the hospital where they dialyzed. Hematocrits were determined on a Coulter Counter^R or Toa Size Max^R and were computed as Hb/mean corpuscular Hb rather than being determined directly. The reason this method was used was because directly determined hematocrit results were not available from St. Paul's Hospital Laboratory due to limited compter memory for laboratory values. For uniformity this method was used for all subjects.

Serum Folate

Frozen serum samples packed in ice were delivered to the St. Paul's Laboratory to have folate determined by RIA (radioimmunoassay) using the BIO RAD Quantaphase kit. Frozen samples were thawed just prior to analysis. The laboratory was advised when the folic acid level was expected to be outside of the normal range. In these cases serum was diluted with the zero standard (blank). Each sample was analyzed in duplicate. A maximum variability of 5% was considered acceptable, and this was achieved with all samples. The mean value for the two determinations was reported.

Whole Blood Folate

Frozen blood samples packed in ice, were delivered to the laboratory at St. Paul's Hospital to have folate determined by RIA (BIO RAD Quantaphase Folate kit). There they were thawed at room temperature, then mixed thoroughly. Next 100 ul volumes were withdrawn and added to 1.0 ml volumes of 0.4% ascorbic acid solution (preservative). These samples were refrigerated for 20 hours. Each sample was gently vortexed with the resulting hemolysate left at 20-25°C for 90 minutes. Samples of hemolysate were diluted as appropriate with whole blood diluent from the BIO RAD kit (the laboratory had been informed in advance when samples were to have folate levels outside of the normal range). Folate levels were determined in duplicate using RIA. A maximum variability of 5% was considered acceptable, and this was achieved with all samples. The mean value for the two determinations was reported.

RBC Folate

Whole blood folate levels were adjusted to yield RBC folate levels using the following formula:

$$\text{RBC folate} = \frac{\text{whole blood folate} - \text{serum folate}\ (1 - \text{hematocrit}/100)}{\text{hematocrit}/100}$$

Serum Zinc

Serum zinc was determined by atomic absorption spectrophotometry. Each sample was run in triplicate and all determined values were within ±5% of each other with a mean of the 3 values taken.

Prior to zinc determination, serum was handled in a filtered air, positive pressure laminar flow hood. Disposable surgical gloves rinsed with quartz distilled and milli-Q demineralized water and dried with white paper towel were worn. Measured portions of serum were transferred to new 25 ml scintillation vials which had been washed as had the original scintillation vials. Serum was diluted in a 1:4 ratio with quartz distilled and milli-Q demineralized water. Pipetting of serum and water was carried out using an SMI micropipettor with glass tips prewashed with the procedure used for the scintillation vials. The teflon tip of the pipettor plunger had been soaked in warm aqua regia for 1 week prior to its use.

Determinations were conducted with a Perkin-Elmer Model 603 Atomic Absorption Spectrophotometer with an HGA-2200 Graphite Furnace and a silicon photodiode temperature sensor. A Perkin-Elmer zinc lamp with pyrolitically coated graphite tubes and argon purge gas was used. It was set at 20 M'A, 213.8 nm and slit 4. Twenty ul portions of the dilute samples were repeatedly introduced into the tube by an autosampler.

Fisher Scientific certified atomic absorption standard so-2-13, 1,000 ppm sequentially diluted to 30, 25, 20, 15, 12.5, 10, 6.25, 5 and 2.5 ppb as well as 3 blanks carried through all steps of sample treatment were also run. Extremely low zinc readings for these blanks indicated that sample contamination was successfully avoided.

3.12 Collection, Washing and Drying of Hair Samples

Hair samples were collected from subjects who did not dye, bleach or perm their hair since these treatments have been shown to decrease hair zinc levels (McKenzie 1978). For each of these subjects, hair was collected from a healthy control matched for sex, hair color and age (within 5 years of study subject) who was not treating their hair or taking any medications.

Hair samples (approximately 60-120 mg) were cut from the occipital region of the scalp as recommended by Gibson (1980). Hair was cut right to the scalp initially. The 2-3 cm taken closest to the scalp was trimmed and transferred to a plastic bag (16 cm x 10.8 cm) which was folded and stapled shut.

Hair washing and drying procedures were based on those of Harrison et al. (1969), Buckley et al. (1984) and Assarian and Oberleas (1977). Preparation of hair samples was carried out in a clean laboratory with a positive pressure, filtered air system. Disposable surgical gloves previously rinsed with distilled deionized water and dried with white paper towel were worn. The work area was covered with white paper towel. A "destatifying gun" was used to destatify the hair sample before it was emptied from its plastic bag into a 120 ml plastic cup with a plastic screw top. Plastic cups had been soaked overnight in 3% HCl, rinsed thoroughly in distilled, deionized water and oven dried covered with white paper towel in a similarly washed plastic container. Hair that clung to the plastic bag and surgical gloves was rinsed into the cup using 10 ml distilled, deionized water while hair that fell onto the paper towel was

emptied into the cup. Seventy-five ml of 1% (v/v) non-ionic detergent was added to the cup which was capped and agitated for 30 minutes on an automatic shaker.

After agitation, hair samples plus detergent were transferred to a 150 ml glass Buchner funnel with a mesh glass filter. The funnels had been soaked overnight in 3% HCl and rinsed thoroughly with distilled deionized water. Prior to soaking, ethanol was run through the funnels under vacuum to dissolve grease which had accumulated on the filter during rinsing of the previous hair sample.

Hair samples were rinsed by running 750 ml distilled, deionized water through the funnel under vacuum. After this rinsing, hair samples were transferred to 25 ml preweighed scintillation vials. These had been washed as those used for the serum zinc samples, dried for 23 hours in an oven set at 68°C and cooled in a desiccator before being weighed on an analytical balance. Hair transfer was carried out using a disposable plastic spoon and knife and the blunt ends of 2 pasteur pipettes "chop stick style." All utensils had been acid washed using procedures used for the plastic cups.

Scintillation vials were placed in an oven set at 68°C and covered with white paper towel. Hair samples were dried for 72 hours before vials were capped, cooled in a desiccator and reweighed to determine hair sample weights.

3.13 Hair Analysis for Zinc

Hair analysis procedures were based on those of Harrison et al. (1969) and Buckley et al. (1984). Hair samples were handled in a filtered air,

positive pressure laminar flow hood. Disposable surgical gloves rinsed with quartz distilled and milli-Q demineralized water and dried with white paper towel were worn throughout.

Each hair sample was treated in its scintillation vial (loosely closed) with 3 ml reagent grade concentrated HNO_3 for 24 hours. At this point 0.5 ml reagent grade concentrated $HClO_4$ was added to complete the digestion. The clear hair solution produced, was slowly evaporated to a 1 ml volume then diluted to 5 ml with quartz distilled and milli-Q demineralized water. Pipetting procedures were as outlined for serum zinc samples.

Zinc analysis was by atomic absorption spectrophotometry and followed the procedures used for serum zinc determinations. Five blanks were carried through all steps of sample treatment (i.e., mock washing, rinsing, drying and digestion) as well as dilution procedures. Extremely low zinc readings for these blanks indicated that sample contamination was avoided.

3.14 Statistical Analysis of Data

Nonparametric tests (Kruskal Wallis k-sample tests) were done manually. All other statistical analysis of data was carried out using CSS Stats software. Statistical significance was assumed at $p \leq 0.05$.

3.14.1 Statistical Analysis of Folate and Zinc Status Data

Before comparing the RBC folate levels of folate-supplemented and unsupplemented subjects, a variance stabilization transformation was performed since the criterion of variance homogeneity was not satisfied.

Next, regressions between length of time on folate supplement and regressions between body weight and RBC folate concentration were done to see if Analysis of Covariance (ANCOVA) was indicated. A correlation ($r=0.70$) was found between RBC folate concentration and length of time on folate supplement so ANCOVA was used with length of time on folate as a covariate to compare RBC folate levels. A nonparametric test (Kruskal-Wallis k-sample test) was also used to compare RBC folate levels.

Before comparing serum zinc levels of zinc-supplemented and unsupplemented subjects, regressions between length of time on zinc supplement and serum zinc and between body weight and serum zinc were done to see if ANCOVA was indicated. No correlations were found so ANOVA was used to compare serum zinc levels and to check for a folate/zinc interaction. The foregoing procedure was repeated after excluding two subjects who had been on the zinc supplement less than four months.

Hair zinc levels of non zinc-supplemented subjects and non zinc-supplemented healthy controls (matched for age, sex and hair color), were compared using a t-test.

Analysis of RBC folates, serum zincs and hair zincs was also done using correlation coefficients. Dietary folate intake (ug/kg body weight [b.w.]) in unsupplemented subjects was examined for correlation with RBC folate level. Dietary zinc intake (mg/kg b.w.) in unsupplemented subjects was examined for correlation with serum zinc concentration. Zinc intake from diet plus supplements (mg/kg b.w.) in all subjects was examined for correlation with serum zinc concentration. Supplemental $1,25(OH)_2$Vitamin D_3 intake (IU/week) in all subjects, as well as in zinc-supplemented and unsupplemented subjects was examined for correlation with serum zinc level. Finally, hair zinc concentration was examined for

correlation with zinc intake (mg/kg b.w. from diet plus supplements) and with serum zinc.

3.14.2 Statistical Analysis of Body Weight and Protein and Energy Intake Data

ANOVA was used to compare percent ideal body weights and energy intakes (expressed as a percent of estimated energy requirement) of the four treatment groups. A nonparametric test (Kruskal Wallis k-sample test) was used to compare protein intakes of the four treatment groups because the criterion of variance homogeneity was not satisfied.

CHAPTER FOUR

RESULTS

4.1 RBC Folate Levels and Dietary Folate Intakes of Treatment Groups

RBC folate levels for the four treatment groups are shown in Table 6. (Individual values are shown in Appendices W and X.) The RBC folate concentrations for each group receiving no folate supplement were within the normal range of 169-707 ng/mL. In contrast, the RBC folate concentrations for both folate supplemented groups were approximately 6.5 to 7 times the upper limit of the normal range.

No significant correlation was found between body weight and RBC folate concentration but a significant correlation ($r=0.70$ $p \leq 0.05$) was found between length of time on folate supplement and RBC folate concentration. The difference between RBC folate concentrations for folate-supplemented and unsupplemented groups was highly significant ($p<0.00001$). This level of significance remained when analysis of covariance was done with number of months of folate supplementation as the covariate. Mean RBC folate levels did not differ significantly between zinc-supplemented and unsupplemented groups ($p \leq 0.05$).

Mean average daily folate intake from diet only (ug/kg b.w.) for all four treatment groups, for all folate-supplemented subjects and all non folate-supplemented subjects and for all study subjects, are shown in Table 6. (Individual values are shown in Appendices V and W.) Differences in dietary folate intakes among the four treatment groups as well as between folate-supplemented and non folate-supplemented subjects were not significant ($p \leq 0.05$). Mean dietary folate intake for all subjects combined was 136 percent of the RNI for folate.

Table 6 Comparison of Treatment Groups' RBC Folate Levels and Average Daily Dietary Folate Intakes Based on Food Frequency Questionnaires (Mean + SD)

Treatment	n	RBC Folate (ng/ml)	Daily Dietary Folate Intake [6] ug/day	ug/kg b.w.*
No Folate, No Zn	5	411 + 116 [a]	269 + 131	4.3 + 2.2
No Folate 22.5 mg Zn/day	4	479 + 197 [a]	317 + 126	5.0 + 2.2
Combined No Folate	9	441 + 150 [a]	287 + 123	4.6 + 2.1
5 mg Folate/ day, No Zn	6	4583 + 813 [b]	219 + 90	3.9 + 2.1
5 mg Folate/ day, 22.5 mg Zn/day	6	5080 + 1624 [b]	236 + 71	3.6 + 1.6
Combined 5 mg folate	12	4832 + 1251 [b]	227 + 78	3.7 + 1.8
ALL SUBJECTS	21		260 + 43	4.2 + 0.6

a,b Figures in the same column not sharing the same superscript are significantly different at $p < 0.00001$

Differences among the four treatment groups in average daily folate intake (ug/kg b.w.) were not significant ($p < 0.05$).

Average daily folate intakes expressed as total ug/day were not compared.

RNI for folate is 3.1 ug/kg b.w.

* mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

Normal RBC folate levels: 169-707 ng/ml (Bio Rad Folate and Vitamin B_{12} Radioimmunoassay Kit)

[6] The food frequency questionnaire used to determine average daily folate intake, was found during its validation, to detect 91 percent of dietary folate consumed. Folate intakes presented, have been adjusted to indicate 100 percent of dietary folate consumed.

4.2 Serum Zinc Levels and Dietary Zinc Intakes of Treatment Groups

No correlations were found between length of time on zinc supplement and serum zinc concentration or between body weight and serum zinc concentration. Mean serum zinc levels for the four treatment groups, for all zinc-supplemented and all non zinc-supplemented and for all study subjects, are shown in Table 7. Serum zinc levels were slightly below the normal range for both groups receiving no zinc supplement and thus for all non zinc-supplemented subjects combined. The 22.5 mg zinc, no folate group had a normal serum zinc concentration while that of the 22.5 mg zinc, 5 mg folate group was slightly below normal. When all zinc-supplemented subjects were combined, serum zinc concentration was at the lower end of the normal range. It is apparent from groups' standard deviations that there was one or more subject in each treatment group with an abnormally low serum zinc concentration. Values for individual subjects are presented in Appendices U and V. The difference between serum zinc concentrations for zinc-supplemented and unsupplemented groups was not significant, nor was the difference between serum zinc concentrations for folate-supplemented and unsupplemented subjects.

No correlations were found between serum zinc concentration and dietary zinc intake (mg/kg b.w.) of non zinc-supplemented subjects nor between serum zinc concentration and zinc intake from diet plus supplements (mg/kg b.w.) of zinc-supplemented subjects. Serum zinc concentration, in all subjects, in zinc-supplemented subjects and in non zinc-supplemented subjects was not correlated with intake of 1,25 $(OH)_2$ vitamin D_3 (IU/week).

Mean average daily zinc intakes from diet only (mg/kg b.w.) for all four treatment groups and for all zinc-supplemented subjects and all nonsupplemented subjects are shown in Table 7. (Individual values are shown in Appendices T and U.) Differences in dietary zinc intakes among the four groups as well as between zinc-supplemented and non zinc-supplemented subjects were not significant ($p \leq 0.05$). Mean dietary zinc intake for all subjects combined was 104 percent of the RNI for zinc for adult males and 78 percent of the RNI for zinc for adult females.

Table 7 Comparison of Treatment Groups' Serum Zinc Levels and Average Daily Dietary Zinc Intakes Based on Food Frequency Questionnaires (Mean + SD)

Treatment	n	5 Hr. Fasting Serum Zn (ug/dl)	Daily Dietary Zinc Intake[§] mg/day	mg/kg b.w.*
No Zn, No Folate	5	62.8 + 14.3	7.39 + 4.45	0.12 + 0.07
No Zn, 5 mg Folate/day	6	62.6 + 18.2	9.12 + 3.09	0.16 + 0.08
Combined No Zinc	11	62.7 + 15.7	8.33 + 3.67	0.14 + 0.08
22.5 mg Zn/day, No Folate	4	73.1 + 17.9	9.40 + 0.17	0.15 + 0.03
22.5 mg Zn/day, 5 mg Folate/day	6	64.1 + 13.8	11.66 + 3.49	0.18 + 0.08
Combined 22.5 mg Zn/day	10	67.7 + 15.3	10.90 + 2.99	0.17 + 0.06
ALL SUBJECTS	21	65.1 + 15.3	9.39 + 1.75	0.15 + 0.03

Differences in serum zinc concentration among the four treatment groups and between all zinc supplemented and all non zinc supplemented subjects were not significant ($p < 0.05$).

Differences among the four treatment groups in average daily zinc intake (mg/kg b.w.) were not significant ($p < 0.05$).

Average daily zinc intakes expressed as mg/day were not compared.

RNI's for zinc are 12 mg (adult males) and 9 mg (adult females).

* mean of post dialysis weights at the 4 dialyses prior to RBC folate determination.

Normal serum zinc level: 65-140 ug/dl (Solomons, 1986).

[§] The food frequency questionnaire used to determine average daily zinc intake, was found during its validation, to detect 87 percent of dietary zinc consumed. Zinc intakes presented, have been adjusted to indicate 100 percent of dietary zinc consumed.

4.3 Hair Zinc Levels of Some Zinc-Supplemented Subjects and Non Zinc-Supplemented Healthy Controls Matched for Age, Sex and Hair Colour

Hair zinc levels of a subgroup of 6 zinc-supplemented subjects and a group of non zinc-supplemented healthy controls are shown in Table 8. Hair zinc level was significantly higher in zinc-supplemented subjects than in healthy controls ($p \leq 0.01$). There was no correlation between hair zinc level of these zinc-supplemented subjects and their serum zinc concentrations or their zinc intakes (mg/kg b.w. from diet plus supplements).

Other study subjects did not undergo hair zinc analysis either because they permed and/or dyed their hair or because problems with hair sample preparation invalidated hair analysis results.

4.4 Body Weights of Subjects in Comparison to Ideal Body Weights (1983 Metropolitan Height and Weight Tables)

Percent ideal body weight for the four treatment groups is presented in Table 9. Differences in percent ideal body weight among the four treatment groups were not statistically significant at $p \leq 0.05$. Percent ideal body weight for each subject taking into account sex, height, frame size and weight are shown in Appendices N and O.

4.5 Mean Daily Energy Intakes of Treatment Groups

Mean daily energy intakes (expressed as total calories and as percent of estimated energy requirement) for each of the four treatment groups are presented in Table 9. Differences in energy intakes (expressed as a percentage of estimated energy requirement) among the four treatment

groups were not statistically significant at $p \leq 0.05$. Mean total daily energy intakes were not compared since groups differed greatly in sex and age distribution. Energy intakes of individual subjects are shown in Appendices R and S.

4.6 Mean Daily Protein Intakes of Treatment Groups

Mean daily protein intake (g/kg b.w.) for each of the four treatment groups is presented in Table 9. Differences in these intakes among the four treatment groups were not statistically significant at $p \leq 0.05$. The daily protein intakes for all four treatment groups exceeded the RNI for protein for females (0.74 g/kg b.w.) and for males (0.82 g/kg b.w.). Protein intakes of individual subjects are presented in Appendices P and Q.

Table 8 Hair Zinc Levels of Some Zinc - Supplemented Subjects and Non Zinc - Supplemented Healthy Controls Matched for Age, Sex and Hair Colour (Mean + SD)

Treatment Group	n	Hair Zinc Level (ug/g)
22.5 mg Zn/day	6	237.99 + 55.10
No Zn	6	127.32 + 24.30

The difference in hair zinc level between zinc - supplemented subjects and healthy controls was significant ($p < 0.01$)

Table 9 Group Mean for Percent Ideal Body Weight (IBW) (1983 Metropolitan Height and Weight Tables) and Average Daily Energy and Protein Intakes Based on 3 Day Food Records (Mean + SD)

Treatment	n	% IBW (avg. for range)	Energy(Kcal) [% req't.]‡	Protein (g/kg b.w.*)
No Folate, No Zn	5	111.0 + 12.7	1360 + 439 [87 + 22]	0.98 + 0.48
No Folate, 22.5 mg Zn/day	4	96.4 + 17.5	1441 + 269 [81 + 20]	0.96 + 0.50
5 mg Folate/ day, No Zn	6	100.7 + 23.2	2034 + 319 [122 + 28]	1.19 + 0.23
5 mg Folate/day, 22.5 mg Zn/day	6	103.8 + 24.4	2080 + 553 [107 + 26]	1.13 + 0.17
All Subjects	**21**	**103.2 + 19.7**	**1729 + 381 [99 + 19]**	**1.07 + 0.11**

Differences among the four treatment groups in percent IBW, percent of energy requirement and protein intake, were not significant ($p < 0.05$).

* if a subject's weight was more than 20% above the upper limit of their IBW range, they were considered obese and mean IBW was used in calculating g protein/kg b.w.

‡ Energy Requirement Determination

- Basal energy expenditures (BEEs) were calculated using the Harris Benedict Equations; males: 66.47 + 13.75 (wt in kg) + 5 (ht in cm) - 6.76 (age); females: 665.1 + 9.56 (wt in kg) + 1.85 (ht in cm) - 4.68 (age). Mean IBW was used for obese subjects.

- BEEs were then multiplied by an activity factor of 1.2 or 1.3 (depending on activity level) to estimate 24 hour energy requirements.

CHAPTER FIVE

DISCUSSION

5.1 Introduction

Dietary energy, protein, folate and zinc intakes are the first results to be discussed in this chapter. This initial section on dietary intakes will give the reader a better perspective for the sections which follow on folate and zinc status of chronic hemodialysis patients. The discussion section of this thesis, is followed by conclusions and related recommendations pertaining to nutritional care of chronic hemodialysis patients.

5.2 Dietary Energy, Protein, Zinc and Folate Intakes

Several recent studies (DeBari et al. 1984, Ramirez et al. 1986a, Ramirez et al 1986b, Sharman et al. 1982, Swainson and Winney 1983) have investigated whether chronic hemodialysis patients require folate supplements. In these studies, subjects were usually described as being clinically stable without specification of what this meant in terms of eating habits. Although prescribed diets were sometimes outlined, actual dietary intakes of energy, protein and folate were never determined. Without knowing what the dietary folate intake of these subjects was, one cannot conclude that the majority of chronic hemodialysis patients do not require supplemental folate. The present study improved upon the foregoing studies, by actually assessing energy, protein and folate intakes of subjects. Since recent studies have suggested that high dose folate supplementation may impair zinc absorption, the present study was also concerned with the effect of such folate supplementation on zinc

status. Accordingly, dietary zinc intakes were determined as a part of zinc status assessment in the present study.

Literature on energy, protein, folate and zinc intakes of chronic hemodialysis patients from the 1960s through to the 1980s, is summarized in Table 10.

TABLE 10. Energy, Protein, Folate and Zinc Intakes of Chronic Hemodialysis Patients with Comparisons to Current Energy and Protein Levels Recommended for Hemodialysis Patients and RNIs for Folate and Zinc

	Energy	Protein	Folate	Zinc
Predialysis Diet in 1960s	High calorie diet prescribed (a,b)	18-22 g/day prescribed (a,b)	Prescribed diet (assayed) -94 ug/day (a)	
Hemodialysis Diet in 1960s through Early 1970s	High calorie diet prescribed (g,h)	50-70 g/day prescribed (a,i,j)	Prescribed diet (assayed) -72 ug/day (a) Prescribed diet (food tables) -50 ug/day (k)	Prescribed diet (food tables) -4.8-10.5 mg/day[1] (c,d,e,f)
Hemodialysis Diet in Late 1970s through 1980s	Actual intakes determined at 26-36 kcal/kg b.w. (l,m)	50-80 g/day (n,o) or 1 g/kg b.w. prescribed (p)	Prescribed diet (food tables) -273 ug/day[2](n) -187-323 ug/day (c,q,r)	Prescribed diet (food tables)[3] (c,d,e,f) -6.9-14.3 mg/day
Mean Dietary Intakes for Subjects in Present Study	1729±381 kcal 28±9 kcal/kg b.w.	1.07±0.11 g/kg b.w.	-260±43 ug/day -4.2±0.6 ug/kg b.w.	9.39±1.75 mg/day
Current Energy and Protein Levels Recommended for Hemodialysis Patients and RNIs for Folate and Zinc	30-35 kcal/kg b.w. (s,t)	1-1.2 g/kg b.w. (s,t)	3ug/kg b.w. (u)	9 mg/day (u) (female adults) 12 mg/day (u) (male adults) *The mixed Canadian diet has been shown to provide 5 mg zinc/1000 kcal (u)

(continued)

Legend for Table 10

1. The investigator in the present study used food tables to determine the zinc content of a 1960s hemodialysis diet (h) providing 60 g of protein and 1750 mg of potassium.

2,3. The investigator in the present study used food tables to determine the folate and zinc content of a 1980s hemodialysis diet providing 72 g of protein and 2700 mg of potassium.

References for Table 10

a) MacKenzie et al. 1968
b) Thiele 1976
c) Nutricom Nutrient Analysis Software
d) Pennington and Church 1980
e) Freeland et al. 1980
f) Murphy et al. 1975
g) Rodwell-Williams 1973
h) Royal Victoria Hospital, Dept. of Dietetics 1970
i) Hampers et al. 1967
j) Siddiqui et al. 1970
k) Whitehead et al. 1968
l) Wolfson et al. 1984
m) Young et al. 1982
n) DeBari et al. 1984
o) Mahajan et al. 1979a
p) Ramirez et al. 1986b
q) Hoppner et al. 1972
r) Perloff and Butrum 1977
s) Gillet et al. 1987
t) B.C. Dietitians' and Nutritionists' Assoc. 1984
u) Bureau of Nutritional Sciences, Department of National Health and Welfare, 1983

Mean energy intake of subjects in the present study (28±9 kcal/kg b.w.) was comparable to the recommended intake of 30-35 kcal/kg b.w. (B.C. Dietitians' and Nutritionists' Assoc. 1984, Gillet et al. 1987) and to previously determined intakes of 26-36 kcal/kg b.w. (Wolfson et al. 1984, Young et al. 1982).

Mean protein intake in the present study (1.07 ± 0.11g/kg b.w.) compared favorably with the recommended intake of 1-1.2 g/kg b.w. (B.C. Dietitians' and Nutritionists' Assoc. 1984, Gillet et al. 1987). This protein intake coincided with the 1g/kg b.w. level recommended during the late 1970s and the 1980s (DeBari et al. 1984, Mahajan et al. 1979a, Ramirez et al 1986b). High protein foods are a major source of dietary zinc. Accordingly, considering similar levels of protein in the 1970s/80s hemodialysis diet and the diet in the present study, similar levels of zinc were expected in these two diets. This was indeed the case with the 1970s/80s hemodialysis diet providing 6.9-14.3 mg zinc/day and the diet in the present study providing a mean of 9.39±1.75 mg/day. Female subjects in the present study had a zinc intake of 7.20±3.30 mg/day representing 80 percent of the RNI of 9 mg/day. The male subjects' zinc intake was 11.02±2.90 mg/day representing 92 percent of the RNI of 12 mg/day.

The group's mean zinc intake of 9.39 mg was provided through an average daily energy intake of 1729 kcalories (i.e., 5.43 mg/1000 kcal). This finding suggests that the diet patients consumed was similar to the typical mixed Canadian diet, which has been shown to provide 5 mg of zinc per 1000 kcalories (Bureau of Nutritional Sciences, Dept. of National Health and Welfare 1983). In conclusion, the diet consumed by subjects provided 80-92 percent of the RNI for zinc which would probably be

adequate for most healthy adults. Whether chronic renal failure and associated hemodialysis treatments or high dose folate supplementation appeared to increase zinc requirements of subjects in the present study, will be discussed later.

Mean folate intake in the present study (260±43 ug/day) was:

1. comparable to the levels of 273 and 187-323 ug/day found when the 1970s/80s hemodialysis diet was analyzed (DeBari et al. 1984, Nutricom Nutrient Analysis Software, Hoppner et al. 1972, Perloff and Butrum 1977).
2. much higher than the 94 ug/day found in the 1960s predialysis diet and the 50-72 ug/day found in the 1960s/70s hemodialysis diet (MacKenzie et al. 1968, Whitehead et al. 1968).

Folate levels in the diets consumed by predialysis and chronic hemodialysis patients in the 1960s and early 1970s were well below the current RNI of 3.1 ug folate/kg b.w. This may account for the frequent macrocytic anemia which led to routine folate supplementation in chronic renal failure patients during this period. When mean folate intake in the present study was expressed in relation to body weight (4.2±0.6 ug/kg b.w.) it was found to exceed the RNI of 3.1 ug/kg b.w. In summary, the diet consumed by subjects in the present study provided adequate folate to meet the needs of healthy adults. However, there is the possibility that chronic renal failure and/or the hemodialysis process increased folate requirements. Whether this appeared to be the case with subjects in the present study will be discussed later.

5.3 RBC Folate Concentration

In the present study, red blood cell folate concentration in chronic hemodialysis patients receiving no folate supplement was in the normal range. In contrast, in chronic hemodialysis patients taking a 5 mg per day folate supplement, RBC folate concentration was about 6.5 times the upper limit of the normal range. No interaction was found between zinc and folate. Specifically, zinc-supplemented subjects did not have significantly lower RBC folate concentrations.

Studies in the late 1960s on chronic hemodialysis patients (Whitehead et al. 1968, Siddiqui et al. 1970, MacKenzie et al. 1968, Hampers et al. 1967) found folate deficiencies which resolved with folate supplementation. However, recent studies on chronic hemodialysis patients (DeBari et al. 1984, Ramirez et al. 1986a, 1986b, Sharman et al. 1982, Swainson and Winney 1983) have shown that a daily supplement of 1 to 5 mg of folate leads to abnormally high RBC folate levels. Ramirez et al. (1986b) found that some but not all patients studied, maintained normal RBC folate levels twelve months after their 1 mg per day supplement was withdrawn.

In the present study, as in previous 1980s studies on chronic hemodialysis patients (Sharman et al. 1982, Swainson and Winney 1983), a 5 mg per day folate supplement resulted in abnormally high RBC folate levels. However, all subjects in the present study who received no supplemental folate had normal RBC folate levels. This result differed from those of 1960s studies (Whitehead et al. 1968, Siddiqui et al. 1970, MacKenzie et al. 1968, Hampers et al. 1967), and from that of Ramirez et al. (1986b). Specifically, the 1960s studies often found signs of folate deficiency in unsupplemented patients while Ramirez et al. (1986b) found

abnormally low RBC folate concentrations in a few of their patients twelve months after withdrawal of a 1 mg per day folate supplement. Differences in dietary folate intakes probably explain the differences in folate status between patients in the present study and in these previous studies. Dietary folate intake in the present study was determined at 260±43 ug per day. The 1960s hemodialysis diet was found to provide only 50-72 ug of folate per day while the diet patients followed prior to starting dialysis provided only 94 ug of folate per day (MacKenzie et al. 1968, Whitehead et al. 1968). Such folate poor diets were likely the cause of patients' folate deficiencies. Ramirez et al. (1986) claimed their patients were prescribed a diet which provided approximately 273 ug of folate per day. However, they never determined patients' actual intakes. Their patients who failed to maintain normal RBC folate levels may well have had poor dietary folate intakes.

Findings of the present study challenge the contention that a low folate diet and folate losses through hemodialysis, make supplemental folate a necessity on a nutritional basis in all chronic hemodialysis patients. This study indicated that clinically stable chronic hemodialysis patients not receiving medications known to increase folate requirements, who consumed approximately 4 ug folate/kg b.w., did not require supplemental folate to maintain normal RBC folate levels. The foregoing results as well as research by Cunningham et al. (1981), suggests that folate losses with hemodialysis are minimal in relation to folate requirements. Folate dialysance was not measured in the present study but Cunningham et al. (1981) found a median loss of 37 ug of folate in 7 hours of dialysis. This would represent about 64 ug of folate in the 12 hours of dialysis per week which was typical for most patients in the

present study. Folate losses through dialysis were probably compensated for by no or minimal urinary folate losses due to anuria or severe oliguria in patients.

Some studies (Simmer et al. 1987, Milne et al. 1984, Gishan et al. 1986) have suggested that folate supplements decrease zinc absorption. However, more recent studies do not support this contention. Keating et al. (1987) showed that administering a 10 mg folate supplement with a 25 mg dose of zinc had no effect on the serum zinc response curve. Butterworth Jr. et al. (1988) found no significant change in plasma or RBC zinc concentrations after four months of supplementing women with 10 mg of folate per day.

Most of the foregoing studies (Simmer et al. 1987, Keating et al. 1987, Milne et al. 1984) assessed the effect of folate supplementation on zinc absorption by monitoring serum zinc concentration for several hours following a 25-50 mg dose of zinc, or by measuring fecal and urinary zinc levels. Butterworth Jr. et al. (1988) assessed folate's effect on zinc status (plasma and RBC zinc concentrations) rather than on zinc absorption. The present study was similar in that serum zinc was measured to try to assess the effect of long term folate supplementation (5 mg per day) on zinc status. In the present study, as in that of Butterworth Jr. et al. (1988), folate supplementation was not associated with lower blood zinc levels.

If serum zinc concentration was considered a reliable indicator of zinc status in chronic hemodialysis patients, it could be concluded that a 5 mg per day folate supplement does not impair zinc status. However, as

will be discussed in the section Serum Zinc Concentration in Zinc-Supplemented versus Non Zinc-Supplemented Subjects:

1. serum zinc concentration may not be reflecting zinc status in individuals with chronic renal failure.
2. alternate methods of assessing zinc status in CRF patients are required.

5.4 Hair Zinc Levels

Initially, subjects from each of the four treatment groups in the present study (no folate, no zinc; 5 mg folate, no zinc; no folate, 22.5 mg zinc; 5 mg folate, 22.5 mg zinc), were to have hair samples analyzed for zinc. Unfortunately, many subjects were excluded from this analysis due to: perming/dying of hair or problems with hair sample preparation which invalidated hair analysis results. Inadequate hair was collected to allow repeat analyses.

As a consequence, the following discussion pertains to mean hair zinc concentrations for six subjects receiving a supplement of 22.5 mg of zinc per day and six healthy controls receiving no zinc supplement. Hair zinc concentration was significantly higher in the hemodialysis subjects than in the healthy controls.

The hair zinc concentration of chronic renal failure patients has been found to be significantly lower than that of healthy controls (Mahajan et al. 1979, Atkin-Thor et al. 1978). Inadequate dietary zinc has been implicated as a cause of zinc deficiency in CRF (Shils and Young 1988), but unfortunately the foregoing studies failed to determine dietary zinc intakes of subjects. Atkin-Thor et al. (1978) induced significant increases in hair zinc concentration when their CRF patients received 178

mg of zinc three times per week. Zinc supplementation has also been found to increase hair zinc levels in healthy adults (Dorea and Paine 1988, Atkin-Thor et al. 1978) and in those with a suspected zinc deficiency.

Unlike the studies of Mahajan et al. (1979) and Atkin-Thor et al. (1978), in the present study, CRF patients who had not received zinc supplements did not undergo hair zinc analysis. Only CRF patients receiving a zinc supplement (22.5 mg/day) underwent hair zinc analysis. Accordingly, it could not be determined from the present study whether a hair zinc concentration higher than that of healthy non zinc-supplemented controls was truly a function of zinc supplementation. However, since zinc supplementation has previously been shown to increase hair zinc levels (Dorea and Paine 1988, Atkin Thor et al. 1978) in adults with and without CRF, elevated hair zinc levels observed in the present study, were likely due to zinc supplementation.

5.5 Serum Zinc Concentration

Serum zinc concentration was below normal in the non zinc-supplemented group and at the low end of the normal range in the zinc-supplemented group. However, the difference in serum zinc concentrations between zinc-supplemented and non zinc-supplemented groups was not statistically significant. No interaction was found between folate and zinc. Specifically, folate-supplemented subjects did not have significantly lower serum zinc concentrations. Several subjects were receiving $1,25(OH)_2$ vitamin D_3 which has been shown to enhance zinc absorption (The Nutrition Foundation 1984) but no correlations were found between serum zinc concentration and supplemental vitamin D_3 dose in the entire group of subjects, zinc-supplemented subjects or non zinc-supplemented subjects.

Abnormally low blood zinc levels have frequently been observed in individuals with chronic renal failure (Condon and Freeman 1970, Halsted and Smith 1970, Burge et al. 1984, Mahajan et al. 1979a, Mahajan et al. 1980). Mahajan et al. (1979b, 1980) found both a 50 mg and a 25 mg zinc supplement significantly increased serum zinc levels in chronic hemodialysis patients.

In these studies of Mahajan et al., subjects served as their own controls going through periods without and with zinc supplementation. The increase observed in serum zinc concentration was highly significant. The present study differed from that of Mahajan et al. in that a zinc-supplemented and a non zinc-supplemented group were compared. The difference in serum zinc concentration between groups was small and not statistically significant. Differences in experimental design between the present study and the previous studies might explain why zinc supplements were less effective at increasing serum zinc in the present study.

First, zinc supplements were given for a period of weeks (6 weeks or more) in the foregoing studies while it was given for 3-132 months in the present study. It is possible that a short term effect was being observed in the previous studies (Mahajan et al. 1979b, Mahajan et al. 1980) with a longer period of adaptation occurring in the present study. Specifically, it is felt that the high blood zinc level resulting when excess zinc is consumed, causes zinc deposition in bone and liver (Mahajan et al. 1980, Prasad, A.S. 1979). Metallothionine production is induced in the liver to bind zinc for storage in the "inert zinc pool" in the body (Mahajan et al. 1980, Prasad, A.S. 1979). This may explain why the serum zinc of zinc-supplemented subjects was not higher than that of unsupplemented subjects in the present study.

A key question arises from this study and others which have found low or low normal serum zinc levels in uremic patients. Specifically, do these serum zinc levels indicate a zinc deficiency or are they a manifestation of chronic renal failure? Patients in the present study did not appear to be overtly zinc deficient in that they did not have any of the obvious deficiency signs such as skin lesions or night blindness. However, whether their serum zinc concentrations indicated a subclinical zinc deficiency needs to be examined. Decreased zinc intake has been cited as a cause of zinc deficiency in CRF (Shils and Young 1988). Although female and male subjects in the present study did not attain 100 percent of the RNIs for zinc, they did reasonably well at 80 and 92 percent respectively. Accordingly, unless their needs were greatly increased above those of healthy individuals, subclinical zinc deficiency would not be expected.

Factors with the potential to increase zinc requirements include: decreased absorption (Shils and Young 1988) and increased losses with hemodialysis treatments. The influence of these factors was not examined in the present study. However, negligible if any zinc would be expected to be lost with dialysis since 40% is bound to a-2 macroglobulin and 55% is bound to albumin (Shils and Young 1988). Another point to consider is the fact that the typical 400-600 ug per day of urinary zinc (Shils and Young 1988) would be conserved since most patients were anuric. Furthermore, there is evidence in the literature to suggest that decreased serum zinc levels in the present study were not an indicator of poor zinc status due to an increased zinc requirement but instead were due to a shift of zinc from serum to the liver. The uremic state and or the secondary hyperparathyroidism often associated with it (David 1977) may be

responsible for this enhanced uptake of zinc by the liver. Specifically, parathyroid hormone has been found to stimulate zinc uptake by the liver (Hirschberg et al. 1985) causing serum zinc concentration to decline. Furthermore, autopsies on uremic patients revealed liver zinc concentrations significantly higher than in healthy controls (Smythe et al. 1982). In conclusion, there is considerable evidence to support the hypothesis that the low serum zinc levels in CRF patients are due to a shift of zinc from serum to other "zinc pools" in the body.

Assessing the zinc status of CRF patients is not straightforward; measurement of several parameters including serum, hair and neutrophil zinc levels has been recommended (Gibson 1988). In the present study, the intention was to measure both serum and hair zinc levels. Unfortunately, hair zinc levels could be measured in only some subjects. Inclusion of neutrophil zinc levels would have improved the present study but unfortunately, the technology for this determination was not available.

Interest in zinc status of CRF patients stems mainly from their frequent complaint of hypogeusia; taste acuity has also been found to improve in such patients after a period of zinc supplementation (Mahajan et al. 1979b, Mahajan et al. 1980). Accordingly, it may have been worthwhile including taste acuity testing in the present study. However, the value of such testing was questioned since many of the subjects were on medications thought to affect taste perception. Since preliminary research (Solomons 1979) suggests that salivary zinc concentration may be low in zinc depleted subjects, it would be interesting to include this measurement along with taste acuity testing in future studies assessing zinc status of CRF patients.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

A quasiexperimental design involving clinically stable chronic hemodialysis patients (henceforth referred to as patients) was used in the present study. Consequently, its findings should not be generalized to the entire population of chronic hemodialysis patients. However, practitioners working with chronic hemodialysis patients should consider the following findings in decisions pertaining to folate and zinc status of their patients.

1. Average daily dietary intakes of patients were:

   energy - 1729±381 kcalories

   protein - 1.07±0.11 g/kg b.w.

   folate - 4.2±0.6 ug/kg b.w. (260±43 ug/day)

   zinc - 0.15±0.03 mg/kg b.w. (9.39±1.75 mg/day)

2. Patients not receiving medications known to increase folate requirements, who consumed approximately 4 ug folate/kg b.w., maintained normal folate stores without folate supplementation (reflected by normal RBC folate levels). Since subjects' folate intakes were higher than the RNI of 3.1 ug/kg, the present study did not establish whether the RNI would be adequate to maintain normal folate stores.

3. Patients who received a supplement of 5 mg of folate per day had RBC folate levels 6.5 to 7 times the upper limit of the normal range of 169-707 ng/mL.

4. Serum zinc concentrations of patients who received a supplement of 22.5 mg of zinc per day were low normal at 67.7±15.3 ug/dL (normal: 65-140 ug/dL) while those of non zinc-supplemented patients were slightly below normal at 62.7±15.7 ug/dL. As discussed previously, there is research suggesting that low serum zinc levels in chronic renal failure patients reflect a shift of zinc from blood to other "zinc pools" in the body rather than poor zinc status.
5. Hair zinc concentration in a subgroup of six patients who received 22.5 mg of zinc per day was significantly higher than in healthy controls (matched for age, sex and hair colour) who received no supplemental zinc. No conclusions regarding zinc status could be drawn from this finding.
6. Serum zinc levels in patients who received a 5 mg per day folate supplement were no lower than serum zinc levels in patients who received no supplemental folate. Since serum zinc concentration does not appear to be a reliable indicator of zinc status in chronic hemodialysis patients, it could not be concluded that a 5 mg per day folate supplement does not impair zinc status.

6.2 Recommendations Regarding Folate Supplementation in Chronic Hemodialysis Patients

Folate supplementation is not indicated on a nutritional basis in chronic hemodialysis patients who are consuming an adequate diet, i.e.: a varied diet providing 1 to 1.2 g of protein per kg b.w. and including 2-3 servings (approximately 1/2 cup portions) of both vegetables and fruits.

However, for patients unable to achieve such an intake either of the following approaches is recommended:

1. A vitamin supplement which provides approximately 4 ug of folate/kg b.w./day should be prescribed.

or

2. Patients' RBC folate levels should be checked every three to four months and measururements should only be taken at least one month after a blood transfusion. Dietary counseling and/or folate supplements should be recommended as indicated.

6.3 Future Research Related to Folate Supplementation in Chronic Hemodialysis Patients

1. The majority of patients in the present study were dialyzed with cellulose membranes (membranes made of natural cellulose which has been dissolved and reformed) or derivatized cellulose membranes (cellulose membranes that have had other groups substituted for hydroxyl groups, e.g.: cellulose acetate). However, some were dialyzed with hydrophobic synthetic membrances (membranes made of a hydrophobic synthetic material such as polyacrylonitrile). Clearance of middle molecules (molecules weighing 350 to 2000 daltons) is higher with these membranes than with cellulose or derivatized cellulose membranes. Patients in this study who were dialyzed with hydrophobic synthetic membranes, had used them for only a short period of their total years on hemodialysis, previously using a regenerated or derivatized cellulose membrane. Accordingly, the present study essentially examined the impact of hemodialysis with cellulose and

derivatized cellulose membranes, on folate status. Hemodialysis using membranes made of hydrophobic synthetics or a combination of hydrophobic and hydrophilic synthetics is the way of the future. These new membranes offer two important advantages: greater biocompatibility and the transport properties required for high flux dialysis. Accordingly, the effect of hemodialysis with these membranes on folate status should be studied.

2. Recombinant erythropoietin is a drug which is now available to some chronic hemodialysis patients with severe anemia. Because it will increase production of red blood cells and reduce transfusions, its effect on folate status should be evaluated.
3. Drugs such as dilantin and acetasalicylic acid have been found to decrease serum folate levels. Their effect on RBC folate levels in chronic hemodialysis patients should be evaluated.
4. Homocysteinemia is thought to be damaging to vascular endothelium and may be a factor in the premature vascular disease which commonly occurs in chronic renal failure patients. High dose folate supplementation has been found to lower plasma homocysteine levels. Long term studies assessing the effects of high dose folate supplementation on plasma homocysteine levels and the incidence of vascular disease should be conducted in CRF patients.

6.4 Recommendations Regarding Zinc Supplementation in Chronic Hemodialysis Patients

Routine supplementation with zinc is not indicated in chronic hemodialysis patients. However, in patients with a poor dietary intake, a trial of zinc supplementation is indicated to try to improve taste acuity

and thus improve food intake. A dose of 25 mg per day is recommended since:

1. this level has been shown to improve taste acuity in chronic hemodialysis patients (Mahajan et al. 1980).
2. levels above 25 mg per day can produce metallic taste, nausea and epigastric distress; levels as high as 150-160 mg per day can result in copper deficiency, anemia due to intestinal interaction of zinc and copper, and gastric erosion (Shils and Young 1988).

6.5 Future Research Related to Zinc Supplementation in Chronic Hemodialysis Patients

1. The mechanism whereby zinc therapy improves taste acuity in some chronic hemodialysis patients has not been elucidated. To gain some understanding of this mechanism, salivary zinc concentration and gustin activity should be determined in conjunction with taste acuity, both before and after a zinc supplementation in chronic hemodialysis patients.
2. At present there is no reliable means of assessing zinc status in chronic hemodialysis patients. Clearly, this area warrants further investigation.

REFERENCES

Anonymous. Report: Excessive folic acid. Am. Family Physician 32: 290-291, 1985.

Assarian, G.S., Oberleas, D. Effect of washing procedures on trace-element content of hair. Clin. Chem. 23: 1771-1772, 1977.

Atkin-Thor, E., Goddard, B.W., O'Nion, J., Stephen, R.L., Kloff, W.J. Hypogeusia and zinc depletion in chronic dialysis patients. Am. J. Clin. Nutr. 31: 1948-1951, 1978.

Bachevalier, J., Botez, M.I. Learning in rats after large doses of folic acid. Nutr. Rep. Internat. 17: 457-462, 1978.

Bastios, P.P., Welsh, S.O., Cronin, F.J., Kelsay, J.L., Mertz, W. Number of days of food intake records required to estimate individual and group nutrient intakes with defined confidence. J. Nutr. 117: 1638-1641, 1987.

B.C. Dietitians' and Nutritionists' Association. The British Columbia Diet Manual, 3rd ed., British Columbia: Hospital Programs, B.C. Ministry of Health, 1984, pp. 308-313.

Bio Rad Folate and Vitamin B_{12} Radioimmunoassay Kit, Bio Rad Laboratories. Mississauga, Ontario.

Block, G. A review of validations of dietary assessment methods. Am. J. Epidemiol. 115: 491-505, 1982.

Boers, G.H.J., Smals, A.G.H., Trijbels, R.J.M. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N. Engl. J. Med. 33: 709-715, 1985.

Brattstrom, L.E., Hardebo, J.E., Hultberg, B.L. Moderate homocysteinemia: A possible risk factor for arteriosclerotic cerebrovascular disease. Stroke 15: 1012-1016, 1984.

Brattstrom, L.E., Israelsson, B., Jeppsson, J.O., Hultberg, B.L. Folic acid: An innocuous means to reduce plasma homocysteine. Scand. J. Clin. Invest. 48: 215-221, 1988.

Briggs, G.M., Calloway, D.H. Bogert's Nutrition and Physical Fitness, 10th ed., New York: Saunders College Publishing/Holt, Reinhart, and Winston, 1979, pp. A7-A17.

Bruce Beck, Produce: A Fruit and Vegetable Lovers Guide. New York City: Friendly Press Inc., 1984, pp. 30, 36,84, 168, 182.

Buckley, R.A., Dreosti, I.E. Radioisotopic studies concerning the efficacy of standard washing procedures for the cleansing of hair before zinc analysis. Am. J. Clin. Nutr. 40: 840-846, 1984.

Buerk, C.A.G., Chandy, E., Pearson, M.S., McCauly, Soroff, H.S. Zinc deficiency: Effect on healing and metabolism in man. Surg. Forum 24: 101-106, 1973.

Bureau of Nutritional Sciences, Food Directorate, Health Protection Branch, Department of National Health and Welfare. Recommended Nutrient Intakes for Canadians, Ottawa: Canadian Government Publishing Centre, 1983, pp. 8, 9, 141.

Bureau of Nutritional Sciences, Food Directorate, Health Protection Branch, Department of National Health and Welfare. Recommended Nutrient Intakes for Canadians, Ottawa: Canadian Government Publishing Centre, 1989, pp. 117, 118, 153, 154.

Burge, J.C., Schemmel, R.A., Park, H.S., Greene, J.A. Taste acuity and zinc status in chronic renal disease. J. Am. Diet. Assoc.: 1203-1209, 1984.

Butterworth Jr., C.E., Hatch, K., Cole, P., Sauberlich, H.E., Tamura, T., Cornwell, P., Soong, S. Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation. Am. J. Clin. Nutr. 47: 484-486, 1988.

Canadian Nutrient File, Health and Welfare Canada, 1979.

Chalmers, F.W., Clayton, M.M., Gates, L.O., Tucker, R.E., Wertz, A.W., Young, C.M., Foster, W.D. The dietary record: How many and which days? J. Am. Diet. Assoc. 28: 711-717, 1952.

Colonotero, G., Wheby, M.S. Disorders of Cobalamin and Folate Metabolism, in: Leavell and Thorup's Fundamentals of Clinical Hematology, 5th ed., ed. O.A. Thorup, Jr. Toronto: W.B. Saunders Company, 1987, pp. 185-204.

Compher, C.W. Nutrition assessment in chronic renal failure. Nutritional Support Services 5: 18-21, 1985.

Compendium of Pharmaceuticals and Specialities, 22nd ed., Ottawa: Canadian Pharmaceutical Association, 1987.

COMPUTRITION Nutrient Analysis Software, Nutricom Inc. 9121 Oakdale Avenue - Suite 201, Chatsworth, California 91311.

Cunningham, J., Sharman, V.L., Goodwin, F.J., Marsh, F.P. Do patients receiving haemodialysis need folic acid supplements? Brit. Med. J. 282: 1582, 1981.

David, D.S. Calcium Metabolism in Renal Failure and Nephrolithiasis, New York: John Wiley and Sons Inc., 1977, p. 33.

DeBari, V.A., Frank, O., Baker, H., Needle, M.A. Water soluble vitamins in granulocytes, erythrocytes, and plasma obtained from chronic hemodialysis patients. Am. J. Clin. Nutr. 39: 410-415, 1984.

Dorea, J.G., Paine, P.A. Making sense of the hair zinc literature: Where do we go from here? Archivos Latino-americanos de Nutricion 38: 93-112, 1988.

Feldman, H.A., Singer, I. Endocrinology and metabolism in uraemia and dialysis: A clinical review. Medicine 54: 345-376, 1974.

Freedman, S., Popio, K., Kredish, N., Heiss, G. Plasma homocysteine and coronary artery disease (abstr.). Am. J. Epidem. 116: 566, 1982.

Freeland, J.H., Lavone-Ebangit, M., Bodzy, P.W. Zinc and copper content of foods used in vegetarian diets. J. Am. Diet. Assoc. 77: 648-653, 1980.

Ghishan, F.K., Said, H.M., Wilson, P.C., Murrell, J.E., Greene, H.L. Intestinal transport of zinc and folic acid: A mutual inhibitory effect. Am. J. Clin. Nutr. 43: 258-262, 1986.

Gibson, R.S. Hair as a biopsy material for the assessment of trace element status in infancy: A review. J. Hum. Nutr. 34: 405-416, 1980.

Gibson, R.S. Personal communication, 1988.

Gibson, R.S. Sources of error and variability in dietary assessment methods: A review. J. Can. Diet. Assoc. 48: 151-155, 1987.

Gillet, D., Stover, J., Espinozzi, N. (eds.) A Clinical Guide to Nutrition Care in Endstage Renal Disease, Washington: Renal Dietitians' Practice Group - American Dietetic Association, 1987, p. 35.

Golden, M.H.N. The Diagnosis of Zinc Deficiency, in: Zinc in Human Biology, ed. C.F. Mills. Great Britain: Springer-Verlag, 1989, pp. 323-332.

Grant, A., DeHoog, S. Nutritional Assessment and Support, 3rd ed., Seattle: A. Grant and S. DeHoog, 1985, pp. 84-115.

Green, D., Stone, N.J., Krumlovsky, F.W. Putative atherogenic factors in patients with CRF. Prog. Cardiovasc. Dis. 26: 133-144, 1983.

Hambidge, K.M. Hair analyses. Ped. Clin. North Amer. 27: 855-860, 1980.

Hampers, C.L., Streiff, R., Nathan, D.G., Snyder, D., Merrill, J.P. Megaloblastic hematopoiesis in uremia and in patients on long-term hemodialysis. N. Engl. J. Med. 276: 551-554, 1967.

Harker, L.A., Slichter, S.J., Scott, R., Ross, R. Homocystinemia: Vascular injury and arterial thrombosis. N. Engl. J. Med. 291: 537-543, 1974.

Harrison, W.W., Yurachek, J.P., Benson, C.A. The determination of trace elements in human hair by atomic absorption spectroscopy. Clin. Chim. Acta 23: 83-91, 1969.

Henderson, T.S., Leung, A.C.T., Shenkin, A. Vitamin status in continuous ambulatory peritoneal dialysis. Peritoneal Dialysis Bulletin, July-September: 143-145, 1984.

Herbert, V. Hematology and the Anemias, in: Nutritional Support of Medical Practice, ed. Howard A. Schneider, Carl E. Anderson, and David B. Coursin. Philadelphia: Harper and Row, 1983, pp. 393-408.

Hildebrand, D.C., White, D.H. Trace-element analysis in hair: An evaluation. Clin. Chem. 20: 148-151, 1974.

Hirschberg, R., vonHerrath, D., Vo, B.K., Bosaller, W., Mauelshagen, U., Pauls, A., Schaefer, K. Parathyroid hormone and 1,25 - Dihydroxyvitamin D_3 affect the tissue concentration of zinc in uremic rats. Nephron 39: 277-279, 1985.

Hoppner, K., Lampi, B., Perrin, D.E. The free and total folate activity in foods available on the Canadian market. Can. Inst. Food Sci. Technol. J. 5: 60-66, 1972.

Hunter, R., Barnes, J. Toxicity of folic acid given in pharmacological doses to healthy volunteers. Lancet 1: 61-63, 1970.

Ibels, L.S., Stewart, J.H., Mahoney, J.F., Neale, F.C., Shell, A.G.R. Occlusive arterial disease in uremic and hemodialysis and renal transplant recipients. A study of the incidence of arterial disease and of the prevalence of risk factors implicated in the pathogensis of arteriosclerosis. Q.J. Med. 46: 197-214, 1977.

Jackson, B., Dujovne, C.A., DeCoursey, S., Beyer, P., Brown, E.F., Hassanein, K. Methods to assess relative reliability of diet records: Minimum records for monitoring lipid and caloric intake. J. Am. Diet. Assoc. 86: 1531-1535, 1986.

Jandl, J.H. Textbook of Hematology, Toronto: Little, Brown and Company, 1987, p. 154.

Kang, S., Wong, P.W.K., Cook, H.Y., Norusis, M., Messer, J.V. Protein-bound homocysteine: A possible risk factor for coronary artery disease. J. Clin. Invest. 77: 1482-86, 1986.

Karkeck, J.M. Improving the use of dietary survey methodology. J. Am. Diet. Assoc. 87: 869-871, 1987.

Keating, J.N., Wada, L., Stokstad, E.L.R., King, J.C. Folic acid: Effect on zinc absorption in humans and in the rat. Am. J. Clin. Nutr. 46: 835-839, 1987.

Kirkpatrick, D.C., Coffin, D.E. The trace metal content of representative Canadian diets in 1970 and 1971. Can. Inst. Food Sci. Tech. J. 7: 56-58, 1974.

Kopple, J.D., Mercurio, K., Blumenkrantz, M.J. et al. Daily requirement for pyridoxine supplementation in chronic renal failure. Kidney Int. 19: 694-704, 1981.

Kopple, J.D., Swendseid, M.E. Vitamin nutrition in patients undergoing maintenance hemodialysis. Kidney Int. 7: S79-S84, 1975.

Lasker, N., Harvey, A., Baker, H. Vitamin levels in haemodialysis and intermittent peritoneal dialysis. Trans. Am. Soc. Artif. Intern. Organs 9: 51-56, 1963.

Leung, A.C.T., Henderson, I.S., Maharaj, D., Thomson, G. Excessive accumulation of folic acid in uremic patients on dialysis. Dialysis and Transplantation 14: 575-577, 1985.

Liener, I.E. Nutritional Value of Food Protein Products in Soybeans: Chemistry and Technology Volume 1, revised ed., ed. A.K. Smith and S.J. Circle. Westport, Connecticut: AVI Publishing Co., 1978, p. 203.

Lowrie, E.G., Lazarus, J.M., Hampers, C.L., Merrill, J.P. Cardiovascular disease in hemodialysis patients. N. Engl. J. Med. 290: 737-738, 1974.

McKenzie, J.M. Alteration of the zinc and copper concentration of hair. Am. J. Clin. Nutr. 31: 470-476, 1978.

MacKenzie, J.C., Ford, J.E., Waters, A.H., Harding, N., Cattell, W.R., Anderson, B.C. Erythropoiesis in patients undergoing regular dialysis treatment (R.D.T.) without transfusion. Proc. Eur. Dialysis Transplant Assoc. 5: 172-178, 1968.

Mahajan, S.K., Gardiner, W.H., Abbasi, A.A., Briggs, W.A., Prasad, A.S., McDonald, F.D. Abnormal plasma and erythrocyte zinc distribution in uremia. Trans. Am. Soc. Artif. Intern. Organs 24: 50-54, 1978.

Mahajan, S.K., Prasad, A.S., Lambujon, J., Abbasi, A.A., Briggs, W.A., McDonald, F.D. Improvement of uremic hypogeusia by zinc. Trans. Am. Soc. Artif. Intern. Organs 25: 443-448, 1979.

Mahajan, S.K., Prasad, A.S., Rabbani, P., Briggs, W.A., McDonald, F.D. Zinc metabolism in uremia. J. Lab. Clin. Med. 94: 693-699, 1979.

Mahajan, S.K., Prasad, A.S., Lambujon, J., Abbasi, A.A., Briggs, W.A., McDonald, F.D. Improvement of uremic hypogeusia by zinc: A double-blind study. Am. J. Clin. Nutr. 33: 1517-1521, 1980.

Mansouri, K., Halsted, J.A., Gambos, E.A. Zinc, copper, magnesium and calcium in dialyzed and non-dialyzed uremic patients. Arch. Intern. Med. 125: 88-93, 1970.

Marginal Comments: Zinc deficiency in man. Am. J. Dis. Child. 130: 359-361, 1976.

Marr, J.W., Heady, J.A. Within-and between-person variation in dietary surveys: Number of days needed to classify individuals. Hum. Nutr.: Appl. Nutr. 40A: 347-364, 1986.

Marumo, F., Tsukamoto, Y., Iwanami, S., Kishimoto, T., Yamagami, S. Trace element concentration in hair, fingernails and plasma of patients with chronic renal failure on hemodialysis and hemefiltration. Nephron 38: 267-272, 1984.

Meadows, N.J., Grainger, S.L., Ruse, W., Keeling, P.W.N., Thompson, R.P.H. Oral iron and the bioavailability of zinc. Brit. Med. J. 287: 1013-1014, 1983.

Milne, D.B., Canfield, W.K., Mahalko, J.R., Sandstead, H.H. Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. Am. J. Clin. Nutr. 39: 535-539, 1984.

Mudd, S.H., Levy, H.L. Disorders of Transulfuration in: The Metabolic Basis of Inherited Disease, ed. J.B. Stanbury, J.B. Wyngaarden, and D.S. Fredrickson. New York: McGraw-Hill, 1983, pp. 522-559.

Mudd, S.H., Skovby, F., Levy, H.L., et al. The natural history of homocystinuria due to cystathionine B-synthase deficiency. Am. J. Hum. Genet. 37: 1-31, 1985.

Murphy, E.W., Wells-Willis, B., Watt, B.K. Provisional tables on the zinc content of foods. J. Am. Diet. Assoc. 66: 345-355, 1975.

Murphy-Chutorian, D.R., Wexman, M.P., Grieco, A.J. Methionine intolerance: A possible risk factor for coronary artery disease. J. Am. Coll. Cardiol. 6: 725-730, 1985.

Myers, R.J., Hamilton, J.B. Regeneration and rate of growth of hairs in man. Ann. N.Y. Acad. Sci. 53: 562-567, 1951.

Neldner, K.H., Hambidge, K.M. Zinc therapy of acrodermatitis enteropathica. N. Engl. J. Med. 292: 879-882, 1975.

NutriCom Nutrient Analysis Software, Smart Engineering Ltd. 1381 Hope Road, North Vancouver, B.C. V7P 1W6.

The Nutrition Foundation. Present Knowledge in Nutrition, 5th ed. Philadelphia: J.B. Lippincott Company, 1980, pp. 169-171, 339, 422-493.

Patrick, J., Dervish, C. Leukocyte zinc in the assessment of zinc status. CRC Critical Reviews in Clinical Laboratory Sciences 20: 95-114, 1984.

Pennington, J.A.T., Church, H. Bowes and Church's Food Values of Portions Commonly Used, 13th ed., Philadelphia: J.B. Lippincott Company, 1980, pp. 169-171.

Perloff, B.P., Butrum, R.R. Folacin in selected foods. J. Am. Diet Assoc. 70: 161-171, 1977.

Polit, D.F., Hungler, B.P. Essentials of Nursing Research: Methods and Applications, Philadelphia: J.B. Lippincott Co., 1985, pp. 382-392.

Powers, D.E., Moore, A.O. Food Medication Interactions, 4th ed., Tempe, Arizona: F-M-I Publishing, 1983, pp. 21, 35, 77, 111.

Prakash, R., Petrie, W.M. Psychiatric changes associated with an excess of folic acid. Am. J. Psych. 139: 1192-1193, 1982.

Prasad, A.S. Zinc in Human Nutrition, Boca Raton, Florida: CRC Press Inc., 1979, pp. 30-40.

Prasad, A.S., Ortega, J., Brewer, G.J., Oberleas, D., Shoomaker, E.B. Trace elements in sickle cell disease. J. Am. Med. Assoc. 235: 2896-2898, 1976.

Prasad, A.S., Rabbani, P., Affasii, A., Bowersox, E., Spivey Fox, M.R. Experimental zinc deficiency in humans. Ann. Internal Med. 89: 483-490, 1978.

Prasad, A.S. Zinc deficiency in man. Am. J. Diseases Children 130: 359-361, 1976.

Prasad, A.S., Cossack, Z.T. Neutrophil zinc: An indicator of zinc status in man. Trans. Assoc. Am. Physicians 95: 165-176, 1982.

Quesenberry, P.J. Origin of the Blood Cells and Architecture of the Bone Marrow, in: Leavell and Thorup's Fundamentals of Clinical Hematology, 5th ed., ed. O.A. Thorup, Jr. Toronto: W.B. Saunders Company, 1987, pp. 10-11.

Ramirez, G., Chen, M., Worth Boyce, H., Fuller, S.M., Butcher, D.E., Brueggemeyer, C.D., Newton, J.L. The plasma and red cell vitamin B levels of chronic hemodialysis patients: A longitudinal study. Nephron 42: 41-46, 1986a.

Ramirez, G., Chen, M., Worth Boyce, H., Fuller, S.M., Ganguly, R., Brueeggemeyer, C.D., Butcher, D.E. Longitudinal follow-up of chronic hemodialysis patients without vitamin supplementation. Kidney Int. 30: 99-106, 1986b.

Reddi, K.K. Serum Ribonuclease of normal persons and patients with renal impairment. Clin. Biochem. 11: 133-134, 1978.

Robinson, C.H. Normal and Therapeutic Nutrition, 14th ed., New York: The MacMillan Company, 1972, p. 582.

Rodwell-Williams, S. Nutrition and Diet Therapy, 2nd ed., Saint Louis: The C.V. Mosby Company, 1973, pp. 558, 559.

Roe, D.A. Drug-Induced Nutritional Deficiencies, 2nd ed., Westport, Connecticut: The Avi Publishing Co. Inc., 1985, pp. 18, 176, 177, 179, 249-254.

Rose, G.A., Wilden, E.G. Whole blood, red cell, and plasma total and ultrafilterable zinc levels in normal subjects and in patients with chronic renal failure with and without hemodialysis. Br. J. Urol. 44: 281-286, 1972.

Roth, H.P., Kirchgessner, M. Zn Metalloenzyme activities: Changes and biochemcial aspects in Zn deficiency. Wld. Rev. Nutr. Diet 34: 144-160, 1980.

Royal Victoria Hospital Dept. of Dietetics. Royal Victoria Hospital Manual of Therapeutic Diets, Montreal 112, P.Q.: 1970, pp. 89-100.

Schneider, Z., Stroinski, A. Comprehensive B_{12}, New York: Walter deGruyter and Company, 1987, p. 343.

Segloni, G.P., Canavese, C., D'Amicone, M., Lamon, S., Talarico, S., Vercellone, A. Zinc copper and magnesium kinetics during desferrioxamine treatment in uremic patients. Nephron 36: 278-279, 1984.

Sharman, V.L., Cunningham, J., Goodwin, F.J., Marsh, F.P. Report: Do patients receiving regular haemodialysis need folic acid supplements? Br. Med. J. 285: 96-97, 1982.

Shils, M.E., Young, V.R. Modern Nutrition in Health and Disease, 7th ed., Philadelphia: Lea and Febiger, 1988, p. 238.

Shrier, S. Hematology, in: Scientific American Medicine, Volume 1, ed. E. Rubentstein, and D.D. Federman. New York: Scientific American Inc., 1983, pp. III-7-III-14.

Siddiqui, J., Freeburger, R., Freeman, R.M. Folic acid, hypersegmented polymorphonuclear leukocytes and the uremic syndrome. Am. J. Clin. Nutr. 23: 11-16, 1970.

Simmer, K., James, C., Thompson, R.P.H. Are iron-folate supplements harmful? Am. J. Clin. Nutr. 45: 122-125, 1987.

Simon, P., Ang, K.S., Meyrier, A., Allain, P., Mauras, Y. Desferrioxamine, ocular toxicity, and trace metals. Lancet 2: 512-513, 1983.

Smythe, W.R., Alfrey, A.C., Craswell, P.W., Crouch, C.A., Ibels, L.S., Kubo, H., Nunnelley, L.L., Rudolph, H. Trace element abnormalities in chronic uremia. Ann. Intern. Med. 96: 302-310, 1982.

Solomons, N.W. On the assessment of zinc and copper nutriture in man. Am. J. Clin. Nutr. 32: 856-871, 1979.

Solomons, N.W. Nutrient Chart Number 1. Dialysis and Transplantation 15: 518, 1986.

Somer, J.B., Aitken, J.M., Abbott, L.K., et al. The influence of pre-transplant etiology of renal disease on lipoprotein lipids in female allograft recipients. Atherosclerosis 37: 1-9, 1980.

STATS-2 Software program by Statsoft, Tulsa, Oklahoma, 1988.

Stuff, J.E., Garza, C., O'Brian-Smith, E., Nichols, B.L., Montandon, M. A comparison of dietary methods in nutritional studies. Am. J. Clin. Nutr. 37: 300-306, 1983.

Sullivan, J.F., Eisenstein, A.B. Ascorbic acid depletion during haemodialysis. J. Am. Med. Assoc. 220: 1697-1699, 1972.

Srivastava, U.S., Nadeau, M.H., Carbanneau, N. Mineral intakes of university students: Zinc content. J. Can. Diet. Assoc. 38: 302-308, 1977.

Swainson, C.P., Winney, R.J. Do dialysis patients need extra folate? Lancet 1: 239, 1983.

Taylor, A. Usefulness of measurements of trace elements in hair. Am. Clin. Biochem. 23: 364-378, 1986.

Thiele, V.F. Clinical Nutrition, Saint Louis: The C.V. Mosby Company, 1976, p. 87.

Tremblay, A., Sevigny, J., Leblanc, C., Bouchard, C. The reproducibility of a three-day dietary record. Nutr. Res. 3: 819-830, 1983.

Valberg, L.S., Flanagan, P.R., Brennan, J., Chamberlain, M.J. Does the oral zinc tolerance test measure zinc absorption? Am. J. Clin. Nutr. 41: 37-42, 1985.

Whitehead, V.M., Comty, C.H., Posen, G.A., Kaye, M. Homeostasis of folic acid in patients undergoing maintenance hemodialysis. N. Engl. J. Med. 279: 970-974, 1968.

Wilcken, D.E.L., Dudman, N.P.B., Tyrrel, P.A., Robertson, M.R. Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: Possible implications for prevention of vascular disease. Metabolism: 37: 697-701, 1988.

Wilcken, D.E.L., Gupta, V.J. Sulphur containing amino acids in chronic renal failure with particular reference to homocysteine and cysteine-homocysteine mixed disulphide. Eur. J. Clin. Invest. 9: 301-307, 1979.

Wilcken, D.E.L., Gupta, V.J. Accumulation of sulphur-containing amino acids including cysteine-homocysteine in patients on maintenance hemodialysis. Clin. Sci. 58: 427-430, 1980.

Wilcken, D.E.L., Gupta, V.J., Betts, A.K. Homocysteine in the plasma of renal transplant recipients. Effects of cofactors for methionine metabolism. Clin. Sci. 61: 743-749, 1981.

Wilcken, D.E.L., Reddy, G.S., Gupta, V.J. Homocysteinemia, ischemic heart disease and the carrier state for homocystinuria. Metabolism 32: 363-370, 1983.

Wilcken, D.E.L., Wilcken, B. The pathogenesis of coronary artery disease: A possible role for methionine metabolism. J. Clin. Invest. 57: 1079-1082, 1976.

Wolfson, M., Strong, C.J., Minturn, D., Gray, D.K., Kopple, J.D. Nutritional status and lymphocyte function in maintenance hemodialysis patients. Am. J. Clin. Nutr. 39: 547-555, 1984.

Young, G.A., Swanepoel, C.R., Croft, M.R., Hobson, S.M., Parsons, F.M. Anthropometry and plasma valine, amino acids, and proteins in the nutritional assessment of hemodialysis patients. Kidney Int. 21: 492-499, 1982.

APPENDIX A

Dear

As you are aware, nutrition plays an important role in health maintenance in those receiving hemodialysis. Exact requirements for some nutrients in adults on regular hemodialysis, are not well established. I am doing a study to help establish requirements for certain vitamins and minerals for individuals like yourself. I need volunteers for this study. The nutritional status of individuals on hemodialysis may be improved through your participation in this study.

Your participation would involve:

1) recording all food and beverages consumed over a 3 day period. You would receive instructions before you start recording.

2) a 30 - 45 minute session with a nutritionist answering questions on how often you eat certain foods. This will be done during a hemodialysis session or after if you prefer.

3) having approximately 30 ml blood taken. You will not be allowed to eat for 5 hours prior to blood collection.If this is a particular problem, for example if you are Diabetic, other arrangements can be made.

4) having a small sample of hair cut near the nape of your neck.

I would appreciate it if you could respond to the statements below by your next dialysis session. Check off statements you agree with and return the form to the Unit Clerk.

Sincerely,

Debbie Reid,R.D.N.

I think I would like to participate in the study. ____
I would like more information before making a decision.____
I am not interested in participating in the study. ____

Name ---------- ---------- (please print)
(first) (surname)

PLEASE RETURN THIS FORM TO THE UNIT CLERK IN THE HEMODIALYSIS UNIT.

Appendix B

Date _____

Verification of Eligibility for the Study HD Unit _____

Subject Name _____ Trt Group_____

Do you dye, bleach or perm your hair?_____

Have you lost any tissue weight in past 6 mths?_____ If yes, how much?_____

Do you use oral contraceptives?_____

Do you receive blood transfusions?_____ If so, when was last one?_____ When do you expect next one?_____

Medications (+ vitamins/minerals)	Start Date	Initial Dose	Present Dose

Additional Comments:

Phone Number:

APPENDIX C

CONSENT FORM

Project Title: Nutritional Status of Adults on Hemodialysis
Investigators: Debbie Reid,R.D.N., Dr.J.Leichter, Dr.J.Price

Information on the Project

The purpose of this study is to learn about specific vitamin and mineral requirements of adults on hemodialysis. The study will be conducted over a three month period. Participants will be asked to:

1) record all food and beverages consumed over a specific 3 day period. Instructions will be given before recording starts.

2) spend 30 - 45 minutes with a nutritionist answering questions regarding how often certain foods are eaten.

3) have approximately 30 ml blood taken just prior to one dialysis session. In the five hours prior to blood collection, food and all fluids other than water must be avoided. If this is a particular problem, for example if you are Diabetic, other arrangements will be made.

4) have a very small sample of hair cut near the nape of their neck.

Debbie Reid, Registered Nutritionist, will answer any questions you have about the study. Confidentiality of records concerning your involvement in this study will be maintained. Only the investigators and your kidney specialist will have access to the results of the study. Dietary results will be shared with your dietitian only with your approval.

Your participation in this study would be greatly appreciated. However, you should not feel obligated to participate. You may withdraw your consent at anytime during the study. Failure to participate in the study or withdrawal from the study will in no way affect your continuing medical care.

CONSENT BY SUBJECT OF RESEARCH STUDY

I have read the foregoing statement concerning the study on nutritional status of adults on hemodialysis and agree to participate in the described study. I acknowledge that I have received a copy of this consent form.

Name of Subject (please print) Signature of Witness

_____ _____

Signature of Subject Date Time AM/PM

_____ _____ _____ _____

APPENDIX D

LIST OF PATIENT MEDICATIONS: LITERATURE (The Nutrition Foundation 1980, Roe 1985, Grant and DeHoog 1985, Powers and Moore 1983, Canadian Pharmaceutical Assoc., 1987, Simon et al. 1983, Segloni et al. 1984) WAS REVIEWED WITH RESPECT TO EFFECTS ON FOLATE AND ZINC METABOLISM

Acetaminophen
Acetaminophen + Codeine
Acetylsalicylic Acid
Adalat
Aldomet
Alka Butazolidine
Allopurinol
Alutabs
Amitryptyline
Amoxil
Amphojel
Atarax
Ativan
Basaljel
Beminal with C Fortis
Benadryl
Benylin
Bethanecol
Bromocriptine
$CaCO_3$
Cafergot
Captopril
Cardizem
Ca Resonium
Catapres
Chlor-tripolon
Cimetidine
Clinoril
Cloxacillin
Colace
Colchicine
Corgard
Darvon
Decadurabolin
Demerol
Desferol Mesylate
Digoxin
Dilantin
Ducolax
1,25(OH)2 Vitamin D_3
Eltroxin
Entrophen
Feldene
Ferrous Sulphate
Gravol
Halcion
Halotestin
Hydralazine
Imodium
Inderal
Indocid
Inferon
Insulin
Intal
Isordil
Kayexalate
Keflex
Lactulose
Lasix
Lomotil
Lopressor
Lorazepam
Maxeran
Meprobamate
Metamucil
Minoxidil
Motilium
Naprosyn
Nitroglycerine
Oral Contraceptives
Panectyl
Perocet
Persantine
Phenobarbital
Pindolol
Prednisone
Premarin
Pyrazinamide
Quinine Sulphate
Ranitidine
Regulex
Rifampin
Riopan
Rivotril
Robalate
Robitussin DM
Seconal
Sennosides A+B
Senokot
Serax
Sinemet
Sinequan
Sulcrate
Talwin
Tapazol
Tegretol
Tenormin
Tolbutamide
Valium
Vancomycin
Voltaren
Warfarin
Yohimbine
Z-Bec

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
BREADS				
white bread	25 g	00-0461	10	0.20
whole wheat bread	25 g	00-0471	14	0.42
rye/pumpernickel	25-32 g	00-0454 averaged values for rye 00-0456 and pumpernickel	7	0.35
whole wheat(ww) roll	35 g	BUNWW	19	0.63
ww hamburger/hotdog bun	40 g	BUNWW	22	0.72
white(wh) roll/croissant	35 g	00-1902 - for roll; assumed folate and zinc in 35 g croissant the same as in 35 g white roll	12	0.28
wh hamburger/hotdog bun	40 g	00-1902	14	0.32
English muffin or crumpet	57 g	00-7458 - for english muffin; assumed folate and zinc in crumpet the same as in english muffin	19	0.44
ww pita pocket	47 g	reference g -folate and zinc values	33	0.85
wh pita pocket	47 g	no values - assumed as with ww pita that folate and zinc values are 1.25 and 1.07 times those in leavened bread made from the same type of flour	24	0.40
whole grain crackers	24 g	WWCRAX - folate value reference d - zinc value	ns	0.73
plain bagel	55 g	no values - assumed folate and zinc in 55 g plain bagel the same as in 55 g white bread	22	0.44
multigrain bagel	55 g	no values - assumed folate and zinc in 55 g multigrain bagel the same as averages of values for 55 g ww, rye and pumpernickel breads	22	0.81

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
SAVOURY GRAIN PRODUCTS				
cooked brown rice	1/2 c	00-1870	8	0.50
cooked white rice	1/2 c	RICE - Folate Value reference e - zinc value	7	0.34
enriched pasta (cooked tender)	1/2 c	00-2159	ns	0.20
whole wheat pasta (cooked tender)	1/2 c	no folate value-assumed insignificant reference d - zinc value	ns	0.45
CAKES, PASTRIES etc				
bran muffin	100 g	CNF values questionable so analysed bran muffin recipe (Joy of Cooking); zinc in flour-reference e, folate in bran-reference b, folate and zinc in other ingredients - CNF	7.3	5.75
ww loaf or muffin	100 g	no folate value-assumed insignificant reference d - zinc value for whole wheat banana bread	ns	1.68
chocolate cake	69 g	00-0559 - folate value reference c - zinc value	4	0.47
Danish pastry	92 g	00-1899 - folate value reference c - zinc value	33	ns
pancake (buttermilk mix-milk + egg added	45 g	00-1457	5	0.27
waffle (mix-milk + egg added)	75 g	00-2417 - folate value no zinc value - assumed the same as for pancake	9	0.45

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
CEREALS				
raw bran	1 Tbsp	reference b - folate value	23	1.3
		BRAN - zinc value		
All Bran types	125 ml	* *zinc values were in general	34	3.5
Bran Flake types	125 ml	* taken from cereal boxes with	11	0.49
Shredded Wheat,	125 ml or	SWHEAT the CNF referred to in a few	10	0.63
Muffets, Weetabix	1 biscuit	cases. A mean value was used		
fortified shredded	175 ml	* for each cereal category.	18	0.75
wheat cereals eg Shreddies		Folate was recorded as 0.06 mg/		
corn cereals eg	125 ml	* 100 g cereal, Canada's approved	9	ns
Frosted Flakes, Corn Bran		level of folate supplementation		
"unpuffed" rice cereals	125 ml	* for cereal.	9	0.24
eg Rice Krispies or Flakes				
"refined cereals" eg	125 ml	*	8	0.24
Alpha Bits, Cheerios,				
Lucky Charms, Fruit Loops				
flaked cereals not in	125 ml	*	8	0.60
previous categories				
eg Special K, Pep, Team,				
Grape Nut Flakes				
Granola	125 ml	HCRUNCH - folate value	14	1.2
		reference e - zinc value		
Puffed Wheat	300 ml	08-0146	ns	0.35
rolled oats (cooked)	125 ml	08-0121	ns	0.38

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
CEREALS Cont'd				
cooked multigrain cereal eg Red River, Sunny Boy	125 ml	08-6045	13	0.28
VEGETABLES and SOUP				
asparagus	125 ml	11-0012	66	ns
avocado	1/2	09-0037	62	ns
beans (green, wax; fresh or canned)	125 ml	11-0053	22	ns
peas (immature green)	125 ml	11-0313	50	ns
beets (diced or sliced; boiled, drained)	125 ml	11-0081	48	ns
beet greens	125 ml	11-0087	11	ns
Bok Choy	125 ml	11-0457 11-0464 no values for bok choy so averaged values for chinese cabbage and cooked spinach	83	ns
broccoli (raw or boiled)	125 ml	11-0090 11-0091 averaged values for raw and boiled	57	ns
brussel sprouts (fresh or frozen, cooked)	125 ml	11-0101 11-0099 averaged values for fresh cooked and frozen cooked	84	ns
green cabbage (shredded; raw or cooked)	125 ml	11-0109 11-0110 averaged values for raw and cooked	29	ns
red cabbage (chopped; raw)	125 ml	11-0112	16	ns

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
VEGETABLES and SOUP cont'd				
chinese cabbage	125 ml	CABCH	25	ns
carrots (raw)	1 medium (59 g)	reference b - folate value	32	ns
carrots (fresh; boiled, drained)	125 ml	11-0125	12	ns
corn on the cob	1 ear (77 g)	11-0168	36	ns
corn (niblets; frozen or canned)	125 ml	reference b averaged values for 11-0172 frozen and canned	30	0.34
creamed corn	125 ml	11-0174	54	0.64
cauliflower (raw or cooked fresh or frozen)	125 ml	11-0135 averaged values for raw 11-0136 and cooked from fresh or frozen state	34	ns
romaine lettuce	1 cup (55 g)	reference b - folate value	98	ns
Crisphread or Butter Lettuce	1 cup (50 g)	LETT averaged values for Crisphread LETTB and Butter lettuces	12	ns
potato (baked, boiled, hash browned, mashed, salad)	100 g	11-0674 averaged values for baked, 11-0363 boiled, hash browned and 11-0367 mashed potatoes 11-0370	12	0.29
french fries	20 fries	11-0405 - zinc value reference b - folate value because CNF value seemed questionable	22	0.38

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
VEGETABLES and SOUP cont'd				
spinach (raw)	250 ml	11-0457	115	ns
spinach (cooked fresh or frozen)	125 ml	11-0458 averaged values for fresh 11-0464 cooked and frozen cooked	124	ns
turnip or rutabaga	125 ml	11-0436 averaged values for turnip 11-0565 and rutabaga	25	ns
squash (winter;baked)	125 ml	11-0644	31	ns
squash (winter; boiled and mashed)	125 ml	11-0491	12	ns
sweet potato or yam (baked and peeled)	114 g	11-0508	26	ns
tomato (raw)	1 medium (143 g)	11-0529	12	ns
tomato (canned)	125 ml	11-0531	10	ns
tomato or vegetable cocktail juice	250 ml	11-0540 averaged values for tomato 11-0578 and vegetable cocktail juices	53	ns
zucchini (raw or boiled)	125 ml	reference b averaged values for raw 11-0478 and boiled	18	ns
mixed frozen vegetables	125 ml	11-0584	17	ns
vegetable soup (with or without meat)	250 ml	SPV averaged values for canned SPM vegetable and canned Minestrone soups	14	0.64

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
VEGETABLES and SOUP Cont'd				
clam chowder (New England or Manhattan)	250 ml	SPCLCM SPCLC averaged values for New England and Manhattan chowders	7	0.89
tomato soup (water or milk base)	250 ml	06-0559 06-0359 averaged values for canned tomato soups reconstituted with water and milk respectively	18	ns
MILK and YOGOURT				
milk (skim, 2%, whole or buttermilk)	250 ml	MLKSK MLK2 MLK BMLK averaged values for the four types of milk	13	1.03
chocolate milk or hot chocolate (made from milk)	250 ml	CMLK HCHOC averaged values for chocolate milk and hot chocolate	13	1.19
yogourt (plain or fruit flavoured)	125 g	01-0117 01-0122 averaged values for plain and fruit flavoured yogourts	14	1.07
FRUITS				
banana (very small 6" - 100g) (medium - 150g)	100 g	09-0040	19	ns
cantaloupe	134 g	09-0181	23	ns
grapefruit (white or pink)	1/2 fruit	09-0116 09-0112 averaged values for white and pink grapefruits	13	ns
grapefruit juice (fresh or canned)	250 ml	09-0124	27	ns
lemonade	250 ml	00-1252	12	ns

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
FRUIT Cont'd				
mandarin orange (fresh)	120 g	09-0218	24	ns
orange (2.5" fruit = 100g) (3.0" fruit = 150g) (3.5" fruit - 235g)	100 g	09-0200	31	ns
orange juice (frozen, reconstituted)	250 ml	09-0215	115	ns
orange juice (canned)	250 ml	09-0207	48	ns
pear (fresh)	169 g	09-0252	12	ns
pineapple juice	250 ml	09-0273	61	ns
raspberries (fresh)	125 ml	09-0306	17	ns
raspberries (frozen)	125 ml	09-0302	35	ns
strawberries (fresh or frozen)	125 ml	09-0316 09-0318 averaged values for fresh and frozen strawberries	14	ns
DESSERTS and NUTRITION SUPPLEMENTS				
pudding or custard (creamy ie not rice or tapioca)	125 ml	00-0948	ns	0.88
chocolate ice-cream or 1 fudgesicle	125 ml (75 g)	reference c - used zinc value for chocolate ice - milk	ns	0.57

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
DESSERTS and NUTRITION SUPPLEMENTS Cont'd				
Ensure	1 can (235 ml)	ENSURE	47	3.8
Ensure Plus	1 can (235 ml)	ENSUREP	49	5.6
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS				
whitefish eg cod, halibut, haddock, sole perch, pike, lingcod, bluefish, mock crab (pollock)	90 g	reference b - zinc value for white varieties of fish averaged folate values for bluefish, cod, halibut, perch, haddock, whitefish and sole; value for sole from reference a, others from CNF 00-430 00-795 00-1104 00-1398 00-1100 00-2468 averaged zinc values for white varieties from reference c and from CNF reference b	11	0.70
trout		TROUT - folate value only; averaged reference c zinc value for white fish and CNF value for canned salmon	11	0.90
salmon (cooked fresh or canned solids/ liquid)	90 g (80 ml)	0-01958 0-01947 averaged folate values for fresh cooked and canned salmons; used zinc value for canned	24	0.85

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS Cont'd				
tuna (canned/drained)	85 g	0-02324	13	0.68
clams or mussels (canned/drained)	84 g (7 clams)	00-0775 - folate value reference 80-zinc value no values for mussels so assumed folate and zinc content as in clams	11	1.4
crab (canned/drained)	78 g (125 ml)	0-02324	16	3.9
lobster (canned/ drained)	73 g (125 ml)	00-1280 - assumed Northern lobster representative of all lobster	12	1.3
oysters (canned)	85 g (4 oysters)	no folate value - assumed the same as for clams reference 77 - zinc value for Pacific canned oysters	11	7.2

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS				
scallops (cooked)	90 g (7 scallops)	00-2024 - folate value reference c - used zinc value for breaded scallops and subtracted 0.09 mg zinc for breading	15	1.20
shrimp (canned)	90 g (28 medium)	00-2045	18	2.2
beef (ground)	90 g	GBEEF	7	5.2
beef (lean roasted or steak)	90 g	13-5091 averaged folate values for RBEEF rump and rib roasts reference c - zinc value	7	5.2
beef liver (fried)	90 g	LIVER	180	3.9
beef kidney (braised)	90 g	00-1160	72	2.7
pork (roast, ham, or bacon; lean portions)	90 g	10-0073 - folate value reference e - averaged zinc values for pork, Boston Butt, ham or picnic and loin (separable lean in all cases)	ns	3.5
pork chop (lean portion)	90 g	10-0042 assumed loin centre cut representative of all pork chops	ns	2.0
pork liver (braised)	90 g	10-0111	147	6.1
veal (cutlets and chops)	90 g	00-2382 assumed loin cut representative of all veal cutlets and chops	5	3.7
lamb (roast; lean portion)	90 g	reference e - zinc value	ns	3.8
lamb chop (lean portion)	90 g	- assumed zinc value as for pork chop since no zinc value available	ns	2.0
chicken (breast meat)	86 g	05-0064	ns	0.86
chicken (dark meat)	88 g	05-0073	8	2.8

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS Cont'd				
roasting chicken or rabbit	90 g	05-0114 - no values for rabbit so assumed same as for chicken (Fe values very similar)	ns	1.4
chicken livers	80 g	05-2028	616	3.5
turkey (dark meat)	90 g	05-0188	8	4.0
turkey (white meat)	90 g	05-0186	ns	1.8
liverwurst or liver pate	30 g (30 ml)	07-0041 - no values for liver pate so assumed same as for liverwurst	9	0.68
weiners	37 g (1 weiner)	07-0022 averaged values for all 07-0023 beef and beef plus pork weiners	ns	0.88
luncheon meat eg Spam, bologna, chicken loaf, salami	28 g	07-0008 assumed values for beef plus pork bologna representative of all luncheon meats	ns	0.55
corned beef, pastrami	90 g	reference c - zinc value for corned beef; assumed this value also representative of pastrami	ns	1.7
cottage cheese (dry curd, 2% or creamed)	1/4 c	01-0014 averaged values for three 01-0015 types of cheese listed 01-2012	7	0.25
Ricotta cheese (from whole or part skim milk)	45 g	01-0037 averaged values for two 01-0036 types of cheese listed	6	0.56
Gruyere and Swiss (block or processed)	45 g	01-0023 averaged values for three 01-0040 types of cheese listed 01-012044	ns	1.7
Mozarella (low fat or regular) or Muenster	45 g	01-1026 averaged values for three 01-1028 types of cheese listed 01-0030	ns	1.2

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used		folate (ug)	zinc (mg)
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS Cont'd					
Cheddar or processed Cheddar	45 g	01-2009 01-2042	averaged values for cheddar and processed cheddar cheeses	6	1.4
Blue or Feta	45 g	01-2004 01-1019	averaged values for Blue and Feta cheeses	16	1.3
Brie or Camembert	45 g	01-0006 01-2007	averaged values for Brie and Camembert	29	1.1
Gouda	45 g	01-022		10	1.8
Provolone	45 g	01-0035		ns	1.5
Monteray Jack, Brick, or Colby	45 g	01-0025 01-0005 01-0011	averaged values for Monteray Jack, Brick and Colby cheeses	8	1.3
egg	1 medium (48 g)	01-2123		31	0.70
tofu	89 g	reference d - zinc value		25	0.65
	piece: 7x6x2 cm	no folate value - see procedures used in estimating folate value following these tables (references c, f)			
peanut butter	15 ml	12-2192		12	0.44
Navy beans (cooked) or Pork and Beans	125 ml	00-0155 00-0156 00-0158	averaged values for cooked white beans, pork and beans in tomato sauce and pork and beans	38	0.85
Kidney beans (cooked)	125 ml	00-0160		47	1.0
Garbanzo beans (cooked)	125 ml	reference b - folate value reference e - zinc value		102	1.4

Continued

Food	Unit	Folate and Zinc Determination: NUTRICOM Codes, Other References and Other Procedures Used	folate (ug)	zinc (mg)
PROTEIN: FISH, MEAT, POULTRY, LEGUMES, CHEESE and NUTS Cont'd				
Lima beans (cooked)	125 ml	00-0177	averaged 45	0.89
Lentils (cooked)	125 ml	00-1254	values 27	0.48
nuts	1 Tbsp (8 g)	00-0008 00-1496 reference a - folate value for cashews reference e - zinc value for cashews	for 7 almonds, peanuts and cashews	0.28

References

a. Hoppner, K., Lampi, B., Perrin, D.E. The free and total folate activity in foods available on the Canadian market. J. Instit. Can. Sci. Technol. Aliment. 5:60-66, 1972.

b. Perloff, B.P. and Butrum, R.R. Folacin in selected foods. J. Am. Diet Assoc. 70: 161-172. 1977.

c. Pennington, J.A.T. and Church, H. Bowes and Church's Food Values of Portions Commonly Used, 13th ed. (Philadelphia: J.B. Lippincott Company, 1980), pp 169-171.

d. Freeland - Graves, J.H., Ebangit, M.L. and Bodzy, P.W. Zinc and copper content of foods used in vegetarian diets. J. Am. Diet Assoc. 77: 648-653, 1980.

e. Murphy, E.W., Wells-Willis, B., Watt, B.K. Provisional tables on the zinc content of foods. J. Am. Diet Assoc. 66: 345-355, 1975.

f. I.E. Liener "Nutritional Value of Food Protein Products," in Soybeans: Chemistry and Technology Volume 1 (revised edition) ed. A.K. Smith and S.J. Circle (Westport, Connecticut: AVI Publishing Co. Inc., 1978), p 203.

g. Computrition Nutrient Analysis Software package

Continued

Estimating the Folate Content of Tofu

No folate value could be found in the literature for Tofu so a value was estimated as follows:

1) Folate values for mature, dry soybeans were found in the literature: 230 ug (f) and 171 ug (c) per 100 g.

2) Thiamin and Riboflavin values were found in the literature for mature, dry soybeans, cooked soybeans and tofu.

	dry soybeans[f]	cooked soybeans[a]	tofu[a]
Thiamin	1.1-1.76 (avg=1.43)mg/100g		
0.21mg/100g	0.06mg/100g		
Riboflavin	0.23 mg/100g	0.09mg/100g	0.03mg/100g

3) Both folate and riboflavin destruction are enhanced by light exposure and both folate and thiamin destruction are enhanced by alkali and oxygen exposure. Accordingly, I would expect destruction of folate to be similar to that for thiamin and riboflavin.

a) Since I don't have a folate value for cooked soybeans I have tried to estimate it by looking at the losses that occurred with cooking for thiamin and riboflavin.
thiamin 0.21/1.43 = 15% retention riboflavin 0.09/0.23 = 39% retention
I think folate is more stable with heating than riboflavin so estimating losses at 60% will if anything underestimate folate retention.
200 ug folate/100g dry soybeans X 0.4 = 80ug folate/100 g cooked soybeans

b) Since I don't have a folate value for tofu, I have tried to estimate it by looking at retention of thiamin and riboflavin during processing from cooked beans to tofu.
thiamin 0.06/0.21 = 29% retention riboflavin 0.03/0.09 = 33% retention average rention = 31%

- Based on 80 ug folate/100 g cooked beans and 31% retention during processing from cooked beans to tofu, the folate content of tofu was estimated at 25 ug/100g.

APPENDIX F

Name: _____
Hemodialysis Unit: _____

3 DAY FOOD RECORD

Please keep a record of everything you eat and drink on the attached form for the following 3 days: _____
_____
_____

Read these instructions carefully before you start to write:

1. Write down everything you eat and drink (including alcohol). Be sure to include all snacks. Record immediately after each meal and snack to ensure accuracy.

2. Write down how much you eat and drink.

a) Use VOLUME measures (cups, Tbsp, tsp or mls) for cereals, rice, pasta, vegetables, canned or sliced fruit, beverages, peanut butter, mayonnaise, salad dressing, butter, margarine, sauces, gravies, soups, sugar, honey, jam etc.

b) Use WEIGHTS (ounces or grams) for meat, fish, poultry, cheese. Use the attached diagrams and labels on packaging to determine weights.

c) Use SIZE for raw fruits, muffins, buns, crackers, cakes, pies, cookies, desserts etc. Give dimensions (eg 1 oatmeal cookie, 2" diameter) or specify small, medium or large based on the attached models.

d) Be specific about TYPE OF FOOD, BRAND NAME IF APPLICABLE and CONTENT OF MIXED DISHES.

For example:

Time	Foods	Amount
Lunch	2% milk	1/2 cup
	regular whole wheat bread	2 slices
	L.S. butter	2 tsp
	Omelette - eggs medium	2
	- cheddar cheese	1/2 ounce
	- vegetable oil	2 tsp
	Digestive cookies	2 cookies

e) Don't forget the extras!
For example: sugar on your cereal
dressing on your salad

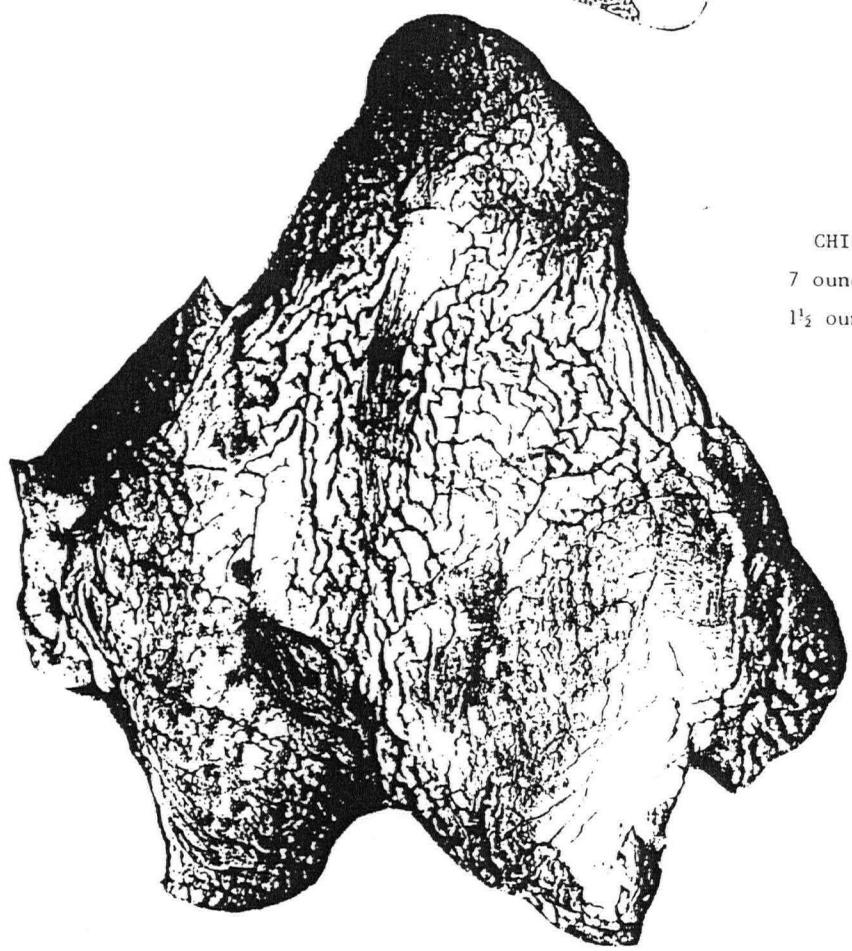
Diagrams of Meat, Poultry, Fish, and Cheese to Help You Estimate Cooked Weights

CHICKEN LEG (cooked)

3 ounces lean flesh

(thigh equals 2 ounces)
(drumstick equals 1 ounce)

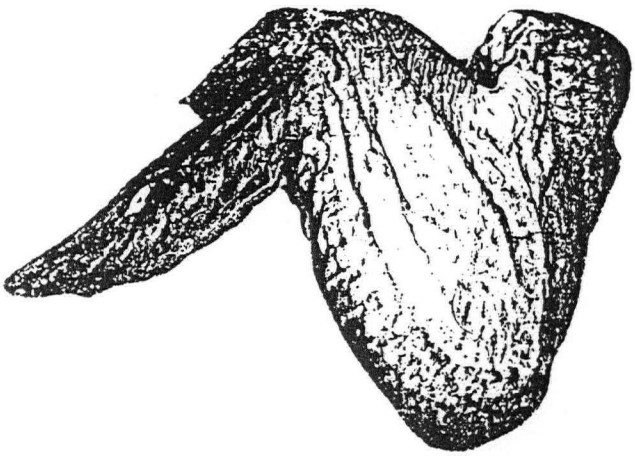
½ ounce skin plus fat



CHICKEN BREAST (cooked)

7 ounces lean flesh

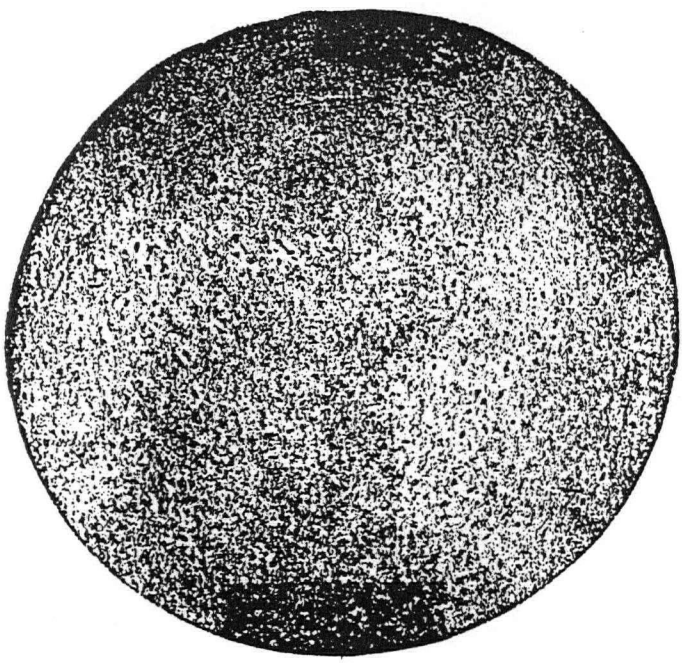
1½ ounces skin plus fat



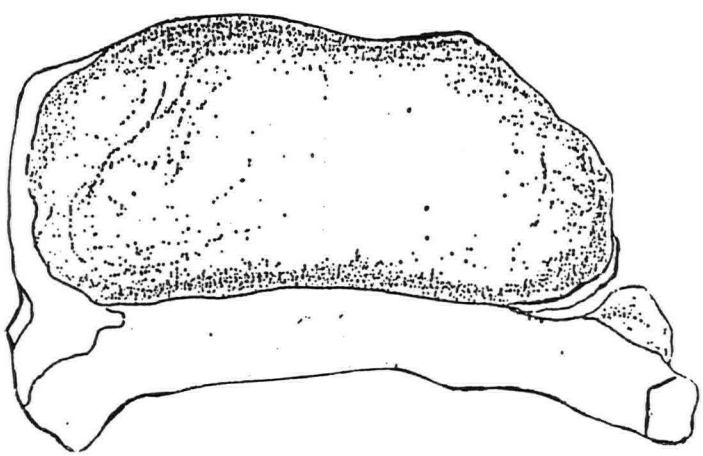
CHICKEN WING (cooked)

½ ounce lean flesh

½ ounce skin plus fat



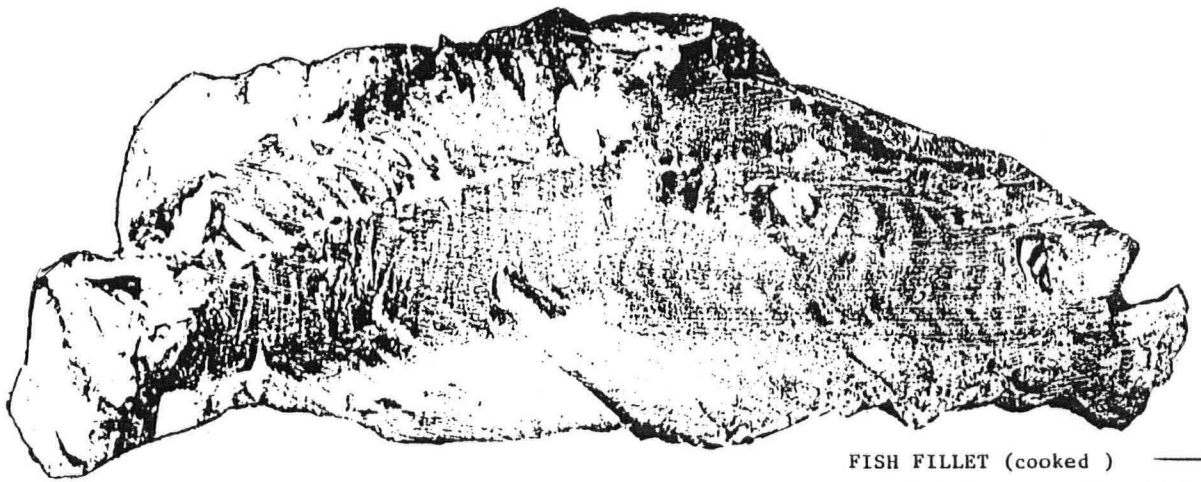
A slice of bologna or other luncheon meat this thick ↘ is equal to 1 ounce.



PORK CHOP (cooked)

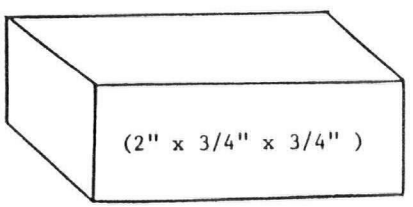
A pork chop this thick ↘ is equal to 3 ounces.

A pork chop this thick ↘ is equal to 2 ounces.



FISH FILLET (cooked)

A fillet of fish about this thick → is equal to 4 ounces.

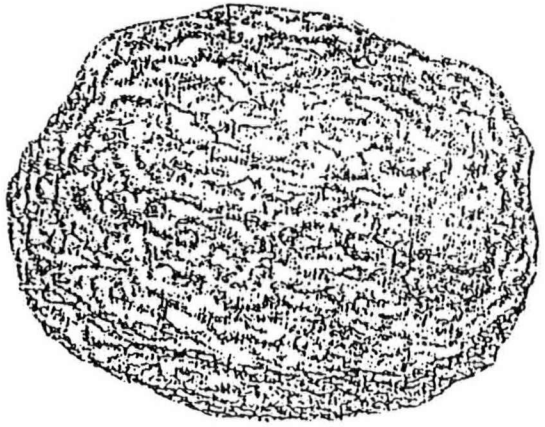


A piece of cheese this size is equal to 1 ounce.

MEAT (cooked)

One slice of meat this thick
is equal to 3 ounces.

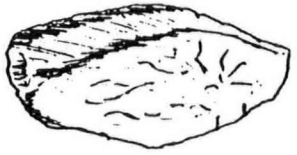
One slice of meat this thick
is equal to 1½ ounces.



HAMBURGER PATTY (cooked)

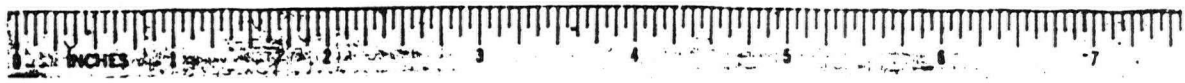
One hamburger patty this thick
is equal to 3 ounces.

One hamburger patty this thick
is equal to 2 ounces.

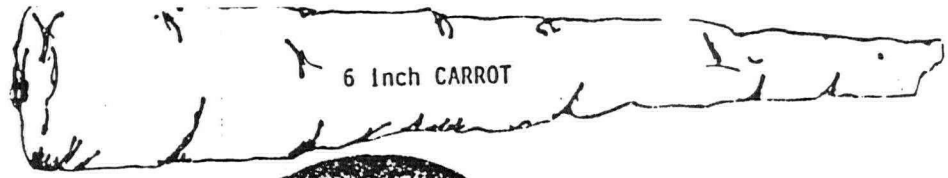


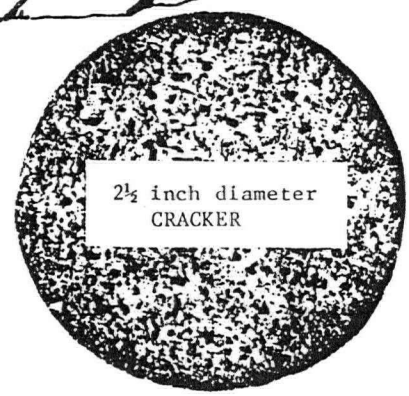
STEWING MEAT (cooked)

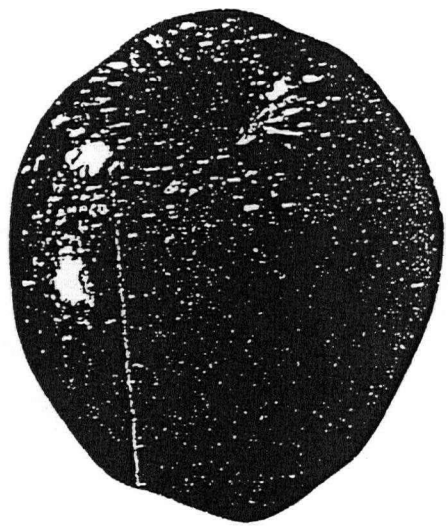
One piece of cooked stewing meat
this size is equal to ½ ounce.



Scale and Diagrams to Help You Determine Sizes of Raw Fruits, Crackers, Cake etc







MEDIUM APPLE

* Use this diagram to describe peaches, oranges, potatoes, tomatoes, etc

MEDIUM MUFFIN

(100 grams)

Day _____ Name: _____

Date: _____

Time	Type of Food	Amount

APPENDIX G

Foods in 3 Day Food Records (Used in Food Frequency Validation) for Which Folate Values Were Taken From Sources Other Than the CNF or Were Estimated

Foods Missing Folate Values	Folate Value Used (ug)	References (see Appendix E) and Estimations Used
BREADS/CEREALS		
2 Tbsp natural bran	46.0	reference b
12 g Crispy Crackers® or Crisp Wheat Crackers®	2.18	used value for 12 g saltines in CNF
1000 ml popcorn	6.98	estimated that 85 ml raw corn yields 1000 ml popped corn and used value for whole grain corn in reference b
BEVERAGES		
682 ml beer	0	assumed folate negligible and assigned a value of 0
200 ml wine	4.40	used value for grape juice in reference b
2 oz rum	0	assumed folate negligible and assigned a value of 0
6 oz diet Pepsi	0	assumed folate negligible and assigned a value of 0
FRUIT		
1/4 kiwi fruit	1.10	used value for 10 ml strawberries in reference b
1/2 papaya	1.50	used value for 150 g mango in CNF
50 g canned mandarins	10.0	used value for 50 g mandarin in CNF
10 g raisins	0.4	reference b

APPENDIX G CONT'D

Foods Missing Folate Values	Folate Value Used (ug)	References (see Appendix E) and Estimations Used
MISCELLANEOUS		
1 small dill pickle	0	assumed folate negligible and assigned a value of 0
1 Tbsp relish	1.5	used value for 10 g cucumber in CNF
2 Tbsp sunflower seeds	16.0	reference b
3 marshmallows	0	assumed folate negligible and assigned a value of 0
10 g semi-sweet chocolate	2.50	used value for milk chocolate in reference b
10 g caramel candy	0	assumed folate negligible and assigned a value of 0
50 g (dry) Mr. Noodle® instant soup	0.54	used value for 109 g cooked enriched macaroni in ref a

APPENDIX H

Foods in 3 Day Food Records (Used in Food Frequency Validation) for Which Zinc Values Were Taken From Other Sources Than the CNF or Were Estimated

Foods Missing Zinc Values	Zinc Value Used (mg)	References (see Appendix E) and Estimations Used
BREADS/CEREALS		
240 g (cooked) egg noodles	1.20	used value for enriched macaroni in reference e
508 g cooked white rice	2.06	reference e
white submarine bun	0.64	used value for 2 white hamburger buns in CNF
30 g Muffets[R] cereal	0.82	used value from cereal box
2 Tbsp natural bran	2.6	reference e
oatmeal muffin -75 g plain muffin	0.45	used value for 75 g pancakes in CNF
-2 Tbsp rolled oats	0.19	value from CNF
-10 g raisins	0.02	reference c
cinnamon bun or sweet roll	0.28	used value for 35 g white hamburger bun in CNF
40 g bran muffin	2.3	analyzed recipe (Joy of Cooking);Zn in flour-ref 80;Zn in other ingredients-CNF
1/2 cup Shreddies[R]	0.48	used value from cereal box
16 g Stoned Wheat Thins[R]	0.06	used value for saltines from reference e
10 g Rye Vita[R] or 10 g wheat cracker	0.22	averaged values for whole wheat crackers-ref e and graham wafers-ref c
40 g Wasa Fibre Crackers[R]	0.89	as for previous crackers
100 g bluberry muffin	0.60	used value for 100 g pancakes in CNF

APPENDIX H Cont'd

Foods Missing Zinc Values	Zinc Value Used (ug)	References (see Appendix E) and Estimations Used
24 g oatcakes	0.34	used value for whole wheat oatmeal raisin cookies in reference d
40 g white bun	0.32	used value for 50 g hamburger bun in CNF
90 g scone	0.64	used value for two 40 g hamburger buns in CNF
74 g waffles	0.44	used value for pancakes in CNF
1 granola bar	0.54	used value for 25 g granola in reference e
169 g Uncle Ben's Converted Rice®	0.68	used value for enriched white rice in reference e
BEVERAGES		
682 ml beer	0.22	reference c
2 oz rum	0.04	used value for brandy in reference c
6 oz diet Pepsi	0.005	reference c
FRUIT		
1 kiwi fruit	0	assumed zinc negligible and assigned a value of 0
juice of 1/2 large lemon	0	assumed zinc negligible and assigned a value of 0
MEAT/ALTERNATES		
120 g lamb	5.07	used value for lamb (roast, separable, lean) in ref e
15 ml peanut butter (sweetened + fat added)	0.45	used value for peanut butter (small amount salt+fat added) in CNF
75 g roast pork + 75 g pork steak	6.33	used value for pork (Boston Butt) in reference e

APPENDIX H Cont'd

Foods Missing Zinc Values	Zinc Value Used (ug)	References (see Appendix E) and Estimations Used
150 g sirloin steak	8.67	used value for roast beef in reference c
15 g salmon (in a curry)	0.14	used value for canned salmon in CNF
60 g smoked salmon	0.57	used value for canned salmon in CNF
120 g stewing beef	6.93	reference c
60 g baked white fish	0.63	reference c
98 g cooked lean hamburger	5.66	used value for cooked regular hamburger in CNF
103 g kidney beans	1.03	used different CNF code for kidney beans with a Zn value
VEGETABLES		
250 ml V-8 juice[R]	0.52	used value for canned tomatoes (solids+liquids) in reference e
150 g brussel sprouts	0.43	averaged values for broccoli and cabbage in reference 77
44 g canned niblet corn	0.18	reference e
110 g Romaine lettuce	0.40	reference c
13 g pickled beets	0.03	averaged values for potato and sweet potato in ref c
15 g cucumber	0.027	used value for zucchini in reference c
140 g potato boiled in skin	0.41	reference c
30 g raw carrot	0.13	reference d
10 g green onion	0.03	reference c
4 radishes	0.12	reference c

APPENDIX H Cont'd

Foods Missing Zinc Values	Zinc Value Used (ug)	References (see Appendix E) and Estimations Used
25 g raw cauliflower	0	assumed zinc negligible and assigned a value of 0
30 g raw broccoli	0.08	used value for frozen broccoli spears in reference c
15 g raw green	0.009	reference c
90 g cooked zucchini	0.16	reference c
MISCELLANEOUS		
50 g chocolate cake	0.34	reference c
12 g tortilla chips	0.17	reference c
1 chocolate doughnut	0.19	used value for 40 g doughnut in reference c
30 g white cake	0.07	reference c
5 g semi sweet chocolate	0.023	used value for 5 g chocolate bar in reference c
2 chocolate covered marshmallow cookies	0	assumed zinc negligible and assigned a value of 0
24 g marshmallows	0	assumed zinc negligible and assigned a value of 0
12 g shredded coconut	0.085	used value for fresh coconut in reference d
4.8 ml cocoa powder	0.024	used value for carob powder in reference d
10 g caramel candy	0	assumed zinc negligible and assigned a value of 0
4 digestive cookies	0.57	used value for oatmeal raisin cookies in reference e
50 g (dry) Mr. Noodle instant soup[R]	0.54	used value for 109 g cooked enriched macaroni in ref e
7 g tomato paste	0.06	used value for canned tomatoes (solids+liquids) in ref c

APPENDIX I Correlation Plot: Average Daily Folate Intake Based on Food Frequency versus Average Daily Folate Intake Based on Three Day Food Record

folate

File: d:\123\folval.prn size: 12 * 2
MISS= -9999.000 LL= 1 UL= 12

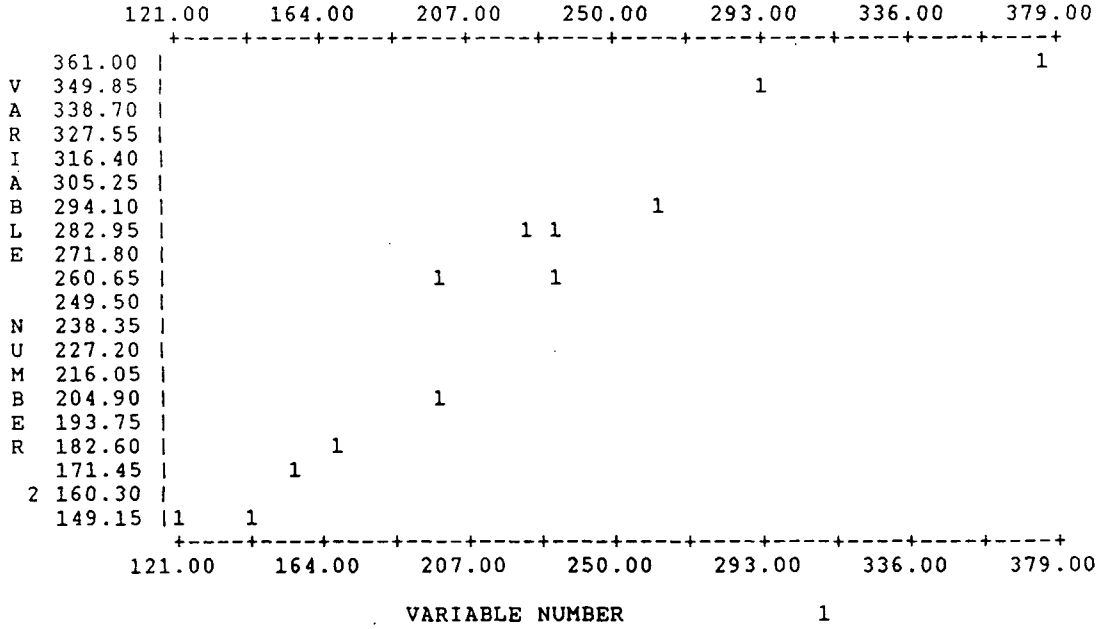
C O R R E L A T I O N:

---

var.:	1	&	var.:	2
M1=	218.8333000		M2=	240.6667000
S1=	71.3134400		S2=	74.5536200
N1=	12		N2=	12

r(10) = .94963 t = 9.582 p =.0000

folate



APPENDIX J Correlation Plot: Average Daily Zinc Intake Based on Food Frequency versus Average Daily Zinc Intake Based on Three Day Food Record

zinc

File: d:\123\zincval.prn size: 12 * 2
MISS= -9999.000 LL= 1 UL= 12

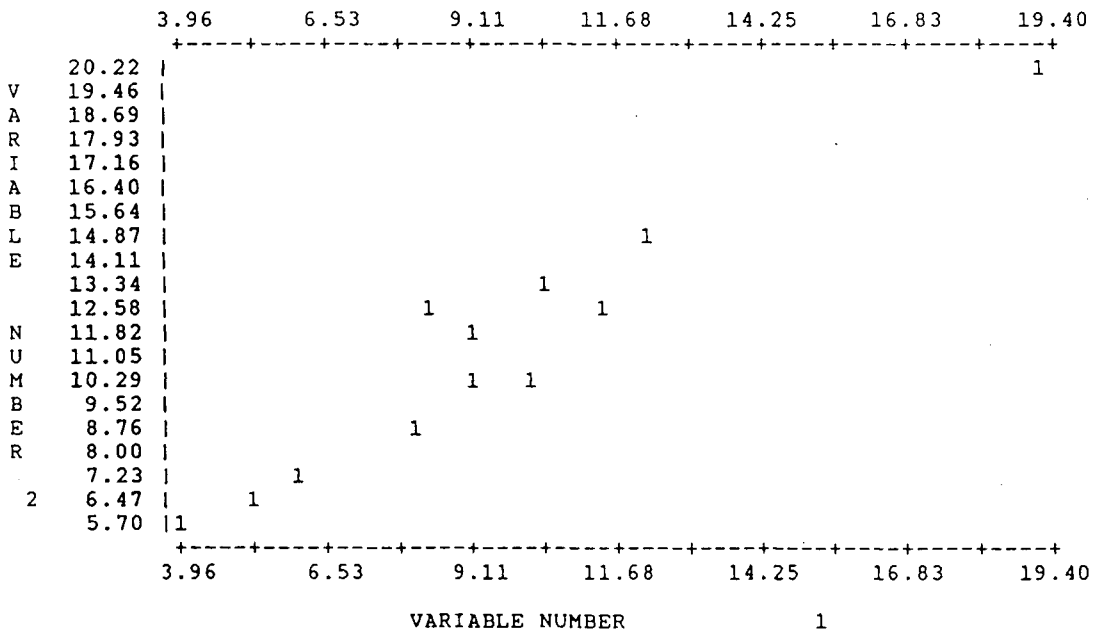
C O R R E L A T I O N:

---

var.:	1	&	var.:	2
M1=	9.5316670		M2=	10.7708300
S1=	3.9973760		S2=	4.1497450
N1=	12		N2=	12

r(10) = .96939 t =12.485 p =.0000

zinc



Appendix K Average Daily Folate Intake as Determined by the Food Frequency as a Percentage of Average Daily Intake as Determined by the 3 Day Food Intake Records

Subject	Food Frequency Folate (ug)	3 Day Record Folate (ug)	% of 3 Day Record Folate Detected by Food Frequency
B.R.	234	257	91
H.R.	143	149	96
M.R.	158	162	98
D.C.	121	138	88
C.S.	202	201	100
R.Mc.	234	281	83
B.C.	294	341	86
S.M.	225	280	80
M.Mc.	201	251	80
J.L.	379	361	105
M.L.	170	178	96
K.S.	265	290	91
			91 + 8 (SD)

Appendix L Average Daily Zinc Intake as Determined by the Food Frequency as a Percentage of Average Daily Intake as Determined by the 3 Day Food Intake Records

Subject	Food Frequency Zinc (mg)	3 Day Record Zinc (mg)	% of 3 Day Record Zinc Detected by Food Frequency
B.R.	5.25	6.28	84
H.R.	10.18	10.26	99
M.R.	8.05	8.64	94
D.C.	4.63	5.50	84
C.S.	3.96	4.94	80
R.Mc.	10.56	12.79	83
B.C.	11.12	11.60	96
S.M.	12.26	14.79	83
M.Mc.	9.14	11.21	82
J.L.	9.12	9.67	94
M.L.	8.64	11.86	73
K.S.	18.74	20.22	93
			87 + 8 (SD)

APPENDIX M Additional Foods East Indian and Chinese Subjects Were Questioned About in the Food Frequency Questionnaire

Food	Unit	Folate and Zn Determination: NUTRICOM Codes, Other References and Other Procedures	folate (ug)	Zinc (mg)
TRADITIOAL EAST INDIAN FOODS				
chapatis	50 g	reference b	36	0.90
eggplant (boiled)	125 ml	11-0210	35	0.73
okra (boiled)	125 ml	11-0279	45	0.54
TRADITIONAL CHINESE FOODS				
lo bok (boiled)	125 ml	11-0431	13	ns
Chinese greens (cooked) other than Chinese cabbage eg bok choy, gai choy, gai lan, wong choy	125 ml	11-0457-Chinese cabbage 11-0464-spinach no values available so averaged values for Chinese cabbage and spinach as an estimate	25 124 75 (average)	ns ns ns

Legend ns - not significant

References

a. NUTRICOM Nutrient Analysis Software

b. Computrition Nutrient Analysis Software

Appendix N Weights of Subjects Receiving No Zinc Supplements in Comparison to Ideal Body Weights (IBW) in 1983 Metropolitan Height and Weight Tables

OF0Z Subjects	Sex	Height (cm)	Frame* Size	Ideal Weight Range (Kg)	Weight** (Kg)	%IBW (Average for Range)
B.A.	M	166.0	small	61.8-64.5	66.9	105.9
D.L.	M	165.7	small	61.8-64.5	64.1	101.4
T.L.	F	146.8	small	46.8-51.4	58.0	118.1
M.M.	F	153.5	large	56.8-63.6	78.1	129.7
M.B.	F	152.3	medium	52.3-58.6	55.4	99.8
				(means + SD)	64.5+8.9	111.0+12.7
5F0Z Subjects						
R.C.	F	165.5	large	63.6-72.3	94.8	139.4
K.H.	F	159.8	small	51.8-57.7	48.4	88.3
N.S.	F	152.2	small	48.2-53.6	44.7	87.8
Sh.S.	F	161.8	small	53.2-59.1	43.5	77.4
S.S.	M	179.3	small	67.7-72.7	82.5	117.5
G.S.	M	179.0	small	66.4-71.4	64.4	93.5
				(means + SD)	63.1+21.5	100.7+23.2

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
0Z - not receiving a zinc supplement

* Frame size was determined on the nondominant arm using an elbow breadth gauge made by the Metropolitan Life Insurance Company

** Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

Appendix O Weights of Subjects Receiving Zinc Supplements in Comparison to Ideal Body Weights (IBW) in 1983 Metropolitan Height and Weight Tables

OF22.5Z Subjects	Sex	Height (cm)	Frame* Size	Ideal Weight Range (Kg)	Weight** (Kg)	%IBW (Average for Range)
H.L.	M	164.9	small	61.8-64.5	59.1	93.5
D.R.	M	172.4	small	64.5-68.6	79.7	119.7
A.K.	F	157.0	small	50.5-56.4	41.3	77.2
M.Be.	F	163.4	small	53.2-59.1	53.4	95.0
				(means + SD)	58.4+16.0	96.4+17.5
5F22.5Z Subjects						
J.B.	M	175.3	small	65.5-68.6	72.5	108.0
D.B.	M	182.0	medium	72.7-79.1	90.5	119.2
T.N.	M	155.2	small	58.2-60.9	50.1	84.1
J.S.	M	184.4	small	70.5-76.4	65.7	89.4
W.H.#	M	159.0	small	60.0-62.7	48.7	79.3
W.W.	M	173.0	medium	68.6-74.1	102.0	142.9
				(means + SD)	71.6+21.5	103.8+24.4

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
22.5Z - receiving a supplement of 22.5 mg zinc/day

* Frame size was determined on the nondominant arm using an elbow breadth gauge made by the Metropolitan Life Insurance Company

** Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

This crippled subject was unable to stand so height was taken in the recumbent position. This measurement was adjusted slightly to account for the fact that his legs wouldn't straighten completely.

Appendix P Protein Intakes of Subjects Receiving No Zinc Supplements (Based on 3 Day Food Records)

g protein/Kg

OF0Z Subjects	Sex	Dialysis Weekday	Nondialysis Weekday	Nondialysis Sat or Sun	Mean for 3 days
B.A.	M	0.79	0.47	0.99	0.75
D.L.	M	1.14	1.94	2.38	1.82
T.L.	F	1.09	0.73	0.73	0.85
M.M.*	F	0.54	0.60	0.60	0.58
M.B.	F	0.98	1.07	0.68	0.91
(means + SD)		0.91+0.25	0.96+0.59	1.08+0.74	0.98+0.48
5F0Z Subjects					
R.C.*	F	0.96	1.03	1.06	1.02
K.H.	F	1.00	2.09	1.43	1.51
N.S.	F	1.17	1.21	0.89	1.09
Sh.S.	F	1.75	0.91	1.70	1.46
S.S.	M	0.88	1.03	0.97	0.96
G.S.	M	1.17	1.34	0.81	1.11
(means + SD)		1.16+0.31	1.27+0.43	1.14+0.35	1.19+0.23

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
0Z - not receiving a zinc supplement

* if a subject's weight (mean of post dialysis weights at the 4 dailyses prior to RBC folate determination) was 120% high end of IBW range (1983 Metropolitan Height and Weight Tables), they were considered obese and mean IBW was used in calculating g protein/Kg

Appendix Q Protein Intakes of Subjects Receiving Zinc Supplements (Based on 3 Day Food Records)

		g protein/Kg			
OF22.5Z Subjects	Sex	Dialysis Weekday	Nondialysis Weekday	Nondialysis Sat or Sun	Mean for 3 days
H.L.	M	0.87	0.83	0.80	0.83
D.R.	M	0.67	0.50	0.41	0.53
A.K.	F	#	#	#	#
M.Be.	F	1.08	1.27	2.19	1.51
(means + SD)		0.87+0.21	0.87+0.39	1.13+0.94	0.96+0.50
5F22.5Z Subjects					
J.B.	M	0.70	0.97	1.41	1.03
D.B.	M	0.85	0.93	0.87	0.88
T.N.	M	1.19	0.65	1.31	1.05
J.S.	M	1.14	1.39	1.40	1.31
W.H.	M	1.25	1.02	1.52	1.26
W.W.*	M	1.35	1.30	1.13	1.26
(means + SD)		1.08+0.25	1.04+0.27	1.27+0.24	1.13+0.17

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
22.5Z - receiving a supplement of 22.5 mg zinc/day

* if a subject's weight (mean of post dialysis weights at the 4 dailyses prior to RBC folate determination) was > 120% high end of IBW range (1983 Metropolitan Height and Weight Tables), they were considered obese and mean IBW was used in calculating g protein/Kg

This subject became very ill and was unable to complete a 3 day food record

Appendix R Energy Intakes of Subjects Receiving No Zinc Supplements (Based on 3 Day Food Records)

OF0Z Subjects	Sex	24 Hr Energy Intake (Kcal); Intake as % of Estimated Daily Requirement * Dialysis Weekday	Nondialysis Weekday	Nondialysis Sat or Sun	Mean for 3 days
B.A.	M	1962(111)	1129 (64)	1654 (93)	1582 (89)
D.L.	M	1710(105)	2335(143)	1897(116)	1981(122)
T.L.	F	980 (71)	945 (68)	1141 (83)	1022 (74)
M.M.	F	857 (61)	1074 (76)	741 (53)	890 (63)
M.B.	F	1329 (89)	1592(107)	1060 (71)	1327 (89)
(means + SD)		1368+470 (87+21)	1415+569 (92+33)	1300+468 (83+24)	1360+439 (87+22)
5F0Z Subjects					
R.C.	F	2352(148)	2305(145)	2597(163)	2418(152)
K.H.	F	1706(125)	2568(188)	1991(146)	2088(153)
N.S.	F	1791(126)	1910(135)	1476(104)	1726(122)
Sh.S.	F	2389(155)	1483 (96)	1760(114)	1878(122)
S.S.	M	1601 (65)	2899(117)	2683(108)	2394 (96)
G.S.	M	1690 (86)	1637 (83)	1780 (90)	1702 (86)
(means + SD)		1922+353 (118+35)	2134+552 (127+38)	2048+488 (121+28)	2034+319 (122+28)

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
0Z - not receiving a zinc supplement
22.5Z - receiving a supplement of 22.5 mg zinc/day

* The Harris Benedict Equation was used to calculate BEE (basal energy expenditure). If a subject's weight (mean of post dialysis weights at the 4 dialyses prior to RBC folate determination) was > 120% high end of IBW range, they were considered obese and mean IBW was used in calculating BEE. Activity level of subjects at the time they participated in the study was determined by their dietitian with BEEs subsequently multiplied by a factor of 1.2 or 1.3 to yield an estimate of 24 hour energy requirement.

Appendix S Energy Intakes of Subjects Receiving Zinc Supplements (Based on 3 Day Food Records)

OF22.5Z Subjects	Sex	24 Hr Energy Intake (Kcal); Intake as % of Estimated Daily Requirement * Dialysis Weekday	Nondialysis Weekday	Nondialysis Sat or Sun	Mean for 3 days
H.L.	M	1321 (84)	1183 (75)	905 (58)	1136 (72)
D.R.	M	1812 (79)	1399 (61)	1406 (62)	1539 (67)
A.K.	F	#	#	#	#
M.Be.	F	1507 (94)	1342 (84)	2091(131)	1647(103)
(means + SD)		1547+248 (86+8)	1308+112 (73+12)	1467+595 (84+41)	1441+269 (81+20)
5F22.5Z Subjects					
J.B.	M	1493 (66)	1171 (52)	2114 (94)	1593 (71)
D.B.	M	3200(132)	3128(129)	2924(120)	3084(127)
T.N.	M	2061(117)	1060 (60)	1831(104)	1651 (94)
J.S.	M	2281(106)	2013 (94)	2574(120)	2289(107)
W.H.	M	2281(179)	1585(125)	1705(134)	1857(146)
W.W.	M	1990 (96)	2028 (98)	1997 (96)	2005 (97)
(means + SD)		2218+561 (116+38)	1831+754 (93+32)	2191+468 (111+16)	2080+553 (107+26)

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
0Z - not receiving a zinc supplement
22.5Z - receiving a supplement of 22.5 mg zinc/day

* The Harris Benedict Equation was used to calculate BEE (basal energy expenditure). If a subject's weight (mean of post dialysis weights at the 4 dialyses prior to RBC folate determination) was > 120% high end of IBW range, they were considered obese and mean IBW was used in calculating BEE. Activity level of subjects at the time they participated in the study was determined by their dietitian with BEEs subsequently multiplied by a factor of 1.2 or 1.3 to yield an estimate of 24 hour energy requirement.

This subject became very ill and was unable to complete a 3 day food record.

Appendix T Average Daily Dietary Zinc Intakes (Based on Food Frequency Questionnaires) and 5 Hour Fasting Serum Zinc Levels of Subjects Receiving No Zinc Supplements

OFOZ Subjects	Average Daily Intake of Zinc from Diet[†] (total mg)	(mg/kg*)	Serum Zinc (ug/dl) NORMAL: 65-140**
B.A.	7.93	0.12	69.1
D.L.	14.14	0.22	60.5
T.L.	7.38	0.13	73.4
M.M.	1.90	0.02	38.9
M.B.	5.62	0.10	72.0
(means + SD)	7.39+4.45	0.12+0.07	62.8+14.3
5FOZ Subjects			
R.C.	6.33	0.07	95.0
K.H.	6.01	0.12	61.9
N.S.	7.45	0.17	70.6
Sh.S.	13.34	0.31	47.5
S.S.	9.36	0.11	53.3
G.S.	12.21	0.19	47.5
(means + SD)	9.12+3.09	0.16+0.08	62.6+18.2

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
OZ - not receiving a zinc supplement

* Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

** Solomons, N., Nutrient chart no. 1, Dialysis and Transplantation 15:518,1986

† The food frequency questionnaire used to determine average daily zinc intake, was found during its validation, to detect 87 percent of dietary zinc consumed. Zinc intakes presented, have been adjusted to indicate 100 percent of dietary zinc consumed.

Appendix U Average Daily Dietary Zinc Intakes (Based on Food Frequency Questionnaires) and 5 Hour Fasting Serum Zinc Levels of Subjects Receiving Zinc Supplements

OF22.5Z Subjects	Average Daily Intake of Zinc from Diet[§] (total mg)	(mg/kg*)	Serum Zinc (ug/dl) NORMAL: 65-140**
H.L.	9.40	0.16	64.8
D.R.	9.23	0.12	86.4
A.K.	#	#	89.3
M.Be.	9.57	0.18	51.8
(means + SD)	9.40+0.17	0.15+0.03	73.1+17.9
5F22.5Z Subjects			
J.B.	6.00	0.08	64.8
D.B.	16.40	0.18	56.2
T.N.	12.54	0.25	54.7
J.S.	11.61	0.18	89.3
W.H.	13.39	0.27	51.8
W.W.	9.99	0.10	67.7
(means + SD)	11.66+3.49	0.18+0.08	64.1+13.8

Treatments - OF - not receiving a folate supplement
5F - receiving a supplement of 5 mg folate/day
22.5Z - receiving a supplement of 22.5 mg zinc/day

* Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

** Solomons, N., Nutrient chart no. 1, Dialysis and Transplantation 15:518,1986

§ The food frequency questionnaire used to determine average daily zinc intake, was found during its validation, to detect 87 percent of dietary zinc consumed. Zinc intakes presented, have been adjusted to indicate 100 percent of dietary zinc consumed.

This subject became very ill and was unable to answer the food frequency questionnaire

APPENDIX V Average Daily Dietary Folate Intakes (Based on Food Frequency Questionnaires) and RBC Folate Levels of Subjects Taking No Folate Supplements

OFOZ Subjects	Average Daily Dietary Intakes of Folate (total ug)	(ug/kg[1])	RBC Folate (ng/mL) NORMAL: 169-707
B.A.	155	2.3	333
D.L.	463	7.2	519
T.L.	345	6.0	332
M.M.	201	2.6	315
M.B.	179	3.2	556
means + SD	269 + 131	4.3 + 2.2	411 + 116
OF22.5Z Subjects			
H.L.	171	2.9	741
D.R.	391	4.9	425
A.K.	*	*	481
M.Be	388	7.3	268
means + SD	317 + 126	5.0 + 2.2	479 + 197

Treatments OF - receiving no folate supplement
5F - receiving 5 mg folate supplement per day
OZ - receiving no zinc supplement
22.5Z - receiving 22.5 mg zinc supplement per day

[1] Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination

* This subject became very ill and was unable to answer the food frequency questionnaire

APPENDIX W Average Daily Dietary Folate Intakes (Based on Food Frequency Questionnaires) and RBC Folate Levels of Subjects Taking 5 mg Folate per Day

5FOZ Subjects	Average Daily Dietary Intakes of Folate (total ug)	(ug/kg[1])	RBC Folate (ng/mL) NORMAL: 169-707
R.C.	120	1.3	4538
K.H.	111	2.3	4379
N.S.	207	4.6	4902
Sh.S.	312	7.2	5961
S.S.	253	3.1	4184
G.S.	312	4.9	3535
means + SD	219 + 90	3.9 + 2.1	4583 + 813
5F22.5Z Subjects			
J.B.	138	1.9	3758
D.B.	320	3.5	3044
T.N.	311	6.2	5518
J.S.	180	2.7	4370
W.H.	226	4.7	6938
W.W.	238	2.3	6853
means + SD	236 + 71	3.6 + 1.6	5080 + 1624

Treatments OF - receiving no folate supplement
5F - receiving 5 mg folate supplement per day
OZ - receiving no zinc supplement
22.5Z - receiving 22.5 mg zinc supplement per day

[1] Weight was taken as the mean of post dialysis weights at the 4 dialyses prior to RBC folate determination