THE EFFECTS OF NUTRITIONAL STATUS ON COGNITIVE FUNCTION

by

L. Valerie Siemens

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ABSTRACT

A pilot study was designed to investigate the association between cognitive and nutritional status in Alzheimer patients. Fifteen Alzheimer subjects, aged 51-78 years and fifteen control subjects, aged 55-81 years volunteered for the study. Cognitive functioning was assessed using the Extended Dementia Scale (EDS), the Mini Mental State Examination (MMSE), and functional status was assessed with the London Psychogeriatric Rating Scale (LPRS). Food intake was recorded for three days by caregivers and control subjects. Nutrient intake and functional blood metabolites of pyridoxine, thiamin, and ascorbic acid were determined for all subjects. Multiple regression analysis was used to show the relationship of dietary and blood parameters to EDS scores. Dietary ascorbic acid, plasma ascorbic acid, and plasma pyridoxal phosphate (PLP) were significant predictors of the EDS scores ($p<0.02$) for the Alzheimer group. Plasma levels of ascorbic acid were a more significant predictor of EDS than PLP levels. A significant increase ($p <0.05$) in mean stimulated transketolase activity was observed in the Alzheimer group, 9.5% compared to 2.9% in the control group. This indicated that there is less thiamin associated with the enzyme in the Alzheimer group. EDS scores correlated with MMSE scores ($r=0.92$). Dietary and blood variables were not predictive of LPRS scores. The results of this study suggest that dietary ascorbic acid, plasma ascorbic acid, plasma pyridoxal phosphate, and thiamin pyrophosphate may be related to the processing and retention of information in elderly subjects who have cognitive deficits.

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CONTENTS

LIST OF TABLES

Chapter I

LITERATURE REVIEW

The elderly constitute a population at risk of developing nutritional deficiencies. Many studies have assessed nutritional intake and blood levels of specific nutrients in this population and found it lacking. In a population of 4277 healthy, elderly persons over the age of 65, 7.2% of women and 5.6% of men exhibited a clinical deficiency of thiamin, and 15.8% of the men were at high risk of developing vitamin C deficiency when serum ascorbate was measured (Health and Welfare, 1973). Thiamin intake was reported to be inadequate in 14.5% of the women, and in 10.7% of the men, whereas vitamin C intake was inadequate in 1.9% of the women and in 4.0% of the men. A more recent study reported biochemical deficiencies of pyridoxine, thiamin, and ascorbate in a population of free-living and institutionalized elderly (Baker et al., 1979).

The clinical effects of these observed inadequate intakes and biochemical deficiencies of thiamin, pyridoxine, and ascorbic acid in the elderly are not known. It has been postulated that the subclinical vitamin deficits noted among the elderly may contribute to cognitive dysfunction (Baker et al., 1979) but little research has been conducted on the relationship of nutritional status to functional memory.

1.1 THE IMPORTANCE OF THE CHOLINERGIC SYSTEM TO MEMORY

The mechanism of age-related memory loss is unknown, but experimental evidence suggests that a disruption in the cholinergic neurotransmitter system (Bartus et al., 1982) is related to memory loss. The neurotransmitter, acetylcholine (ACh), is released at neuronal synaptic junctions during times of nervous excitation. ACh is synthesized from choline and acetyl coenzyme A by choline acetyltransferase(CAT) and is hydrolyzed by acetylcholinesterase(AChE). Anticholinergic drugs have been used extensively to investigate the function of ACh in memory. Much of the information implicating a cholinergic function in memory loss has been reported in pharmacological studies.

The relationship of the cholinergic system of the brain to memory and cognitive functions was investigated in human volunteers using the drugs scopolamine, methscopolamine bromide, and physostigmine (Drachman and Leavitt, 1974). Scopolamine, an anticholinergic agent, crosses the blood-brain-barrier (BBB) and produces amnestic symptoms. Methscopolamine bromide acts peripherally and does not affect the CNS. Physostigmine is an anticholinesterase agent prolonging the action of ACh. The subjects used in this experiment were divided into three groups: 1) drug treated subjects consisted of 20 male and 20 female students (aged 19-25 years), 2) normal controls consisted of 23 male and female students aged 18-26 years, and 3) 24 normal male and female volunteers aged 59-89 years. All drugs were administered subcutaneously in a dose of 1.0 mg. A series of tests were administered to evaluate immediate memory span, memory storage and retrieval and nonmemory cognitive ability.

Subjects receiving scopolamine showed impairment of memory storage, retrieval, and nonmemory cognitive functions. Methscopolamine and physostigmine did not produce any significant changes in memory or other cognitive functions. When compared to normal young subjects, the aged subjects showed significant impairment of memory storage ($p < 0.05$). When memory and cognitive profiles of aged subjects were compared to those of the young scopolamine treated subjects, functions were similarly preserved or impaired in both groups. In the subjects aged 59-89 years, the differences in memory storage were significantly less ($p < 0.05$) when compared to normal young subjects. When the young scopolamine treated group and the elderly group were compared to normal controls, significant impairment of cognitive function was observed ($p < 0.05$) for both groups.

The similarity of the changes in cognitive function observed in the aged subjects and in the younger scopolamine treated subjects imply that the cholinergic system is involved in memory function. Drachman and Leavitt (1974) hypothesized that the cognitive and memory function in aging may reflect some relatively specific disorder of cholinergic neurotransmitter function such as impaired synthesis, release, or receptor uptake of acetylcholine.

The role of acetylcholine in memory function was also studied by Potamianos and Kellett (1982). Thirteen non-psychiatric, non-demented patients aged 75 to 92 years took part in the experiment. A double-blind trial investigated the effects of benzhexol (a centrally acting drug) and a placebo. The tests used to measure memory function included a word list, a short story, a new word learning test, digit span, and

orientation. Subjects were given 2 mg of benzhexol or the placebo and tested one-and-a-half hours after the treatment. Treatments were given on two consecutive days. Paired t-tests showed significant differences between the placebo group and the benzhexol treated groups in three psychological tests: the word list, short story, and new word learning test. Benzhexol significantly decreased performance ($p < 0.01$) in all three tests. The authors stated that the results contributed to the importance of the cholinergic system in the formation and storage of new memories.

Potamianos and Kellett (1982) stated that the tests used to measure memory were not standardized, and suggested that well-standardized tests are needed to assess cognitive function in persons over the age of 70. Such tests would enable cognitive function to be validly assessed in this age group.

More recent research has documented a decrease of ACh in senescent mice (Gibson and et al., 1981). Two strains of male mice at 3 (n=11), 10 (n=11), and 30 (n=11) months of age were injected wth U-^{14}C glucose or $^{2}H_4$-choline. U-^{14}C glucose labelled the acetyl moiety of ACh, whereas the $^{2}H_4$-choline labelled the choline moiety of ACh. The incorporation of either of these precursors into ACh provided a direct method for assessing the dynamics of the cholinergic system (Gibson et al., 1981). The concentration of ACh in whole brains was determined by gas chromatography/mass spectrophotometry, and the incorporation of U-^{14}C glucose was determined by liquid scintillation. The synthesis of ACh from either precursor decreased by 10 months and decreased with increasing age of the mice. In one strain (C57BL), the rate of ACh

synthesis from U^{14}glucose declined 49.3 and 68.6% in the 10 and 30 month animals when compared with the 3 - month old mice. In the other strain (BALB/c), the estimated rate of ACh synthesis declined 42.3 and 65.1% at 10 and 30 months, respectively. The incorporation of $^{2}H_4$choline into ACh declined with senescence as well. In the BALB/c strain, ACh synthesis decreased by 50.4 and 75.9% in the 10 and 30 month old mice compared to the mice at 3 months of age. Depressed ACh synthesis in senescent mice was correlated with behavioral deficits as measured by a string test (r=0.98). The string test is a standardized test which provides an index for experimentally-induced CNS dysfunction and is specific for the detection of behavioral changes associated with thiamin deficiency (Barclay et al. 1981). Scores for 10 and 30 month old mice of both strains were 35-42 and 77-78% lower than those of the 3 month old animals.

This study demonstrated that reduced ACh synthesis occurs in senescent mice and is correlated with the development of behavioral deficits (Gibson et al., 1981). Whether these results can be applied to elderly humans remains to be determined. Pharmacological manipulation of the cholinergic system in aged humans results in memory impairment (Drachman and Leavitt, 1974, Potamianos and Kellett, 1982). Taken together, these findings support the hypothesis that memory is dependent upon cholinergic function. The behavioral changes in senescent mice and the memory impairment which sometimes occurs in humans as a consequence of aging may be attributed to a decrease in ACh metabolism.

Nutrients have been identified as components intrinsic to brain function. Insufficient intakes of thiamin have been associated with reduced

ACh synthesis (Barclay et al., 1981). Ascorbic acid has been shown to affect the release of ACh from synaptic vesicles (Kuo et al., 1979). Inadequate intakes of pyridoxine may indirectly reduce ACh synthesis and/or release by producing changes in the structure of brain cells (Root and Longenecker, 1983). Therefore, vitamin deficiencies of thiamin, pyridoxine,and ascorbic acid due to decreased metabolic function might interfere with cholinergic transmission in the brain which could clinically be manifested as memory loss.

1.2 EFFECT OF THIAMIN ON THE CENTRAL NERVOUS SYSTEM

Thiamin plays an important role in intermediary metabolism and in the maintenance of the nervous system. The active form of thiamin is the coenzyme, thiamin pyrophosphate (TPP), which is involved in carbohydrate oxidation, glycolysis, the TCA cycle, and the hexose monophosphate shunt. Thiamin in the nervous system exists as TPP, thiamin monophosphate (TMP), thiamin triphosphate (TTP), and free thiamin. Although TMP, TTP, and free thiamin have no coenzyme function, they have been reported to have neurophysiological activity (Lipton et al., 1979).

When thiamin intake is inadequate, a peripheral and a central effect has been observed on the nervous system. Peripheral neuropathy (dry beri-beri) occurs as a result of primary thiamin deficiency, but is also associated with decreased intakes of other B-vitamins (Tanphaichitr and Wood, 1984). Experimentally induced CNS lesions associated with thiamin deficiency have been described in rats, pigeons, and monkeys (Prickett, 1934, Alexander, 1940, Rhinehart et al., 1949).

Thiamin deficiency can be induced experimentally with antimetabolites such as oxythiamine and pyrithiamine. The action of antimetabolites on whole animals was reviewed by von Muralt (1962) and performed by Gurtner using ^{35}S-thiamin. Von Muralt conducted experiments using isolated nerve fibers from frogs and demonstrated that antimetabolites of thiamin decreased nervous excitability, whereas thiamin-like substances such as hydroxyethylthiamine restored nervous excitability.

The effects of the antimetabolites oxythiamine (OT) and neopyrithiamine (NPT) were studied by Gurtner. Seventy-five rats were divided into four treatment groups. Group 1 received a thiamin-free diet, group 2 received 250 ug NPT plus 400 ug thiamin injected intraperitoneally (IP), group 3 received 10 mg OT plus 400 ug thiamin injected IP, and controls were maintained on a thiamin-free diet with 40 ug thiamin supplement. Results showed that neurological symptoms of cramps and paralysis occurred in 73 % and 93% respectively, of the animals in the NPT group, as compared to 20% and 46%, respectively, of the animals in the thiamin deficient group. No neurological symptoms were observed in the OT group. When ^{35}S-thiamin was given to rats and frogs, free thiamin and TMP increased in stimulated peripheral nerves. The increase in free thiamin and TMP was attributed to the hydrolysis of TPP. These findings gave conclusive evidence that thiamin has a definite role on the transmission of nervous impulses which is separate from its coenzyme function.

Changes in central nervous system function due to thiamin deficiency are clinically manifested in the Wernicke-Korsakoff syndrome; a condition primarily confined to alcoholics. Wernicke-Korsakoff's syndrome is composed of two entities: Wernicke's disease and Korsakoff's psychosis.

The former is a neurological disorder characterized by ocular abnormalities, ataxia, and global confusion. Korsakoff's psychosis is characterized by memory loss and learning impairment. Since most patients present with both components, the disease is known as the Wernicke-Korsakoff syndrome (Victor et al., 1971).

Victor and associates reported that thiamin administration prevented the progression of Wernicke's disease. They observed 254 hospitalized alcoholics with Wernicke-Korsakoff's syndrome from 1950 to 1961. Undernutrition was defined as gross dietary inadequacy or weight loss of a least 20 lbs in the year preceding the illness, and occurred in 147 cases (84%). The dietary inadequacies were descriptive in nature and were obtained by patient reports. These reports indicated that a balanced meal had not been consumed for a period of several months. Meals were skipped and alcohol had been substituted for food. No biochemical tests were performed to assess nutritional status. Therefore, nutritional deficiency could not be assessed specifically.

It was reported that 2 to 3 mg of thiamin were sufficient to modify the ocular signs, but 50 mg daily administered intravenously and intramuscularly were more beneficial in reversing both the ocular symptoms and ataxia. The reversibility of the ocular abnormalities and the ataxia suggest that there is a biochemical abnormality which has not yet caused significant structural changes in the brain. Victor and co-workers stated that patients with Korsakoff's psychosis who present with ocular and ataxic signs should be promptly treated with thiamin to prevent the development of the irreversible memory changes and learning impairment. Scores on the Wechsler Memory Scale have indicated that patients with Korsakoff's psychosis are severely impaired with respect

to the acquisition and retention of new information (Victor et al.,1959, Butters and Cermak, 1980). The memory loss is resistant to thiamin treatment, and 80% of all Korsakoff patients will show little improvement in memory with vitamin supplementation. The authors stated that amnesia will persist as a chronic, lifelong disorder in the Korsakoff patient.

Because thiamin reversed the opthalmoplegia, ataxia, and nystagmus of Wernicke's disease it was hypothesized that these neurological effects were due to an altered biochemical reaction (Victor et al., 1971). Thiamin supplementation did not consistently reverse the amnesia in Korsakoff patients, and thus a structural lesion was implied. Pathological examination of brains of patients with Korsakoff's psychosis demonstrated diencephalic lesions. The intensity and extent of these lesions may account for the failure of the amnestic symptoms to respond to thiamin (Victor et al., 1971).

It has been hypothesized that a lack of thiamin in the diet of the alcoholic may induce the diencephalic lesions characteristic of Korsakoff's psychosis (Freund, 1973) and may be indirectly responsible for the memory loss. However, alcohol could directly exert a neurotoxic effect and reduce the absorption or metabolism of thiamin to produce amnestic symptoms (Butters and Cermak, 1980).

Long term alcohol consumption has been directly related to reductions in dendritic spines in the hippocampi of mice (Riley and Walker, 1978). The hippocampus has been implicated as the memory repository in humans, thus alcohol may produce memory impairment in humans by reducing dendritic spines as seen in mice. Therefore, a reduced intake of

thiamin or an inability to metabolize thiamin may produce changes in the hippocampus which would be manifested as memory loss. Extensive research in this area is needed to confirm this hypothesis.

A lack of thiamin has been linked to mental changes in psychiatric in-patients (Carney et al., 1979, Carney et al., 1982). In a study by Carney and co-workers (1979), 154 psychiatric patients aged 15-83 years($\bar{x}$=51.3) with a history of inadequate dietary intakes were investigated as part of normal clinical care. Dietary, physical, and psychological histories and examinations were completed on all subjects. Serum pyruvate (SP), and red cell transketolase (TK) were measured and thiamin status was estimated. Decreased activity of transketolase indicated poor thiamin status. Thiamin deficiency was defined as a TK activity coefficient greater than 1.3. The activity coefficient is equivalent to the activity of enzyme after thiamin was added (TPP effect/ units of enzyme activity per gram of hemoglobin).[1] Serum pyruvate levels greater than 79 umol/L were also indicative of thiamin deficiency. Twenty-three patients showed increased TK activity coefficients, 42 had raised SP values, and 58 patients had elevations of both values. When these patients were grouped together as 'low thiamin' group and comparisons made to patients considered to have adequate thiamin nutrition, significant differences ($p < 0.05$) were observed with respect to one or more signs of malnutrition. The symptoms of malnutrition were: weight loss greater than 3.2 kg, angular stomatitis, cheilosis, red raw tongue, and nutritional edema. Significantly more ($p <$

[1] The stimulation of TK activity with the addition of TPP is compared to TK activity without the addition of the coenzyme. The enhancement of TK activity may then be expressed in terms of an activation coefficient or more commonly as a percent, known as %TPP or the TPP effect.

0.01) alcoholics, drug addicts, schizophrenics, and endogenous depressives showed evidence of low thiamin status than did the controls.

A different population of psychiatric patients was studied by Carney et al. (1982), and they confirmed the earlier findings that thiamin is associated with mental changes. Diet histories and erythrocyte transketolase (ETK) activity were determined on 172 psychiatric in-patients. Thiamin deficiency was defined the same way as in the previous study. The nutritional status for pyridoxine (aspartate transaminase or AST) and riboflavin (glutathionine reductase or GR) was also assessed. Normal ranges of activity coefficients for GR were 1.0-1.25 as defined by Williams (1976), and normal ranges for AST were 1.0-1.75 as defined by Carney and associates (1982). Values exceeding the upper limits indicated deficiency. Twelve percent of the patients were deficient in more than one vitamin, and of these, 30% were thiamin deficient. Patients who had a TK activity coefficient > 1.3 were significantly ($p < 0.05$) more likely to have schizophrenia and alcoholism than patients who were considered to have riboflavin and pyridoxine malnutrition.

The data presented by Carney et al.(1979) and Carney et al.(1982) suggests that thiamin deficiency, as defined by TK activity coefficients > 1.3, may have some etiological significance to mental symptoms. These studies did not propose a mechanism of the role of thiamin in metabolism and the contribution to the development of specific mental symptoms. It may be that mental disorders could alter the requirement for thiamin and/or absorption of thiamin, thereby accounting for the increased TK activity coefficients. Whether the thiamin deficiency is a result of the mental illness or is a causative factor in the genesis of the mental

symptoms remains speculative at this time. Suffice to say, these findings indicate that thiamin deficiency is related to abnormal CNS function.

The role of thiamin relative to the confused state in elderly orthopaedic patients was assessed by Older and Dickerson (1982). Thiamin status was estimated by the active form of thiamin, thiamin pyrophosphate(TPP). Values of 0-14% TPP were associated with saturation of tissues with thiamin, values of 15-25% represented a risk with respect to deficiency, and values of > 25% indicated severe thiamin deficiency.

In group I (17 females, 13 males), patients underwent arthroplasty and were in good thiamin status as %TPP values were within the normal limits prior to surgery. A significant rise in the TPP effect was observed 48 hours later but returned to normal by 14 days post-operatively. Five patients who were noticeably confused after the operation showed a considerable increase in %TPP, ranging from 25% to 56%. These values began to decrease two days after surgery, which suggested that there may have been an increased requirement for thiamin in these patients after the operation.

Group II (30 females, and 4 males) underwent surgery for a femoral neck fracture. For 14 days post-operatively, patients were noticeably deficient in thiamin as indicated by %TPP values between 20 and 25%. The authors observed that patients who were confused and sleepy had the higher TPP values. The authors suggested that the patients who had femoral neck fractures may have been thiamin deficient before the surgery, based on the findings of an earlier study (Older and Dickerson,

1980). In that study, voluntary food intake of 19 women (mean age = 78) was measured at 3, 7, and 14 days after surgery. Food was weighed before and after each meal was served. Mean intakes of thiamin for post-operative days 3, 7, and 14 were 571, 603, and 555 ug, compared to the recommended intake of 700 ug.

Older and Dickerson (1982) hypothesized that elderly orthopaedic patients who consume diets which are marginal in thiamin could become deficient due to the increased requirement as a result of increased metabolism following surgery. Decreased thiamin status was observed in patients who were noticeably confused, and this post-operative confusion was attributed to inadequate dietary thiamin.

However, confusion and the dietary intake of the subjects was not quantitated, thus objective studies could assess the role of thiamin upon post-operative confusion more specifically. As well, studies with elderly subjects are needed to assess the requirement of dietary thiamin.

The effects of decreased thaimin intake and status on behavior and ACh metabolism in male Wistar rats was investigated by Barclay et al.(1981). Thiamin deficiency was induced by a thiamin deficient diet in conjunction with the thiamin antagonists oxythiamine (OT) and pyrithiamine (PT). Oxythiamine acts peripherally, whereas PT accumulates in the brain and produces neurological symptoms associated with thiamin deficiency, for example, ataxia. Rats were divided into four groups which consisted of ad-libitum controls, pair-fed controls, oxythiamine-treated, and pyrithiamine-treated animals. Ad libitum fed controls and

pair-fed controls received 0.1 mg/kg of thiamin hydrochloride (HCl) daily by IP injections. Oxythiamine-HCl treated rats received 40 mg/kg IP, and the PT treated group received 0.5 mg/kg IP. Behavior was assessed by a string test which is used to measure behavioral changes related to thiamin deficiency. Thiamin status was determined by measuring TK activity. ACh levels and synthesis were measured by U-^{14}C-glucose and $^{2}H_4$-choline injected via jugular cannulae of polyethylene tubing. After one day of treatment with PT and a thiamin free diet, 37.5% of the rats performed poorly on the string test. By day 12, 89.6% of thiamin deficient (PT) rats, but only 8.1% of the pair-fed controls had decreased string test scores. The OT treated rats did not have decreased string test scores, which implies that there is a CNS effect related to thiamin deficiency (Barclay et al., 1981).

Decreased ACh synthesis was reported in the late stages of PT treatment. The addition of an acetylcholinesterase inhibitor, physostigmine, improved low scores on the string test in 69.2% of the PT treated rats. The authors suggested that the reversal of neurological impairment by physostigmine and the decrease in ACh synthesis implicate a central cholinergic dysfunction in the pathophysiology of thiamin metabolism.

Similar effects of pyrithiamine(PT) and oxythiamine(OT) on ACh levels were reported in the rat brain (Vorhees et al., 1978). Three groups of 20 to 25 day old rats were fed a thiamin deficient diet which contained less than 20 ug/kg/day. This represented a severe thiamin deficiency as the requirement for proper growth and reproduction is 5 mg/kg diet (National Research Council, 1978). The rats were injected subcutaneously with one of the following: 40 mg/kg oxythiamine-HCl plus 0.2 mg/kg

thiamin-HCl/day, 1 mg/kg neopyrithiamin-HBr plus 0.2 mg/kg thiamin/day, or 0.2 mg/kg thiamin-HCl/day. The group of rats receiving only the 0.2 mg/kg thiamin-HCl served as the controls. PT treatment resulted in a significant decrease in ACh levels in all brain regions examined: the medulla-pons, midbrain, diencephalon, corpus striatum, and the hippocampus. OT treatment resulted in decreases in the medulla-pons and striatum. ACh utilization was significantly reduced in the midbrain, striatum, and hippocampus in the PT group, while OT reduced ACh utilization only in the striatum. PT produced the neurological symptoms associated with thiamin deficiency: ataxia, incoordination, and opisthotonus. The combined PT treatment and dietary deficiency of thiamin produced neurological symptoms which were not observed in the OT treated and control groups also fed the thiamin deficient diet (Vorhees et al., 1978). These results suggest that neuropathy induced by thiamin avitaminosis due to PT involves the cholinergic system.

Evidence to support the hypothesis that thiamin is linked to cholinergic activity has been recently published by Micheau et al.(1985). This study investigated the effects of a thiamin derivative, sulbutiamine, on memory and hippocampal cholinergic acitivity in mice. Fifty-four male mice of the BALB/c strain, aged 14-16 weeks were assigned to three groups: 18 mice were given 300 mg/kg of sulbutiamine in 0.2 mL of a gum arabic emulsion (15% w/v); 17 mice received only the 0.2 mL of the gum arabic emulsion; and 19 mice received no treatment. The last two groups represented the controls. The sulbutiamine and the gum arabic groups received the treatments orally by intragastric intubation for a 10 day period.

Behavior was assessed using a Skinner box. When the mice pressed the lever, they were rewarded with a food pellet. All lever presses were registered on a pen recorder. The criteria of learning was defined as the speed of acquisition of the lever-press response in a single session of 30 minutes. The criteria of memory consisted of a 15 minute acquisition session followed by a retention session which occurred 30 minutes and 24 hours later.

Neurochemical analysis was performed on six animals in each group. Sodium-dependent high affinity choline uptake (SDHACU) was measured in aliquots of resuspended crude synaptosomal pellets of hippocampi.

Results showed that memory significantly improved ($p < 0.01$) in the sulbutiamine treated group as compared to the untreated group. The authors concluded that sulbutiamine improved long term memory of a partially learned response. Results from the neurochemical analysis showed a significant increase ($p < 0.025$) in choline uptake over a four minute period in the dorsal hippocampi of the sulbutiamine treated mice as compared to the two control groups. Thus, sulbutiamine enhanced both memory and choline uptake.

The authors postulated that the increase in choline uptake may have been related to thiamin metabolism, in that thiamin is necessary for the synthesis of the acetyl moiety for ACh formation. Mention has been made of studies demonstrating a reduction in ACh synthesis in animals where metabolism was inhibited by thiamin deficiency (Barclay et al.,1981, and Vorhees et al.,1978). An alternative hypothesis was that the observed increase in choline uptake by hippocampal neurons was the result of an

increase in the synthesis and the release of ACh by septohippocampal neurons (Micheau et al.,1985).

However, these results should be interpreted with caution. Long term memory was defined as the retention of a task over a 24-hour period. A better measure of long term memory retention would be to test the mice after a longer interval, for example, 72 hours or one week. Furthermore, the authors state that 300 mg/kg of sulbutiamine is a large dosage, and no mention is made of a normal dosage for mice, or if there were any adverse side effects. Thus, another approach to test the facilitaiton of sulbutiamine on memory would be to administer varying amounts of thiamin or its derivatives.

Despite these problems, the results of this investigation give conclusive evidence which links thiamin metabolism to cholinergic activity. However, research in animals and in humans needs to be designed to determine a causal link between thiamin metabolism, cholinergic activity, and memory.

The data presented from rat studies implicate a cholinergic impairment associated with thiamin malnutrition. It is possible that the mental changes associated with the Wernicke-Korsakoff syndrome, in psychiatric patients, and in the post-operative confusion in the elderly may be pathological expressions of impaired cholinergic activity due to thiamin malnutrition.

1.3 EFFECTS OF PYRIDOXINE ON THE NERVOUS SYSTEM

The term, vitamin B_6, includes a number of forms of the vitamin: pyridoxine, pyridoxal, and pyridoxamine (Dakshinamurti, 1982). The major form of vitamin B_6 occurring in the tissues is the coenzyme pyridoxal 5'-phosphate (PLP). The coenzyme is a cofactor in metabolism, in particular amino acid metabolism. Pyridoxine has a number of effects on nervous system function in several species. Ataxia, altered mobility and alertness, abnormal head movements and convlusions have been reported in the chicken, duck, turkey, rat, guinea pig, pig, cow, and human when pyridoxine malnutrition was identified (Dakshinamurti, 1982).

Hyperirritability and convulsive seizures in four young infants aged 5 weeks to 4 months were reported to be due to a deficiency of pyridoxine in a commercial liquid formula (Coursin, 1954). Vitamin assays showed that the formula contained less than 60 mg of pyridoxine per litre. The recommended intake of pyridoxine for infants is 0.015 mg/g protein per day which represents a requirement of 0.36 mg of pyridoxine per day (Health and Welfare, 1983). Thus, the amount of pyridoxine reported in the formula by Coursin is below the requirement. Sleep-induced electroencephalographic measurements assessed the status of the central nervous system, and showed abnormal electroencephalograms for all subjects. When the infants were given an evaporated milk formula the symptoms disappeared, presumably due to the presence of sufficient amounts of pyridoxine in the evaporated milk. However, the amount of pyridoxine in the evaporated milk formula was not quantitated in this investigation. When a pharmacological dose of 100 mg of pyridoxine was given intramuscularly to one infant, the EEG became normal

five minutes after injection. Coursin stated that this was one of the first actual recordings of a neurological response to pyridoxine.

Pyridoxine appears to be a factor in depression. Depression associated with the use of oral contraceptives has been linked to pyridoxine deficiency (Baumblatt and Winston, 1970, Adams et al., 1973). Baumblatt and Winston (1970) investigated the response of women to pyridoxine supplements whose symptoms of depression were thought to be related to oral contraceptive medication. Symptoms were emotional lability and irritability, depression, fatique, mild paranoid ideation, difficulty with concentration, and sleep disturbance. From a total of 58 women, 44 (75.8%) had complete resolution of symptoms after receiving a therapeutic dose of 50 mg of pyridoxine during three menstrual cycles. The requirement of pyridoxine for adult women is 1.1 mg/day (Health and Welfare, 1983).

The results of this study may be confounded by several variables. Firstly, all women received pyridoxine and therefore a possible placebo effect could not be ruled out in the resolution of depressive symptoms. Secondly, pyridoxine status in terms of intake or blood levels was not measured, thus it is difficult to ascertain whether the observed effects of pyridoxine on depression were due to a functional deficiency of the vitamin or to an increase in requirement. Lastly, the depressive symptoms were volunteered by the women and gave a qualitative, rather than a quantitative description of depression.

The effect of pyridoxine supplementation on depression in oral contraceptive users was observed in a study by Adams et al.(1973).

Women who were using contraceptives and attending a clinic were screened for depression using the Beck Scale, which is a valid self-rating questionnaire. A score of greater than 23 indicated moderate to severe depression. Women who were judged to be depressed by factors other than depression were eliminated from the study. Depressive symptoms included pessimism, irritability, dissatisfaction, lethargy, and loss of libido. Twenty-two women whose symptoms were attributed to OC use took part in the study, and 11 of these subjects showed biochemical evidence of pyridoxine deficiency. Pyridoxine deficiency was defined by the presence of two or more of the following: a urinary HK/HA ratio > 2.25 (HK=3-hydroxykynurenine, HA=3 hydroxyanthranilic acid), urinary 4-pyridoxic (4-P) acid > 2.5 g/24 hours, erythrocyte alanine transferase (Al-AT) stimulation > 40%, and aspartate aminotransferase (Asp-AT) stimulation > 90%. With OC use, the tryptophan metabolites, HA and HK, increase in the urine. Several enaymatic reactions in the tryptophan pathway require PLP as a coenzyme. Low urinary excretion of 4-pyridoxic acid is a measure of pyridoxine deficiency as this compound is a major excretory product of pyridoxine. The activity of the Al-AT and Asp-AT enzymes decrease due to unavailable pyridoxine. In a double-blind crossover trial, a pharmacological dose of 40 mg of pyridoxine was administered daily to the 22 subjects for a 2 month period. Of these, 11 subjects who showed biochemical evidence of pyridoxine deficiency responded to pyridoxine supplementation. The authors observed a significant improvement in depression ($p < 0.01$) in these subjects as measured by a decrease of 5 points or more in the Beck scores.

Wynn et al. (1975) confirmed these results in a double-blind cross-over trial of 39 women who received oral contraceptives and were diagnosed as depressed. Nineteen women showed biochemical evidence of pyridoxine deficiency as defined by Adams et al.(1973). A significant improvement in Beck scores ($p < 0.05$) was observed after the administration of pyridoxine.

Further evidence that depression may be associated with pyridoxine deficiency has been supplied by the work of Carney et al.(1979). Thiamine and pyridoxine status was examined in 154 newly admitted psychiatric patients. Complete dietary, physical, and psychiatric histories were obtained for all subjects. Pyridoxine deficiency was determined by measuring the activity of erythrocyte aspartate transaminase (AST). An AST activity coefficient > 1.75 represented increased stimulation of the enzyme and indicated depleted pyridoxine stores. Sixteen patients had increased AST values and of these, 9 had symptoms of depression. Elevated AST was found in significantly more depressives than in control patients ($p < 0.001$). The authors concluded that endogenous depression may be linked to pyridoxine avitaminosis.

More recently, Carney et al.(1982) confirmed previous results and suggested that affective changes are characteristic of pyridoxine deficiency. As in the earlier study, psychiatric and dietary histories and physical examinations were obtained for 172 patients. Clinical pyridoxine deficiency was based on observed classical symptoms related to changes in the skin, hair, finger nails, and tongue, and the presence of cheilosis, angular stomatitis, edema, trophic ulcers, and peripheral neuropathy. Ten percent of the patients were reported to have pyridoxine deficiency, but it is unclear whether this diagnosis was solely

due to elevated AST activity, or to a dietary deficiency of pyridoxine or to the presence of clinical symptoms. The authors stated that patients who showed biochemical and clinical evidence of pyridoxine deficiency were more likely ($p < 0.05$) to have depression than those patients who were diagnosed with thiamin deficiency. Since patients with affective disorders exhibited both riboflavin deficiency (glutatione reductase activity coefficient > 1.25, Williams, 1976) and pyridoxine deficiency (AST activity coefficient > 1.75, Carney et al., 1979), one cannot conclusively state that these disorders were due to pyridoxine deficiency per se. Carney et al.(1982) concluded that both pyridoxine and riboflavin deficiencies may have a primary role in the etiology of affective disorders, which are assumed to be synonymous with depression.

Even though these findings appear to support the association of pyridoxine nutritional status and depression, it remains to be shown whether a causal relationship exists between a vitamin deficit and the observed depression (Stewart et al., 1984). In addition, affective disorders may not be related to a deficiency of one vitamin as demonstrated by Carney et al.(1982), but probably encompasses a range of vitamin deficits. Irrespective of these inherent problems, the research findings reported do provide evidence that pyridoxine is associated with depresssion. Pyridoxine in the active form, PLP, is a cofactor in the catabolism of amino acids in the nervous system. Pyridoxine-dependent enzymes are necessary for the synthesis of neurotransmitters such as serotonin, dopamine, and norepinephrine (Dakshinamurti, 1982). Serotonin and dopamine have a role in the maintenance of normal mood (Thomson, 1978).

It has been postulated that a deficiency of PLP may result in inadequate synthesis of these monoamines, thereby producing depression (Stewart et al., 1984).

Evidence linking pyridoxine deficiency to learning in rats was provided by Sloane and Chow (1964). These investigators studied the effects of pyridoxine deficiency on the initial acquisition of behavior. The pyridoxine deficient group was fed a diet ad libitum which did not contain pyridoxine. The control diet included 30 mg/kg pyridoxine-HCl. The controls were divided into two groups: one fed, ad libitum, the basal diet plus pyridoxine, the other was pair-fed in amounts sufficient to maintain the animals at the same weight as the rats fed the pyridoxine free diet. Total urinary xanthurenic acid estimated relative pyridoxine deficiency. Deficient animals had mean values of 5.2 ±1.1 mg xanthurenic acid, whereas controls had mean values of 1.1 ±0.4 mg. Ataxia and acrodynia were observed in the deficient animals, but none of these animals experienced convulsions.

Acquired behavior was assessed in both groups of 5 littermate pairs by avoidance and escape responses to electric shock by pressing a lever (Sloane and Chow, 1964). Control animals performed significantly better ($p < 0.05$) than the deficient animals both for the avoidance and escape measures.

In a second experiment, 7 pairs of control and pyridoxine deficient rats were evaluated with respect to the time taken to learn to press a lever to obtain water after water deprivation. In 5 of the pairs, the pyridoxine deficient animals learned significantly more slowly ($p < 0.05$) than the control animals.

Evidence linking pyridoxine deficiency to impairment of mental function was provided by Gantt et al.(1959). The effect of pyridoxine deficiency on conditioned reflexes was examined in dogs and rats. Four adult dogs were maintained on a stock diet which did not contain all vitamin requirements. Pyridoxine deficiency was induced by omitting pyridoxine from the diet. Retention of conditional reflex was tested in pyridoxine deficient dogs and consisted of differentiation of two tones, each one octave apart. Impaired differentiation in dogs occurred 4-15 days after vitamin depletion; but this loss was never complete and returned to normal after the dogs were returned to an adequate diet (Gantt et al., 1959). Rats were divided into three groups of 5-10 animals: group I received a diet from natural food sources, group II received a synthetic diet which was nutritionally adequate, and group III received the same diet as group II, but lacked pyridoxine. Formation of conditional reflex was tested and consisted of maze running to obtain food. Gross behavioral alterations and marked physiological changes were observed in pyridoxine deficient rats. Maze running time and errors in the pyridoxine deficient animals was 7 minutes and 33 seconds with 9.8 errors compared to control values of one minute 38 seconds with 3.8 errors.

The authors reported that rats showed much greater disturbances in general behavior and conditional reflex (CR) disturbances than did the dogs, possibly due to the fact that the rats were young when the pyridoxine deficiency was initiated. Furthermore, the relative weight loss for the rats was greater than for the dogs.

The authors concluded that pyridoxine deficiency was related to a loss of reflex function. There was a decrease in retention of CR in dogs, and decreased CR in rats. Also, Gantt and co-workers equated the loss of CR function in dogs with inadequate pyridoxine intake to the loss of mental function in man. Results of an earlier study (Gantt and Muncie, 1942) demonstrated a loss of CR in three alcoholic patients with Korsakoff's psychosis. Thus, pyridoxine appears to be necessary for normal conditional reflex function which is the basis of mental performance (Gantt et al., 1959). A mechanism by which pyridoxine exerted this effect was not proposed. Perhaps a stronger association of pyridoxine to mental function could have been demonstrated in the animal studies conducted by Gantt and co-workers if more animals had been studied, if pair-fed controls had been used, if the sample size had been equal for both species, and if reliable biochemical indices of pyridoxine status had been determined and correlated to CR impairment.

The association of pyridoxine deficiency in rats with histological changes in the brain was documented by Morré and Kirksey (1980). Maternal pyridoxine deprivation was linked to neurological changes in the progeny during CNS development. Weanling rats were fed 1.4 or 20.0 mg pyridoxine-HCL/kg during growth, gestation, and lactation. Pups were maintained on the same maternal diets. In 15-day old pups who were fed 1.4 mg pyridoxine, Purkinje cell degeneration in the cerebellum was greater when compared to the pups fed 20.0 mg. At fifty days of age, the group fed 20.0 mg of pyridoxine in the diet had greater dendritic thickness and complexity of basilar and apical dendrites. The authors postulated that since pyridoxine is involved in protein synthesis, a

deficiency of the vitamin may prevent the synthesis of enzymes and structural proteins essential for dendritic growth.

More recently, a functional role of pyridoxine in the maintenance of cell structure in the brain has been demonstrated (Root and Longenecker, 1983). Weanling male albino rats were fed diets which did not contain pyridoxine. Pyridoxine deficiency caused axonal swellings in the hippocampus and a decrease in dendritic arborization. Pyramidal cells from the cerebral cortex of rats fed pyridoxine deficient diets showed no basal dendrites when compared to controls who consumed 2.5 mg/kg pyridoxine-HCl. Root and Longenecker (1983) stated that the rat brain cells in rats fed pyridoxine free diets are similar in appearance to abnormal cells found in the brains of Alzheimer patients as reported by Scheibel (1978). These authors further stated that the changes seen in the brains of pyridoxine deficient rats suggest that nutritional deficiency is capable of causing marked accleration of the type of cellular deterioration seen in human aging. The authors postulated that a deficit of a combination of nutrients over a lifetime could cause sufficient damage to neuronal processes to account for functional impairment associated with aging, and acceleration of this type could cause memory loss.

1.4 EFFECT OF ASCORBIC ACID ON THE NERVOUS SYSTEM

Ascorbic acid (AA) deficiency has been shown to be associated with specific psychiatric symptoms, particularly depression and irritability. In addition, animal studies have reported that acetylcholine (ACh) release may be improved in AA containing media. Ascorbic acid has been

shown to influence the release of ACh from isolated synaptic vesicles in 3 animal species studied by Kuo et al. (1978). Addition of 10 uM of AA in the presence of ATP, Mg^{++}, and Ca^{++} at 20°C resulted in a progressive increase in the release of ACh from the synaptic vesicles over a 20 minute interval. Addition of 2.5 uM of AA resulted in the release of 50-60% of ACh in synaptic vesicles from the rat brain on incubation for 20 minutes in the presence of ATP, Mg^{++}, and Ca^{++}. The maximum release was observed with 5 uM of AA.

Although the brain has the highest concentration of ascorbic acid of all tissues except for the adrenals and the pituitary, the function of ascorbic acid in the human brain is not known. Kuo and associates also reported that norepinephrine release from rat synaptic vesicles was enhanced by AA. The research by Kuo et al.(1978) points to a physiological function of ascorbic acid in the release of norepinephrine and in the release of ACh, which is essential to memory function.

The effects of ascorbic acid supplementation on 40 chronic psychiatric male patients was investigated by Milner (1963). In a double blind study, 20 men received a placebo preparation and 20 received 1g AA per day for 3 weeks. Urine specimens were collected for 24-hour periods during the experiment, and urinary excretion of AA was quantitated. The number of 24-hour periods for urine collection was not specified. The mean urinary excretion of AA in the placebo group was 15.2 mg per 24 hours. Milner stated that this value represented the lowest limit of normal as defined by a minimum daily excretion of 20 mg. Estimated normal excretion rates are 45-50 mg/day (Health and Welfare, 1983). Mean urinary excretion values for the treatment group were not reported.

Psychological tests included a self-rating scale, the Minnesota Multiphasic Personality Inventory or MMPI, and the Wittenborn Psychiatric Rating Scale. Significant ($p < 0.01$) improvement in scores related to depression using both scales were reported in the group receiving the AA supplement. Milner (1963) suggested that AA tissue saturation resulted in an overall improvement in personality functioning. An estimate of tissue saturation was derived by plotting urinary excretion in mg/24 hr against the number of days supplemented. When the body pool reached a maximal level, urinary excretion increased. The mean time for saturation to be completed was 6 days, which indicated a deficiency state of AA. This is in contrast to normal subjects who can be saturated within 24-48 hours with 1 g of AA/day (Milner, 1963). The psychological and physical improvement observed in these patients after saturation with AA indicated that their diets had been inadequate in the vitamin, which contributed to a deficiency state. However, no quantitation of dietary AA was obtained prior to the study, nor was it assessed during the study.

Behavioral effects of ascorbic acid deficiency were described by Kinsman and Hood (1971) in five healthy male prisoners. The subjects were assessed by a series of behavioral tests during vitamin depletion and repletion. The subjects were deprived of AA for periods of 84-97 days following a control period where they were fed a solid soy protein based diet which contained 2.5 mg AA and was supplemented with 75 mg of AA (Hodges et al., 1971). The depletion phase consisted of a liquid diet which did not include any AA. Energy intake was adequate. The men received a mineral supplement as well, but the authors noted that this

supplement was inadvertently omitted from the diets during the depletion period. Biochemical tests of ascorbic acid status included daily urinary excretion, and weekly plasma and whole blood determinations. L-ascorbate 1-^{14}C in a dose of 0.86 mg was given orally on day 23, and was measured in urine, feces, and breath. Estimations of body pool size of AA were derived by dividing the spectrophotometric activity of urinary AA by the total radioactivity remaining in the body (Baker et al.,1971). Body pool size and whole blood levels were separated using the median to identify high and low ascorbate subjects. Scores for behavioral measures were separated according to the high and low levels of body pool size and blood AA. Body pool sizes were 1314 mg and 161 mg, and whole blood levels were 1.65 mg/100 mL and 0.44 mg/100 mL.

Functional assessment involved a physical fitness test. Impaired performance on this test was associated with lower levels of AA, and was due to scorbutic arthropathy or neuropathy or both (Kinsman and Hood, 1971). The Digit Symbol Substitution Test was the only mental function task out of a total of 9 areas of mental function to show a significant decrease ($p < 0.05$) with smaller body pool sizes. For tests of psychomotor function, measures of hand-arm dexterity also decreased with smaller body pool sizes. Four scales of the MMPI (Hypochondriasis, Hysteria, Depression, and Social Introversion scales) showed significant ($p < 0.05$) increases from high to low body pools of AA. Scores for three MMPI scales (Hypochondriasis, Depression, and Hysteria) were plotted against the log body pool of AA. As the AA level increased, scores for each of the MMPI scales increased. It should be noted that the scores for the MMPI are influenced by somatic complaints and may reflect poor general health.

Personality changes occurred when whole blood AA decreased from 1.21-1.17 mg/100mL ,or decreased from 761 mg to 561 mg for body pool ascorbate. Performance changes occurred when whole blood AA decreased from 0.67-0.14 mg/100 mL and when body pool AA decreased from 190 to 63 mg. The impairment in psychomotor performance occurred at lower levels of AA than the observed personality changes. This was attributed to a reduced arousal level. The personality changes associated with AA deficiency corresponded to the clinical neurotic triad of the MMPI (Hypochondriasis, Depression, and Hysteria) (Kinsman and Hood, 1971). These authors stated that the elevation of the neurotic triad, together with the Social Introversion Scale indicated a depressed and withdrawn individual concerned about his bodily state. The authors concluded that the behavioral changes observed in their study were dependant upon changes in AA levels in blood and whole body pools.

It should be noted that Kinsman and Hood observed behavioral changes in five men who had been totally deprived of ascorbic acid until deficiency symptoms appeared. These results cannot therefore be generalized to the population as a whole, since a total lack of dietary ascorbic acid in the diet is not a common nutritional problem. Furthermore, it is possible that the omission of mineral supplementation during the first 34 days of the 84-97 day depletion period (Hodges et al.,1971) created an additional variable in the study.

The findings of Milner (1963) and Kinsman and Hood (1971) indicated that severe ascorbic acid restriction (Kinsman and Hood, 1971) or a borderline scurvy condition (Milner, 1963) is associated with behavioral alterations. These psychological changes suggest that a deficit of ascorbic acid has an effect on the central nervous system.

1.5 ROLE OF VITAMINS IN FUNCTIONAL CAPACITY OF ALZHEIMER'S DISEASE

Alzheimer's Disease is classified as an idiopathic dementia, ie. a dementia of unknown cause. Since it appears that vitamins are associated with CNS function, it is of interest to apply this information to a disease state in which memory loss is identified. It has been postulated that some cases of idiopathic dementia are due to undernourishment of the brain with respect to water-soluble vitamins (Spector et al., 1979); notably thiamin, pyridoxine, and ascorbic acid. These vitamins are transported from the blood into the CSF via the choroid plexus, which forms part of the blood-brain-barrier. When the choroid plexus malfunctions, transport of water-soluble vitamins from the blood into the CNS is impaired. Consequently, the brain becomes depleted of these nutrients, resulting in dysfunction or degeneration of neurons (Spector et al., 1979). Spector and associates speculate that all of the pathological alterations observed in AD brains could be due to inadequate central nutrition.

This hypothesis is substantiated by some of the literature that has been already cited. Thiamin deficiency had definite CNS effects, and may adversely affect ACh metabolism. Pyridoxine deficiency in rats produces histological changes in the brain which are similar to the dendritic alterations seen in AD. Ascorbic acid may have a physiological role in the release of ACh, which in turn appears to function in memory. Furthermore, ascorbic acid enhances the release of norepinephrine (Kuo et al., 1979), and functions as a reducing agent for the enzyme ß-monooxygenase which catalyzes the hydroxylation of dopamine to norepinephrine. Thus, ascorbic acid is related to the synthesis and stability of ACh and norepinephrine in the brain. Studies have shown

conclusively that there is a loss of cholinergic neurons in Alzheimer's Disease which accounts for the observed memory loss. There is not similar data to support the hypothesis that deficits in the noradrenergic system are involved in the pathology of AD (Davies, 1983). Therefore, this review has focused on the role of the cholinergic system in memory function. Deficiencies of thiamin, pyridoxine, and ascorbic acid singly or combined, could be responsible for the impaired cognitive performance, specifically memory loss, in AD.

1.6 ALZHEIMER'S DISEASE AS A MODEL OF MEMORY LOSS

The two regions of the brain which are important for memory are the amygdala and the hippocampus. Cellular abnormalities occur in these regions of the brain as well as in the cerebral cortex, and are diagnostic of AD. Neuropathological examination of the brains from Alzheimer patients show two distinct lesions: neurofibrillary tangles which accumulate within the cell bodies of neurons, and senile or neuritic plaques which consist of abnormal neurites (primarily axon terminals)(Coyle et al., 1983). Since senile plaques and neurofibrillary tangles occur with high frequency in the hippocampal region of Alzheimer brains, it is thought that the memory loss in AD is due to the presence of these structures caused by brain cell loss (Coyle et al., 1983).

In addition to the senile plaques and neurofibrillary tangles observed in the hippocampal regions of Alzheimer brains, dendritic changes have also been noted. Pyridoxine deficiency in the rat has been shown to produce histological changes in the brain which have some simi-

larity to the histological changes observed in the brains of Alzheimer patients (Root and Longenecker, 1983). These changes include loss of dendritic spines and irregular swelling of cell bodies (Scheibel and Scheibel, 1981). Scheibel and Scheibel stated that the hippocampus is particularly vulnerable to these pathological alterations, which result in the eventual disappearance of the entire cell dendrite complex. Reduction in the number of dendrites and extent of dendritic arborization in AD has also been reported by Mehraein et al.(1975) and by Buell and Coleman (1979). Scheibel and Scheibel (1981) postulated that the progressive loss of ensembles of hippocampal pyramids and the resulting loss of enormous numbers of synaptic connections may be responsible for the deficits in geographic and temporal orientation seen in AD.

Research has shown that cholinergic terminals in the hippocampus are important for memory function, and some of the cognitive deficits of Alzheimer's Disease (AD) may be a direct result of a decrease in the ACh-mediated transmission of impulses (Wurtman, 1985).

The above hypothesis is substantiated by research carried out by Davies (1978), who measured the activity of two enzymes concerned with the metabolism of ACh, the biosynthetic enzyme choline acetylase (choline acetyltransferase or CAT) and the degradative enzyme acetylcholinesterase (AChE). The most significant decrease ($p < 0.005$) in CAT was observed in the hippocampal regions of 21 AD brains, with a mean reduction of 91.2%. AChE was also significantly reduced to a mean of 78.4% in the hippocampus ($p < 0.005$). Significant reductions in CAT and AChE were also observed in the cerebral cortex of AD patients compared to controls. The results indicated that extensive loss of two enzymes associated with cholinergic neurons occurrred in AD (Davies, 1978).

Based on these results, Davies proposed that extensive degeneration of cholinergic neurons occurs in AD.

Support for the cholinergic hypothesis in AD has been given by Whitehouse et al.(1982). These authors observed that the neurons of the nucleus basalis of Meynert (nbM); the major source of extrinsic cholinergic innervation to the cerebral cortex, decreased by as much as 80% in AD brains. The decrease in the number of neurons in the nbM of these patients is congruent with the reduction in the concentration of specific cholinergic presynaptic markers (Whitehouse et al., 1982) as previously described in AD by Davies (1979). More recently, Wurtman (1985) has stated that the observed levels in CAT activity reflects the loss of choline or ACh releasing terminals in the hippocampus and cerebral cortex of Alzheimer patients.

1.7 EFFECTS OF NUTRITION ON COGNITIVE FUNCTION

Recently, interest in the role of nutrients and memory function in the elderly has been stimulated by Goodwin et al.(1983). This study related cognitive function with intake and blood values of vitamins. Healthy, non-institutionalized men and women older than 60 years took part in this experiment (n=260). Nutritional status was evaluated by three - day food records and by chemical determination of blood levels of specific nutrients. Cognitive status was measured by a non-verbal test of abstract thinking (Halstead-Reitan Categories Test or HRCT) and by a memory test (Russell revision of the Wechsler Memory Test or WMT). Coefficients of correlation between cognitive function and total intake of specific nutrients showed no significant associations. When coefficients of correlation between blood levels of specific nutrients and the

results of cognitive testing were compared, blood levels of riboflavin and ascorbic acid correlated positively with verbal memory, r=.14 and .15, respectively ($p < 0.05$). Goodwin and associates found that the results of the correlations between nutritional intake or blood levels and results of cognitive testing were inconclusive. The relationship of mean scores on the HRCT for the bottom 5%, bottom 10%, and top 90% with respect to total intake was determined. There was a trend for those subjects in the bottom 5% or 10% of intake in all nutrients to do poorly on the test when compared to the rest of the population, but was only significant ($p < 0.05$) for ascorbic acid and folic acid. Analogous data for the WMT were compiled, and showed that those subjects in the bottom 5% or 10% of intake did poorly compared to the remaining 90% of the population. The mean scores for the HRCT for subjects in the bottom 5%, bottom 10%, and the top 90% were compared to blood levels of specific nutrients. Subjects with low blood levels of ascorbic acid, riboflavin, B_{12}, and folic acid scored lower on the test when compared to the remaining 90% of the population. Analagous data was reported for the WMT. Statistically significant deficiencies in performance were observed in subjects with low blood levels of ascorbic acid and B_{12}. Normal ranges of blood levels for these vitamins were not defined.

Goodwin et al.(1983) concluded that there is an association between poor performance on cognitive tests and low intakes and serum levels of riboflavin, folate, vitamin B_{12}, and ascorbic acid. Such an association suggests that there may be a contribution of nutritional status to cognitive function in a healthy, elderly population; thereby giving credence to the hypothesis that nutrition does play a role in memory. Alternatively, the authors stated that poor cognitive status may be

responsible for the development of poor nutritional status, as subjects with impaired cognition might be less capable of maintaining adequate nutrition. As the subjects in this study had normal cognitive function, the effect of memory on nutritional status could not be determined.

Goodwin and coworkers failed to demonstrate conclusively a relationship between nutrient intake or blood level and cognitive status; indicating that further research is required to definitively link cognitive function with nutritional status.

Therefore, a pilot study was undertaken in our laboratory to investigate the effect of nutritional status on memory using a group of subjects who had a diagnosed memory deficit, Alzheimer's Disease. The nutritional status and memory function were also assessed in a control group of age-matched subjects. Since it appears that ascorbic acid, pyridoxine, and thiamin are involved in specific biochemical reactions which are related to memory, it was decided to attempt to relate the dietary and functional forms of the vitamins to memory performance.

Chapter II

MATERIALS AND METHODS

2.1 SUBJECTS

Fifteen subjects diagnosed as having Alzheimer's Disease (AD) were screened by Dr. M.O. Agbayewa, Department of Psychiatry, University of Manitoba, using the DSM III criteria for Primary Degenerative Dementia (American Psychiatric Association, 1980). The DSM III criteria are shown in Appendix A. Fifteen subjects who did not have a diagnosis of Alzheimer dementia were age-matched to the fifteen individuals with Alzheimer's Disease and served as control (C) subjects. Both the Alzheimer and control groups were selected from volunteers who expressed interest in participating in this study. The study was publicized in the media and gerontologists in the city of Winnipeg were asked to refer patients. All subjects, except one, lived in their own homes. A family member volunteered this subject in response to a media request for subjects and it was not appropriate to refuse his participation. Table 1 depicts the characteristics of the sample population.

Individuals who met the DSM III criteria for Alzheimer's Disease were screened for the following:

1) Physical illness or psychiatric illness which could impair food intake by patients. Thus, the subjects had to consume at least two meals per day. It was recognized that dementia may affect the nutrient

TABLE 1

Description of Subjects

	Females	Males	Age Range
AD	7	8	51-78
C	12	3	55-81

intake of some Alzheimer patients; consequently, any subjects with diagnosed nutritional disorders were excluded from the study. 2) Must be at least 50 years of age, 3) No diagnosis of multi-infarct dementia or alcoholic dementia according to the DSM III criteria, 4) No use of medications known to influence absorption, excretion, or metabolism of pyridoxine, thiamin, and ascorbic acid.

All participants gave the researchers permission to contact their personal physicians. All subjects were required to give informed consent before inclusion in the study. When individuals were unable to give informed consent by reason of cognitive incapacity, the next-of-kin was asked to give consent. Consent forms used in this study are shown in Appendix B.

2.2 COGNITIVE TESTING

Three measures of cognitive functioning were employed in this study. Where possible, the scales were administered to all subjects in their homes. The Extended Dementia Scale (EDS) was developed by Hersch (1979) from the Mattis Scale (Coblentz et al., 1973), and was used to discrimi-

nate between the demented and non-demented individuals in this project. The EDS consists of 23 test items serially measuring changes in cognitive function. Criteria used to measure cognitive function include orientation, arithmetic skills, intellectual assessment, and memory impairment. After administration of the EDS, subjects were assigned scores to a maximum of 250 points.

The Mini-Mental State Examination (MMSE) was used in conjunction with the EDS to detect cognitive impairment (Anthony et al., 1982). Several tasks comprise the MMSE and the maximum score is 30 points. Cognitive functions that are measured in this test include orientation to time and place, recall, short-term memory, ability to perform serial subtraction or reverse spelling, constructional abilities, and use of language.

The London Psychogeriatric Rating Scale (LPRS) is a scale used to provide global assessment of an individual's level of functioning, including mental status (Hersch et al., 1978). The LPRS consists of a 36-item questionnaire where the answers are scored 0, 1, or 2. A higher score reflects a greater degree of impairment in an individual's level of functioning (Hersch et al., 1978). The LPRS was completed by the care-givers of the Alzheimer subjects. The control group did not receive the LPRS.

2.3 DIET RECORD

The control group and the care-givers of the Alzheimer subjects were instructed to record food intakes in household measures for a consecutive three-day period. Three-day food records were chosen because they give reliable estimates of food intake for elderly people (Yearick et al., 1980). A copy of the instructions for completing the food records is included in Appendix C.

The food records were analyzed for energy and pyridoxine, thiamin, and ascorbic acid by use of the computer nutrient data bank (Canadian Nutrient File, Health and Welfare, 1986) at the University of Manitoba.

2.4 BLOOD SAMPLES

The blood metabolites which were assessed were the functional forms of thiamin (thiamin pyrophosphate) and pyridoxine (pyridoxal 5'-phosphate), and ascorbic acid.

Approximately 30 mL of blood was taken from all subjects after a 10-12 hour fast. The blood was separated for the analysis of red blood cell transketolase, plasma pyridoxal phosphate, and ascorbic acid. Measurement of erythrocyte transketolase activity and ascorbic acid were performed in the Department of Foods and Nutrition laboratory, University of Manitoba, the same day the sample was taken. Plasma pyridoxal phosphate was quantitated using samples that had been frozen at -20°C.

2.4.1 Vitamin Assays

2.4.1.1 Plasma Ascorbic Acid

Plasma ascorbic acid determination was performed using the method described by Lynch et al.(1969) as adapted from Roe and Kuether (1943). In this method, plasma ascorbic acid is oxidized to dehydroascorbic acid which changes to diketogulonic acid in acid pH. This compound couples with 2, 4,-dinitrophenylhydrazine to form a brown hydrazone which undergoes molecular rearrangement in strong acid to form a red compound whose absorbance is compared with that of ascorbic acid standard similarly treated.

2.4.1.2 Thiamin Assessment by Erythrocyte Transketolase Activity

Thiamin adequacy was assessed by measuring erythrocyte transketolase activity (ETK), and its stimulation by in-vitro addition of the coenzyme, thiamin pyrophosphate (TPP) (Warnock, 1975). In individuals with adequate thiamin nutriture, incubation of ETK with sufficient TPP will produce maximum enzyme activity (Hoorn et al., 1975). Individuals who have poor thiamin status will exhibit a greater activation of ETK when TPP is added. The enhanced enzyme activity is expressed in percent and is referred to as the TPP effect (Sauberlich, 1967).

Activity of ETK was determined by ultraviolet spectrophotometry based on the method adapted from Smeets and Muller (1971) and Bayoumi and Rosalki (1976). This assay is NADH-dependent, and the rate of NADH oxidation measured spectrophotometrically at 340 nm, is proportional to transketolase activity. Previous results in this laboratory have shown

that ETK activity and the TPP effect decreased 5-15% after 24 hours either at 4°C or at -20°C, and continued to decrease slowly and variably over time. Therefore, it is recommended that the enzyme assay be performed immediately after the preparation of the hemolysate.

2.4.1.3 Plasma Pyridoxal 5'-Phosphate Assessment

The functional form of pyridoxine is pyridoxal 5'-phosphate (PLP), and a radioenzymatic method adapted from Camp et al.(1983) was used to assay PLP in the plasma. This method quantitated the amount of [^{3}H]-tyramine from [^{3}H]-tyrosine. Tyrosine decarboxylase is the enzyme which decarboxylates tyrosine to tyramine. The reaction is dependent on the PLP concentration, therefore, the amount of tyramine formed is directly proportional to the amount of PLP in the plasma.

Certain points in the published method were unclear, and communication with V.M. Camp resulted in the following modifications:

1. tyrosine decarboxylase preparation: an additional step of dialysis after purification was necessary and the concentration was diluted 10-fold instead of eighteen-fold.
2. [^{3}H]-tyrosine preparation: a "carrier" of buffer with cold tyrosine added was used instead of buffer alone to dilute the [^{3}H]-tyrosine.
3. 0.5M borate buffer, pH 10.5: the NaCl and Na_2CO_3 concentrations were reduced from 6.8M and 0.7M to 2.1M and 0.2M respectively, and the tyramine concentration was increased from 95.4 uM to 730 uM.

The centrifugation step in the extraction of [^{3}H]-tyramine from the assay mixture was eliminated in this laboratory because the phases separated satisfactorily after standing for a few minutes.

2.5 STATISTICAL ANALYSIS

The 1984 and 1986 editions of the Statistical Analysis System (SAS) computer multiple regression program was used to analyze the data.

F-tests determined whether the independent variables differed significantly in the Alzheimer group compared to controls. A stepwise variable selection technique was used to describe the best model for the prediction of EDS scores. Both linear and quadratic terms for all nutrients were examined for significant contributions to the EDS scores.

The mathematical linear regression model is as follows:

$$y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{4i} + e_j$$

where: $e_j \sim N(0,\sigma)$

$\beta_0 = 117.4280$	y = EDS
$\beta_1 = -0.8496$	X_1 = Dietary ascorbic acid
	$i = 1 \ldots n_K$
$\beta_2 = 9.0521$	X_2 = Plasma ascorbic acid
$\beta_3 = 2.5368$	X_3 = Plasma pyridoxal phosphate
$\beta_4 = -0.06696$	X_4 = Plasma pyridoxal phosphate

Chapter III

RESULTS

3.1 NUTRITIONAL FACTORS

Dietary ascorbic acid (DC), plasma ascorbic acid (BC), and plasma pyridoxal phosphate (PLP) were significantly related to the EDS score ($F=5.16$, $p < 0.02$). The statistical analysis is shown in Appendix D. The prediction equation for EDS based on the regression analysis was:

$$EDS=117.4280 - -0.8496(DC) + 9.0521(BC) + 2.5368(BB_6) - -0.06696(BB_6^2)$$

For this equation, the r^2 is equal to 0.67, indicating that 67% of the variability in the EDS score can be attributed to its linear regression on dietary and blood ascorbic acid and plasma pyridoxal phosphate.

The contribution of pyridoxal phosphate to the prediction of EDS score is both linear and quadratic. Dietary ascorbic acid had a limited linear contribution which appeared to decrease after a maximal point. The plasma ascorbic acid coefficient of 9.05 was greater than the pyridoxine coefficient of 2.54 and consequently had more predictive value for the EDS.

Individual dietary intakes and plasma ascorbate levels are given in Appendix E. Dietary intake of ascorbic acid for the AD group ranged from an average of 60.9 to 158.6 mg/day, and for the control group from 49.7 to 186 mg/day. All subjects in the study consumed diets which contained the recommended amounts of ascorbic acid, 45 mg/day for females, and 60 mg/day for males (Health and Welfare, 1983). Plasma

ascorbic acid levels for the AD and control subjects ranged from 0.5 ug/mL to 12.5 ug/mL and 4.0 ug/mL to 14.1 ug/mL respectively. No significant differences were observed between the AD group and the control group with respect to dietary intake or plasma levels of ascorbate.

Dietary intake of pyridoxine and plasma 5'-phosphate levels for all subjects are presented in Appendix E. The range of plasma PLP for the AD group was 2.8-62.5 ug/L and for the control group was 1.5-135.0 ug/L. These ranges were not significantly different between the two groups.

The mean dietary thiamin individual intake and %TPP values for all subjects are shown in Appendix E. The mean %TPP value in the AD group (9.5%) was compared to the mean %TPP value in the control subjects (2.9%) and was significantly different as determined by a t-test (α=0.05, $p < 0.05$). Eleven of the 15 AD subjects and 14 of the 15 control subjects had %TPP values in the normal range of 0-14%. Three AD subjects and one control subject had %TPP values in the range of 15-24% and were thus considered to be marginally deficient. One AD subject had a %TPP value greater than 25% which represented severe thiamin deficiency. No significant differences were observed between the AD group and controls with respect to dietary intake of thiamin.

3.2 COGNITIVE ASSESSMENT

The mean EDS and MMSE scores for the AD group and the control group are presented in Table 2. These results show that the AD group scored lower on both measures of cognitive function. The EDS and the MMSE were highly correlated (r=.92) as shown in Appendix D. Individual scores on

TABLE 2

Mean EDS and MMSE Scores

	AD	C
	$\bar{x}$±sd	$\bar{x}$±sd
EDS	104.2±55	238.2±7.8
MMSE	11.5±8.0	28.4±1.1

the LPRS for the AD subjects are shown in Table 3.

TABLE 3

LPRS Scores

Subject	Score
AD1	27
AD2	22
AD3	6
AD4	12
AD5	41
AD6	36
AD7	26
AD8	6
AD9	17
AD10	9
AD11	12
AD12	34
AD13	47
AD14	27
AD15	20

Chapter IV

DISCUSSION

The coefficient of dietary ascorbic acid on EDS scores is designated as a negative value. This suggests that there is an effect of ascorbic acid on cognitive ability in the AD subjects at a maximal point of intake. The effect then decreases and exerts no further effect. This concept agrees in principle with the fact that there is an optimal requirement for ascorbic acid. It has been established that there is a saturation effect of tissues with ascorbic acid (Basu and Schorah, 1982). Consequently, there is an optimal amount of ascorbic acid which has a metabolic effect. Plasma ascorbic acid was a significant predictor of EDS scores. No values have been determined for a normal range of plasma ascorbic acid for this population, but values of 4-15 g/mL are considered to be a normal range for the methodology used in this study (Lynch et al.,1969). Nutrition Canada (1973) defined serum ascorbic acid levels of < 2.0 mg/mL to be at high risk for ascorbic acid deficiency, and values of 2.0 to 4.0 mg/mL for moderate risk of ascorbic acid deficiency. In this study, only one AD subject fell in the high risk group and one in the moderate risk group.

Goodwin et al.(1983) noted that low levels of dietary intake and low serum levels of ascorbic acid were associated with poor cognitive function. However, it is not possible to make these comparisons in the present investigation as the AD subjects had cognitive impairment and

the subjects studied by Goodwin and associates had normal cognitive function. Furthermore, the subjects in the present study had normal intakes and plasma levels of ascorbic acid. Studies by Milner (1963) and Kinsman and Hood (1971) have established the occurrence of definite psychiatric symptoms associated with ascorbic acid deprivation. These studies suggest that ascorbic acid has a role in the maintenance of CNS function. It is of interest to note that the highest concentration of ascorbate in the human brain occurs in the hippocampus and the amygdala (Mefford et al.,1980); two areas of the brain mainly associated with memory function.

Ascorbic acid has been shown to affect the release of ACh. Cholinergic terminals in the hippocampus have been implicated in memory function, thus memory loss in Alzheimer's Disease may be a direct result of a reduction in the transmission of nervous impulses mediated by ACh (Wurtman, 1985). In an in-vitro experiment, Kuo et al.(1979) demonstrated that concentrations of ascorbic acid in the media were related to the release of ACh from isolated synaptic vesicles in three species. These results suggest that ascorbic acid has a physiological function in the CNS, and may indirectly enhance memory by stimulating the release of ACh, which by itself appears to intimately linked to memory function. Since the findings in this study indicate that both dietary and plasma ascorbic acid have an effect on memory function in Alzheimer's Disease, this effect may be manifested at the synapses of the cholinergic neurons in the hippocampus.

The identification of the quadratic term in the linear regression equation for pyridoxal phosphate suggests that pyridoxal phosphate has a

positive effect upon memory to a maximal point followed by a decreasing effect. The quadratic relationship of PLP to EDS is expected as PLP functions as a coenzyme and would be expected to show typical Michaelis-Menten kinetics.

Normal values have not been identified for plasma PLP, however, a normal reference range has been suggested by Tietz (1986) to be 5-23 ug/L, and normal values of 0-20 ug/L have been reported by Hamfelt (1967). In this investigation, four out of the 30 subjects had values in excess of the normal ranges which were 45.4, 62.5, 60.0, and 135.0 ug/L. At present, all of the high plasma values observed here cannot be explained. In one case, the individual was taking supplements of pyridoxine which may have contributed to the high plasma PLP of 135 ug/L. Plasma PLP concentration increases with increasing pyridoxine intake (personal communication, Dakshinamurti, 1987). This relationship has been documented in rats (Lumeng et al.,1977) and in human adolescents (Tanphaichitr and Pakpeankitvatana, 1985). However, the effects of pyridoxine supplementation on plasma PLP has not been assessed for an elderly population. One error which may have contributed to the elevated PLP values may be that some of the control subjects or caregivers did not report supplements of dietary pyridoxine.

Pyridoxal phosphate appears to play a role in the maintenance of the cellular structure of the CNS (Morré and Kirksey, 1980, Root and Longenecker, 1983). The dendritic changes in Alzheimer's Disease could contribute to memory loss in that there would be a concomittant decrease in the number of functional neurons and synapses involved in neurotransmission. Pyridoxine transport may also be affected in AD and the brain

may become undernourished due to the failure of the choroid plexus (Spector, 1979). If this is the case, a deficiency of pyridoxal phosphate in the brain region associated with memory, specifically the hippocampal formation, could produce dendritic changes which would lead to the degeneration of neurons and subsequent memory loss.

Unlike dietary intake of ascorbic acid, dietary pyridoxine did not contribute to the EDS score of the AD group. This population consumed adequate amounts of pyridoxine, and appear to synthesize the active form since the blood ranges are normal. Individuals with Alzheimer's Disease have a decrease in the number of functioning cells. Since pyridoxine is involved in protein synthesis, there may be an increased requirement for PLP to maintain cell function. In order to assess pyridoxine requirements in Alzheimer's Disease a study to determine requirements would be necessary.

In this study, the significant differences between the mean TPP effect in the AD subjects and in the control subjects indicated that the AD subjects as a group had poor thiamin nutritional status. Erythrocyte transketolase (ETK) activity decreases in thiamin deficiency. When thiamin pyrophosphate (TPP) is added to thiamin deficient red blood cells, ETK activity is restored. This change in enzyme activity is measured as the TPP effect or %TPP. The TPP effect is directly related to the severity of the biochemical defect and is considered to serve as a functional evaluation of thiamin adequacy (Brin et al. ,1965). Of the four AD individuals, 3 were classified in the marginal thiamin deficient category, and one was in the severe thiamin deficient category. Although the sample size is small, the results are in agreement with the

findings of other studies which demonstrate the existence of thiamin deficiency based on inadequate intakes and/or reduced ETK activity in the elderly population (Brin et al., 1965, Hoorn et al., 1975, Vir and Love, 1977).

The present study found no significant differences between dietary intake of the AD group as compared to control subjects. All subjects in the present study were consuming an average intake of at least 0.40 mg/1000 kcal thiamin daily which meets the recommendations (Health and Welfare Canada, 1983). Thus, the decreased thiamin status in the AD group compared to the control group cannot be attributed to a decrease in dietary thiamin intake.

Malabsorption cannot be identified as a specific problem in this group. Folic acid deficiency, magnesium deficiency, and alcohol consumption may contribute to thiamin malabsorption (Vir and Love, 1977). An attempt was made to screen patients with malabsorptive disorders from participating in the study. Folic acid and magnesium nutritional status were not assessed, thus it was not possible to test whether these factors affected thiamin status. Alcohol consumption cannot be considered to contribute to the reduced thiamin status in the AD group, as there was negligible reported alcohol consumption in this group.

The decrease in stimulated transketolase activity observed in the AD subjects compared to the control subjects could be related to a metabolic defect in AD. It is possible that the AD subjects are unable to synthesize the apo-transketolase due to a decrease in the number of functioning cells. The metabolic defect may involve an error in gene

expression which would result in a reduction in protein synthesis (Crapper McLachlan and Lewis, 1985). These authors stated that positron emission tomography has revealed an overall reduction in protein synthesis in Alzheimer brains, but does not identify which protein groups are affected.

It has been shown that CAT activity is also reduced in AD, and a reduction in activity of pyruvate dehydrogenase complex (PDHC) in Alzheimer brains has been reported (Sorbi et al., 1983). It is of interest to note that the PDHC system is involved in the oxidation of pyruvic acid to acetyl-CoA and requires TPP as a cofactor.

Crapper McLachlan and Lewis (1985) cited results of studies which indicated reductions of total RNA and mRNA and alterations in chromatin structure in brains of individuals who were diagnosed with Alzheimer's Disease. Although there is not any experimental data to indicate an alteration in transketolase structure, two hypotheses are possible. There could be a reduction in transketolase synthesized, or the presence of an abnormal enzymatic protein which does not bind effectively to the coenzyme. If this is the case, additional dietary thiamin may be required to restore enzymatic activity.

The Extended Dementia Scale (EDS), the Mini Mental State Examination (MMSE), and the London Psychogeriatric Rating Scale (LPRS) were the psychological tools used in this study to measure memory and functional impairment in Alzheimer's Disease. The EDS is a validated instrument which differentiated non-demented individuals from demented individuals and assesses the degree of dementia (Hersch, 1979).

The MMSE was also used to assess cognitive impairment. It requires less time and is easier to administer than the EDS. It is a reliable instrument (r=0.89) and at a cut off point of 23/24 has a sensitivity of 87% and a specificity of 82% (Anthony et al., 1982). In this study, the MMSE correlated well with the EDS, indicating that the MMSE could be used instead of the EDS to assess cognitive impairment. It should be noted that two AD patients were arbitrarily given a score of "0" on the MMSE because they were untestable, and one AD subject received a score of 26 out of a possible 30. The present findings support those of Anthony and co-workers (1982) who stated that the MMSE should not be used as the sole criterion to assess cognitive function.

The LPRS was administered to the AD group to provide a global assessment of the level of functioning and mental status (Hersch et al., 1978) as determined by a caregiver. Higher scores indicate less functional ability, and the three subjects with the highest scores, 47, 41, and 36, had just been admitted to extended care facilities. The rest of the subjects were still free-living and had lower LPRS scores. These findings agree with those of Hersch and co-workers who demonstrated that the LPRS can be used as a guide to monitor changes. The LPRS did not correlate with the MMSE nor with the EDS. These findings are contrary to those of Hersch (1979) who demonstrated a high correlation between LPRS score and EDS scores in 104 individuals. A correlation between the LPRS and the EDS may not have been observed in the present study due to the small sample size and the fact that the MMSE and the LPRS were not administered at the same time for all subjects, and were administered by different people.

Chapter V

CONCLUSION

The results of this study suggest that dietary ascorbic acid, plasma ascorbic acid, pyridoxal phosphate, and thiamin pyrophosphate may be related to cognitive function in Alzheimer subjects. These results do not implicate the role of these nutrients as a causative factor in Alzheimer's Disease but rather one of functional capability. It is possible that the AD subjects have an increased requirement for ascorbic acid, pyridoxine, and/or thiamin for the activation of enzymes. The nutritional requirements of the elderly population as a whole are not known. Furthermore, the nutritional requirements in memory function are not well defined. The results of the present investigation suggest that requirements of ascorbic acid, pyridoxine, and thiamin be determined for an elderly population who are free from disease. Additional studies are required to investigate the effects of aging per se on cognitive function and how functional capacity might be maintained in the elderly with nutrition intervention.

The Extended Dementia Scale (EDS), Mini Mental State Examination (MMSE), and the London Psychogeriatric Rating Scale assessed functional capacity. The EDS and the MMSE are highly correlated and since the MMSE is a less time consuming instrument it may be a more useful assessment tool for large population studies of aging.

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Appendix A

DIAGNOSTIC CRITERIA FOR PRIMARY DEGENERATIVE DEMENTIA

Diagnostic Criteria for Primary Degenerative Dementia

I. A. Loss of intellectural abilities of sufficient severity to interfere with social or occupational functioning.

B. Memory impairment.

C. At least one of the following:

(1) impairment of abstract thinking, as manifested by concrete interpretation of proverbs, ability to find similarities and differences betweeen related words, difficulty in defining words and concepts, and other similar tasks

(2) impaired judgment

(3) other disturbances of higher cortical function, such as aphasia (disorder of language due to brain dysfunction) apraxia (inability to carry out motor activities despite intack comprehension and motor function), agnosia (failure to recognize or identify objects despite intact sensory function), "constructional difficulty" (e.g., inability to copy three-dimensional figures, assemble blocks, or arrange sticks in specific designs)

(4) personality change, i.e., alteration or accentuation of premorbid traits.

D. State of consciousness not clouded (i.e., does not meet the criteria for Delirium or Intoxication, although these may be superimposed).

E. Either (1) or (2):
(1) evidence from the history, physical examination, or laboratory tests, of a specific organic factor that is judged to be etiologically related to the disturbance

(2) in the absence of such evidence, an organic factor necessary for the development of the syndrome can be presumed if conditions other than Organic Mental Disorders have been reasonably excluded and if the behavioral change represents cognitive impairment in a variety of areas.

II Insidious onset with uniformly progressive deteriorating course.

III Exclusion of all other specific causes of Dementia by the history, physical examination and laboratory test.

From: American Psychiatric Association, (1980)

Appendix B

CONSENT FORMS

CORRELATION BETWEEN NUTRITIONAL STATUS AND MEMORY

Authorization for Release of Medical Information I

I, _____, a volunteer participant in the above-named study, hereby authorize my physician, Dr. _____ _____ to provide the researchers (Drs. Agbayewa and Bruce of the University of Manitoba) with information on my medical status relevant to this study.

Dated at _____ the _____ day of _____ 19 ____.

_____ _____
Witness Signature

CORRELATION BETWEEN NUTRITIONAL STATUS AND MEMORY

Authorization for Release of Medical Information II

I, _____, having consented on behalf of _____ my kin who is judged unable of informed consent; hereby authorize Dr. _____ to provide the researchers (Drs. Agbayewa and Bruce of the University of Manitoba) with information on _____'s medical status relevant to this study.

Dated at _____ the _____ day of _____ 19____.

_____ _____
Witness Signature

CORRELATION BETWEEN NUTRITIONAL STATUS AND MEMORY

Consent Form I

I, _____ hereby consent to participate in the above-named study. The purpose, procedures and risk of which have been explained and are clear to me.

I understand that:

1) The purpose of the study is to explore the relationship between nutrition and memory.

2) My participation in this study will involve interviews with the investigators (Drs. Agbayewa and Bruce, of the University of Manitoba) and/or their assistants. Information about my nutritional intake and general health will be obtained.

3) I will be requested to complete dietary records of my food intake.

4) The investigators will further ask me to respond to certain questions which will help them in determining my intellectual abilities at time of interview.

5) A venous blood sample will be collected to determine my nutrient level on one occasion. There are no known risks involved in participating in this study except for the minimal risk of blood sample collections.

I further understand that I can refuse to participate or withdraw from this study at any time without any prejudicial effects.

Any information obtained during this study will be kept confidential.

Dated at _____ this _____ day of _____ 19___.

_____ _____

Witness Signature

_____

Address

CORRELATION BETWEEN NUTRITIONAL STATUS AND MEMORY

Consent Form II

I, _____ being a next of kin/committee of _____ who is judged unable to give an informed consent, consent to _____'s participation in the above study. The purpose, procedures and risks of the said study have been explained, and are clear to me.

I understand that:

1) The purpose of the study is to determine the relationship between nutrition and memory.

2) Participation in this study will involve interviews with the investigators (Drs. Agbayewa and Bruce) and/or their assistants. Information about nutritional intake and general health will be obtained.

3) The spouse/relative will be requested to complete dietary records of food intake and a questionnaire which requests information on the individuals intellectual functioning-capabilities and disabilities.

4) The investigators will further request answers to certain questions which will help them in determining his/her intellectual abilities at time of interview.

5) Blood samples will be collected to determine nutrient levels on one occasion. There are no known risks involved in participating in this study except for the minimal risk of blood sample collection.

I further understand that I can refuse to participate, or withdraw from this study at any time without any prejudicial effects.

Any information obtained during this study will be kept confidential.

Dated at _____ this _____ day of _____ 19 __.

_____ _____
Witness Signature

_____
Address

Appendix C

FOOD RECORD

Suggested Way of Measuring Foods.

Food	Measurement
- Milk, etc.(whole, 2%, skim, in tea,coffe,on cereal)	- cups, tablespoons, teaspoons or ml.
- Cereals (dry, cooked, presweetened)	- cups, tablespoons or ml.
- Potatoes (mashed, boiled fried, chips)	- cups, small or large size, number of fries.
- Bread (white, brown, whole wheat, rye)	- slices, large or small loaf.
- Biscuits, rolls, buns	- number, size and type.
- Meat	- slices, ounces, dimensions.
- Fruit	- number and size, or cups.
- Vegetables	- cups or number, eg. 1 carrot.
- Sugar	- teaspoons or tablespoons or ml.
- Condiments (jam,jelly, ketsup etc.)	- tablespoons or teaspoons, ml.
- Sweets (candies, chocolate)	- number of pieces or size of package.
- Beverages (soda pop, alcoholic beverages,juices)	- cups or ounces/ml (low calorie produce)

Directions for Completing the Three-Day Food Record

- Please use the attached pages to record your food and beverage intake for the days indicated.
- It is important that you record everything you eat and drink, at home and away from home.
- Describe each food item and the amount eaten.
- Indicate if the food was eaten raw or cooked (e.g. fried, boiled, baked) and how it was served (e.g. with gravy, sauces, dressings).
- Do not change your eating pattern during the three days.
- Record any vitamin and mineral supplement you consume.

Sample Food Record Identification Number 014

List below all the food you eat and drink on Tuesday June, 11 1985

Food Item plus Additional Toppings	Amount	Method of cooking	Description - size - brand - flavor
Breakfast			
orange juice	½ cup or 4oz		frozen McCains
cornflakes	⅔ cup		Kelloggs
milk	½ cup or 4oz.		2%
sugar	1 tsp		white granulated
coffee	1 cup 8oz		
sugar	1 tsp		white granulated
milk	1 tbsp		
Lunch			
Vegetable soup	1 cup	boiled	Campbells - canned made with 1 can water
soda crackers	3		2x2" inches
Egg Salad Sandwich			
bread	2 slices		white enriched
egg	1 large	boiled	
mayonaise	1 tsp.		Kraft
butter	1 tbsp		
lettuce	1 leaf		5" x 4" iceburg lettuce
buttermilk	1 cup		

Did you take any vitamin or mineral supplement today? yes If yes, please indicate the brand and dosage. 1 One-A-DAY PLUS IRON

... continued Identification Number 014

List below all the food you eat and drink on Tuesday June 11, 1985

	Food Item plus Additional Toppings	Amount	Method of cooking	Description - size - brand - flavor
	peppermint candy	5 candies	—	
	Orange	1 small		fresh 2½" diameter
Dinner	Ham	1 slice	baked	4 inches in diameter
	Peas	½ cup	boiled	frozen
	potatoes	1 large	baked	skins removed 4" in diameter
	butter	1 tsp		
	Carrots	10 small	boiled	baby carrots ⅔ cup
	Coffee	1 cup		
	milk	1 tbsp		2% butter fat
	sugar	1 tsp		white
	Chocolate Cake	1 piece	baked	2"x3" with icing
Snack				
	Digestive cookies	2		Peak Frens Brand 2½" in diameter
	tea	2 cups		
	milk	2 tsp		2%
	1 beer	12 oz		Labatts lite 4% alcohol

Did you take any vitamin or mineral supplement today? _____ If yes, please indicate the brand and dosage. _____

Appendix D

STATISTICAL ANALYSIS

DEP VARIABLE: EDS TRMT=ALZHEIMER

EXTENDED DEMENTIA SCALE

ANALYSIS OF VARIANCE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PROB>F
MODEL	4	28480.62050	7120.15512	5.159	0.0162
ERROR	10	13800.27950	1380.02795		
C TOTAL	14	42280.90000			

ROOT MSE	37.14873	R-SQUARE	0.6736
DEP MEAN	104.2	ADJ R-SQ	0.5430
C.V.	35.65137		

PARAMETER ESTIMATES

VARIABLE	DF	PARAMETER ESTIMATE	STANDARD ERROR	T FOR H0: PARAMETER=0	PROB > |T|	VARIABLE LABEL
INTERCEP	1	117.42802	54.80210090	2.143	0.0578	INTERCEPT
DC	1	-0.84965681	0.39942958	-2.127	0.0593	DIETARY VITAMIN C
BC	1	9.05207695	3.18371591	2.843	0.0175	BLOOD VITAMIN C
BB6	1	2.53684985	3.10470897	0.817	0.4329	BLOOD PLP
BB6_2	1	-0.06696122	0.04919063	-1.361	0.2033	

OBS	ACTUAL	PREDICT VALUE	STD ERR PREDICT	LOWER95% PREDICT	UPPER95% PREDICT	RESIDUAL
1	44.0000	41.7823	27.2100	-60.8196	144.4	2.2177
2	80.5000	80.5760	13.9779	-7.8626	169.0	-0.0760
3	120.5	105.5	24.7991	6.0074	205.1	14.9707
4	91.0000	106.5	13.3629	18.5385	194.5	-15.5038
5	134.5	123.1	15.7712	33.1987	213.0	11.3777
6	186.0	149.8	14.5943	60.8342	238.7	36.2343
7	136.0	127.7	14.9249	38.5453	217.0	8.2512
8	116.0	160.5	21.1061	65.2669	255.7	-44.4665
9	177.0	179.1	23.6527	81.0195	277.3	-2.1462
10	91.0000	136.8	14.2183	48.1479	225.4	-45.7765
11	83.0000	75.9505	29.4115	-29.6240	181.5	7.0495
12	101.5	83.0904	19.7316	-10.6341	176.8	18.4096
13	8.5000	11.7273	35.6065	-102.9	126.4	-3.2273
14	14.0000	67.9214	22.4980	-28.8479	164.7	-53.9214
15	179.5	112.9	15.8400	22.9094	202.9	66.6071

SUM OF RESIDUALS	2.34479E-13
SUM OF SQUARED RESIDUALS	13800.28
PREDICTED RESID SS (PRESS)	26864.08

TRMT=ALZHEIMER

VARIABLE	N	MEAN	STD DEV	SUM	MINIMUM	MAXIMUM
EDS	15	104.20000	54.955112	1563.0000	8.5000000	186.00000
MINI	15	11.53333	7.989875	173.0000	0.0000000	26.00000

PEARSON CORRELATION COEFFICIENTS / PROB > |R| UNDER H0:RHO=0 / N = 15

	EDS	MINI
EDS	1.00000	0.92260
EXTENDED DEMENTIA SCALE	0.0000	0.0001
MINI	0.92260	1.00000
MINI MENTAL STATE	0.0001	0.0000

Appendix E

DIETARY AND BLOOD PARAMETERS

Dietary Intake and Plasma Ascorbic Acid

Dietary Ascorbic Acid (mg)		Plasma Ascorbic Acid (ug/mL)	
AD	C	AD	C
102.1	184.0	0.5	5.7
125.8	162.1	6.5	11.5
60.9	49.7	2.3	14.1
94.0	62.7	6.4	5.6
109.4	54.2	10.1	9.5
83.0	57.9	9.5	13.5
111.7	102.5	10.6	7.9
93.9	186.0	12.5	12.0
68.1	112.5	10.6	4.6
84.7	107.3	7.9	5.0
114.2	127.7	8.7	11.6
146.8	174.8	8.3	9.8
109.7	108.6	10.0	12.8
158.6	118.0	8.1	4.0
131.4	93.9	10.7	11.9

Dietary Intake of Pyridoxine and Plasma Pyridoxal 5'-Phosphate

Dietary Pyridoxine (mg)		Plasma Pyridoxal 5'-Phosphate (ug/L)	
AD	C	AD	C
1.7	1.9	2.8	3.1
2.3	1.8	5.1	135.0
1.6	1.0	10.3	5.4
1.5	1.4	5.0	6.0
1.4	1.2	3.1	8.0
1.3	1.1	8.6	3.9
1.4	1.3	4.1	60.0
1.9	1.0	4.3	5.4
1.7	1.3	16.5	1.5
1.6	1.7	11.0	9.1
1.3	1.5	45.4	6.0
2.0	1.8	7.5	9.8
1.8	1.1	62.5	17.3
1.1	1.9	5.5	6.5
1.4	1.9	4.6	8.0

Dietary Intake of Thiamin and % TPP values

Dietary Thiamin (mg)		%TPP	
AD	C	AD	C
1.00	1.70	29.90	-5.30
1.40	1.20	18.40	7.90
2.20	1.10	0.00	-9.00
1.70	0.80	5.10	10.70
1.50	1.60	18.80	12.60
1.20	0.78	15.20	6.20
1.30	0.90	4.10	1.60
1.20	1.30	6.50	-12.00
0.90	1.00	0.00	5.30
1.20	1.70	5.30	20.40
1.20	1.00	3.50	0.44
1.50	1.30	11.60	0.00
1.70	0.90	9.50	-3.30
1.00	1.30	5.10	5.10
1.40	1.30	9.30	3.60
$\bar{x}$±sd 1.42±0.34	1.18±0.30	9.49±0.15	2.95±0.42