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A Relationship Between Vitamin B_{12}, Folate, Ascorbic Acid, and Mercury Metabolism

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To the Graduate Council:

I am submitting herewith a thesis written by Nancy Elizabeth Zorn entitled "A Relationship Between Vitamin B_{12}, Folate, Ascorbic Acid, and Mercury Metabolism." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Human Ecology.

John T. Smith, Major Professor

We have read this thesis and recommend its acceptance:

Betsy Haughton, Michael H. Sims, Roy Beauchene

Accepted for the Council:
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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Betsy Haughton

Michael H. Sinn

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Accepted for the Council:

Vice Provost and
Dean of The Graduate School

A RELATIONSHIP BETWEEN VITAMIN B_{12}, FOLATE, ASCORBIC ACID, AND MERCURY METABOLISM

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Nancy Elizabeth Zorn

August 1988

DEDICATION

To my earthly father, William C. Zorn, who passed away the week this endeavor began but who lives daily in my memories. He instilled in me the belief that I could attain any goal.

And to my Heavenly Father who gives me Eternal Life. He provides the spiritual guidance to attain His goals for me.

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As is true is so many circumstances of life, there are many people who have been helpful in achieving this degree. The list is too lengthy to mention everyone but a few individuals deserve special recognition.

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ABSTRACT

The effect of megadoses of vitamin B_{12}, folate, and vitamin C on the *in vivo* methylation of mercuric chloride was studied in guinea pigs. The incorporation of high levels of vitamin B_{12}, folate, and vitamin C resulted in a decrease in both inorganic mercury and methylmercury concentrations in all tissues except the lung and heart compared to controls. However, percent methylmercury levels tended to increase with vitamin treatment.

The addition of megadoses of vitamin B_{12} fed either singularly or in combination with the other vitamins resulted in increased methylmercury concentrations in the liver, spleen, and kidney tissues of the guinea pig. Moreover, percent methylmercury levels increased with B_{12} treatment in the liver, heart, and kidney. Incorporation of high levels of folate into the dietary regime also affected the mercury methylation process particularly in the liver, heart, kidney and hair tissues. However, this effect was observed most often in animals fed both B_{12} and folate. Vitamin C appears to play a synergistic role with vitamin B_{12} and/or folate in the methylation of mercury.

Auditory brainstem responses (ABR) produced results which supported the theory that vitamin B_{12} treatment can potentiate the mercury effect in the auditory pathways of the guinea pig. Wave latencies were longer and threshold values were higher in animals fed megadoses of B_{12}. Interpeak latency means were also longer in animals treated with vitamins and mercury compared to untreated controls.

Rats fed diets supplemented with either methionine or cysteine exhibited differences in methylmercury levels. Both methionine and cysteine supplementation resulted in a significant increase in percent methylmercury levels in the liver and kidney.

These data indicate that megadoses of vitamin B_{12}, folate, and/or vitamin C may alter the methylation process of mercury *in vivo*. In addition, methionine or cysteine supplementation can effect the metabolism of mercuric chloride possibly via transmethylation processes.

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CHAPTER I

INTRODUCTION

Mercury has been recognized as a hazardous environmental pollutant since the outbreak of mercury poisoning in Minamata Bay, Japan, in the 1950's. People were poisoned as a result of consuming fish which had acquired high concentrations of methylmercury due to contamination of local waters with industrial waste (1,2). Mass poisonings were reported in Iraq during 1971-72 (3). Grain which was treated with mercurial seed dressings was issued to Iraq farmers in 1956 and 1960 for planting but economic conditions resulted in its improper use as food (3,4). Several outbreaks of poisoning due to the use of treated seeds for food were subsequently reported (2,4,5).

Despite these events, mercury is still widely used in the production of certain chemicals, fungicides, insecticides, paints, and electrical equipment (6,7). In addition, many industrial waste products contain mercury and are discarded in oceans, rivers, lakes, and landfills (6,7). A more recent concern is the level of mercury exposure as a result of dental amalgams. Thus, the general public continues to be exposed to mercury from a number of rather widespread sources. As a result, many toxicological studies have been conducted to explain the distribution, mode and site of action and elimination of mercury in animals.

Mercury is toxic in either the metallic or divalent form; however, the chemical form and dose alters the selection of the target organ (7). It has long been known that mercury and its organic form, methylmercury,

are neurotoxic. Although there have been a large number of neurological studies conducted using animal models, only a few have explored the visual and otological deficits caused by mercury.

Methylmercury has been shown to be highly toxic to mammalian systems. The biological formation of methylmercury requires the coenzyme methyl-cobalamine (vitamin B_{12}) (8). Additionally, it is logical to assume that the regeneration of methyl-cobalamine would require one of the forms of tetrahydrofolate (folacin) (9). Therefore, it appears conceivable that individuals who consume megadoses of these vitamins, B_{12} and folacin, would increase their potential to form methylmercury. It has also been shown that the addition of ascorbic acid to an incubation mixture enhances methylmercury-induced lipid peroxidation in the mitochondria (10). Additionally, studies have shown that ingestion of megadoses of vitamin C with other vitamins permits increases in the level of metabolically active cobalamin (vitamin B_{12}) as well as other vitamins (11). The purpose of this investigation was to examine if there is a relationship between consumption of megavitamins and mercury retention and metabolism.

Research Hypothesis

Since this project consisted of three distinct investigations, there were three separate research questions:

1) Ho: The ingestion of megadoses of vitamin B_{12}, folate, or ascorbic acid will not result in increased methylation and retention of mercury as methylmercury in the liver, heart,

spleen, kidneys, brain, muscle, lungs, blood or hair of the guinea pig.

Ha: The ingestion of megadoses of vitamin B_{12}, folate, or ascorbic acid will result in increased methylation and retention of mercury as methylmercury in the liver, heart, spleen, kidneys, brain, muscle, lungs, blood or hair of the guinea pig.

2) Ho: A casein-based diet supplemented with methionine or cysteine will not result in increased methylation and retention of mercury as methylmercury in the liver, heart, spleen, kidneys, brain or lungs of rats.

Ha: A casein-based diet supplemented with methionine or cysteine will result in increased methylation and retention of mercury as methylmercury in the liver, heart, spleen, kidneys, brain or lungs of rats.

3) Ho: A diet containing megadoses of vitamin B_{12}, folate, or ascorbic acid will not effect mercury metabolism in guinea pigs as demonstrated by altered auditory brainstem responses.

Ha: A diet containing megadoses of vitamin B_{12}, folate, or ascorbic acid will effect mercury metabolism in guinea pigs as demonstrated by altered auditory brainstem responses.

CHAPTER II

REVIEW OF LITERATURE

Mercury Assessment Measurements

The best non-invasive indices of exposure to methylmercury are levels of mercury in hair and blood (12). Studies in Scandinavia on the metabolism of trace amounts of radiolabeled methylmercury by humans have shown that methylmercury is completely (more than 95%) absorbed from food and is rapidly distributed throughout the body. These studies also allowed calculation of the half-life of mercury in humans (about 70 days) and provided information regarding the relative concentration of mercury in the various parts of the body (12). Additional studies have shown the relationship between ingestion of methylmercury from contaminated fish and mercury levels in the blood and hair (12).

Blood measurements are of great diagnostic and prognostic significance because there is an equilibrium between tissue concentrations and blood level (13). However, the central nervous system (CNS) stands out as an exception to this. Both the brain and cerebellum may still contain significant amounts of methylmercury even several years following exposure and while the kidneys, liver, and blood have returned to normal values. Therefore, the CNS appears to accumulate methylmercury over much longer time spans than any other organ and consequently, the degree of concentration in the CNS is not reflected reliably by blood measurements (13).

Due to several unique properties, hair has gained a more important role in the assessment of both mercury exposure and body burden. First, hair contains a number of sulfhydryl groups which act as binding sites for mercury ions and radicals (13). As a general rule, the mercury concentration in hair is 250-300 times higher than that in blood. Therefore, the higher concentrations yield more reliable measurements (14). Secondly, since hair growth resembles an extrusion process with a mean growth rate of 1 cm/month, analysis of segments (1 cm long) of hair can provide mean monthly blood concentrations of mercury throughout one year prior to sampling (13,14).

Urine sampling has also been used as a method of determining inorganic mercury exposure. However, the mercury content of urine displays such wide individual variation, depending on fluid intake, type of food and many other factors, that it is impossible to derive any meaningful definition of normal values and any definite conclusions about the body burden (13). While the mercury content of urine yields little information about the body burden, it has occasionally been used as a diagnostic tool. For example, following a sudden and known ingestion of mercury salts, the urinary content serves as an indicator of uptake. Alternatively, when a patient is given treatment for removal of a high body burden of mercury, the urinary content will reflect tissue release of incorporated mercury and thus, the effectiveness of the therapy (13).

Health Risks

In 1984, the United States Environmental Protection Agency (15) estimated that the average daily retention of mercury for the adult population was 153 nanograms per day for atmospheric Hg which is the predominant form of elemental mercury vapor. This is for the general population or one which is not occupationally exposed to the element. Retention of organomercurials mainly as methylmercury from fish was estimated at 3666 nanograms per day. Consumption of inorganic mercury compounds derived from nonfish dietary products was predicted to be 2000 nanograms per day with 5 nanograms per day consumed in drinking water (15).

However, it was suggested that these estimations should be considered highly provisional since their derivation was predicated on an approach totally at variance with the "real world" situation in which human beings are exposed simultaneously to many interacting environmental chemicals. In addition, these estimates are seriously influenced by such factors as degree of environmental pollution peculiar to each geographic zone, ambient temperature, humidity, sex, race, dietary habits, prevalence of disease and conditions of stress (15).

Another important source of the human burden of mercury which was not considered in these estimations is the widespread daily use in some black communities, both in developed and third world countries, of depigmentation soaps and other cosmetic products which contain 3-5% mercuric salts. These mercuric salts, which are applied to the skin as ointments in suitable lipid vehicles, are readily absorbed and retained in the body (16).

In recent years, a number of articles (17,18,19) have indicated that fish consumption is beneficial in prevention of cardiovascular disease. However, studies have also shown that fish contaminated by mercury spills, particularly methylmercury, can present a potential human health hazard.

A recent study was conducted by the Food and Drug Administration (FDA) (12) to evaluate fish consumption in the United States and calculate possible exposure to methylmercury. A consumption study which was commissioned by the Tuna Research Foundation in 1973-74 was used to determine the intake of fish and shellfish in the U.S. The average consumption of total seafood per individual was 18.58 ounces per month or approximately 18 g/day with 61.5% of the individuals sampled eating tuna. Mercury residue levels in domestic fish sampled by the FDA were then used to calculate the level of exposure to methylmercury. According to OSHA, the maximum permissible exposure limit for mercury is 0.1 mg/m^3 and the threshold value for organic compounds is 0.001 ppm (20). At this level, the U.S. fish consumption data did not indicate any cause for concern of methylmercury poisoning for the average American (12).

However, studies conducted in other countries have shown slightly different results. A human metabolic study done in Japan examined the intake of tuna in relation to mercury concentrations in human hair (21). It was clearly shown that an increase in the mercury concentration in the hair was approximately proportional to the amount of tuna flakes consumed. It was, thus, suggested that those populations consuming large quantities of fish should be monitored in order to prevent mercury

poisoning (21). However, a recent study (22) examining methylmercury exposure and mortality in Japan found that there was no appreciable difference in life expectancy in populations of methylmercury-exposed areas as compared to control areas. However, the authors suggested that further investigation was needed to provide more insight into the delayed effect of methylmercury exposure on human health (22).

A study done by Mason in 1987 surveyed the mercury content of eel and roach taken from 67 sites in Great Britain (23). On the average, eels contained significantly more mercury than roach. It was also determined that concentrations of metals in eels may more closely reflect environmental levels and could possibly be used as an indicator species. Twenty-five percent of the eels sampled exceeded recommended standards for human consumption. While freshwater fish do not figure much into the diet of most Britons, there is a growing export of eels to mainland Europe and, therefore, it may be advisable to initiate a program of monitoring freshwater fish for heavy metals (23).

Dermelj et al. (24) measured the levels of mercury, methylmercury, and selenium in scalp hair of populations from Mediterranean areas. Populations which consumed minimal amounts of seafood had very low concentrations of total mercury and methylmercury and a high ratio of mercury to methylmercury. However, populations from Yugoslav and the Greek coastal areas had elevated mercury levels in scalp hair with most of the mercury as methylmercury. This population had a high consumption of fish, shellfish, and other edible sea products. This study indicated that the elevated concentration levels of mercury in the scalp hair of these populations can be "directly attributed to the higher consumption"

of seafood products (24). This concept has been supported by research done in New Guinea (25), the United Kingdom (26), and Spain (27).

Another concern that has been investigated recently is the level of mercury exposure which may result from dental amalgams, particularly among dental professionals. Silver-amalgam is still the cheapest, most durable, and most extensively used restorative material in dentistry for repair of decayed posterior teeth. Mercury accounts for about 50% of this alloy by weight (16). Sources of mercury exposure which exist in a dental office include accidental spills, poor mercury hygiene, manual mulling to express excess mercury from freshly mixed amalgam, mechanical amalgamators, ultrasonic amalgam condensors, failure to use high-vacuum suction while removing old amalgam restorations and improper dry heat sterilization of amalgam-contaminated instruments (28).

A recent comprehensive assessment of the magnitude of mercury exposure in 4,272 US dentists focused on urinary levels of mercury (28). Urinary mercury levels ranged from 0-556 μg/liter with a mean level of 14.2 μg/liter. It was noted that urinary mercury levels were related to geographic location within the country, dental specialty, type of air-conditioning in the practice, duration of working hours, and, especially, number of amalgam restorations placed or removed weekly, years in a dental specialty, and years in the current office facility. For example, a four-fold increase in urinary mercury level was observed in general practioners compared to orthodonists, and more than 31% of the dentists with urinary mercury levels in excess of 50 μg/liter practiced more than 40 hours per week (28). These results were supported by a more recent study which showed a significantly higher

blood level of mercury in 205 practicing dentists compared to non-dental controls (29).

One study also indicated that subjects with dental amalgams had unstimulated mercury vapor concentrations significantly higher than basal levels of control subjects with no amalgams (30). With chewing, these levels increased six-fold over unstimulated mercury levels, or a 54-fold increase over levels observed in control subjects (31). Therefore, it has been concluded that dental amalgam mercury makes a major contribution to total daily dose levels.

Site of Toxicity

Studies have shown that inorganic mercury and methylmercury are distributed in the body at different sites. Mitsumori et al. (32) treated rats of both sexes with various levels of methylmercury chloride and determined that the hair had high levels of mercury, while subsequently lower levels were found in the kidney, blood, liver, and nervous tissues, respectively (32). Blood biochemical examinations revealed that cholesterol and urea nitrogen increased which may have indicated toxic renal damage. These researchers found sex differences in clinical signs, mortality, body weight, hematology and blood biochemistry within the treated groups (32).

Thomas et al. (33) determined that the distribution of mercury is affected by the sex of the animal. Female Long-Evans rats were able to excrete organic mercury more rapidly than their male counterparts. In fact, whole body clearance of mercury was faster in females than in males. It was determined that female rats had significantly higher mean

percentages of the methylmercury dose located in the kidney and brain, while males had higher mean percentages of the dose in pelt and whole body (as organic or total mercury). A sexual difference was also shown in accumulation or retention of inorganic mercury in the nervous system. The reported sexual differences in methylmercury toxicity may be explained by these sexual differences in distribution and retention of organic and inorganic mercury following methylmercury exposure (33).

Alexander and Aaseth (34) found that the organ distribution of mercury was altered with administration of cysteine or glutathione. A rapid uptake of mercury in the kidney and a depressed content in the liver and blood was observed in female rats given an intravenous injection of methylmercury chloride premixed with glutathione or cysteine as compared to animals given methylmercury alone. Following exposure to methylmercury, the physiological formation of a methylmercury-glutathione complex takes place mainly intracellularly. Since the liver has the ability to synthesize glutathione, it is a primary site for the conjugation of methylmercury and glutathione. Additionally, the liver cells are able to export large amounts of this complex into the bile which reduces the liver content of methylmercury. The addition of cysteine increases the amount of methylmercury binding since it probably increases the intracellular levels of glutathione. It was noted that this complex can be reabsorbed from the gut and is rapidly extracted from the blood plasma by the kidneys (34).

A recent study by Atchison et al. (35) examined the effect of methylmercury at the nerve terminals. It has been shown previously that methylmercury blocks nerve-evoked release of acetylcholine, while

spontaneous quantal release of acetylcholine is first increased then decreased (35,36). These changes in spontaneous quantal release of transmitter are measured electrophysiologically as alterations of miniature endplate potential (MEPP) frequency (36). This is not due to impaired postjunctional sensitivity (37) or exhaustion of releasable transmitter stores (38). The present study investigated the idea that methylmercury might suppress nerve-evoked transmitter release by blocking the nerve terminal calcium channels which open during depolarization and permit calcium to enter which then triggers the release of transmitter. It was shown that methylmercury blocked the depolarization-induced calcium influx irreversibly and noncompetitively at the nerve terminals in both the central and peripheral nervous system. The block of calcium uptake by methylmercury was shown to differ from that of mercury in two ways. First, methylmercury reduces the total influx of calcium through a voltage-dependent pathway less effectively than inorganic mercury. Second, inorganic mercury more potently and efficaciously blocks the slow phase of calcium uptake, while methylmercury appears to block the fast phase of calcium uptake more effectively. However, the efficacy of methylmercury as a blocker is greater for the slow than fast components (35).

This study was later supported by Levesque and Atchison in 1987 (36) in which it was shown that methylmercury-induced stimulation of quantal release of acetylcholine at the neuromusclular junction was not blocked by agents which depolorize the mitochondrial membrane. However, the quantal release was blocked by an agent which blocks calcium influx specifically via the so-called "uniporter". Therefore, simple

inhibition of mitochondrial function does not prevent methylmercury from causing an increase in minature end-plate potential (MEPP) activity. It was suggested that methylmercury may interact with the mitochondria to induce the release of bound calcium stores into the nerve terminal cytoplasm. This increase in free ionized cytosolic calcium in turn is thought to promote the increased quantal discharge of acetylcholine observed as increased frequency of MEPP's (36).

Neurotoxicity

Although it has been well established that mercury and its derivatives are severe neurotoxins (1,3), the exact molecular basis of organic mercurial-induced neurotoxicity in humans and animals is largely unknown. Methylmercury, in contrast to the inorganic forms, passes with ease through physiologic barriers, such as the blood-brain barrier, blood-testes barrier, and placenta (39). Neuropathologic observations reveal the cortex of the cerebrum and cerebellum are selectively involved with focal necrosis of neurons, their lysis, and phagocytosis, and replacement with increased numbers of supporting glial cells. These changes are most prominent in the deeper fissures such as the visual cortex and insula (39).

Omato et al. (40) demonstrated an inhibition of amino acid incorporation associated with possible defects in the cytosol fraction and microsomes isolated from brains of mercury-treated animals. However, they concluded that the primary site of organic mercurial action is not the protein synthetic per se. It was suggested that the neurotoxicity of mercury may be associated with other intracellular

processes coupled with the formation and/or regulation of the protein synthetic apparatus (40).

Cheung and Verity (41) compared the perturbation of brain protein synthesis by methylmercury chloride in vivo and in vitro. These researchers found that stimulation of amino acid incorporation following in vivo administration of methylmercury was apparently associated with the ribosome fraction, while in vitro preincubation of the postmitochondrial supernatant with methylmercury produced stimulation associated with the pH 5 enzyme fraction. A model was proposed to explain the effects of methylmercury on brain protein synthesis which involves the existence of an inhibitor (or regulator) of protein synthesis that is highly sensitive to methylmercury. A more recent study by Cheung and Verity (42) identified the locus of mercurial inhibition of translation as a perturbation in the aminoacylation of tRNA which is not associated with defective initiation, elongation, or ribosomal function (42).

The distribution and concentration of mercury in various areas of the brain have been studied by several investigators (43,44), but comparatively few microanatomical studies have been directed towards revealing the pathogenesis of the neurological lesions in mercury intoxication. Moller-Madsen and Danscher (45) examined the localization of inorganic mercury in the central nervous system of the rat. Adult Wistar rats of both sexes were given mercuric chloride in the drinking water at a dose level of 20 mg $HgCl_2$/liter distilled water ad libitum during an 8-month period. The animals were subsequently sacrificed and coronal sections of the brain and cervical spinal cord were examined

according to a histochemical technique based on a physical development process that makes mercury deposits visible (45).

It was found that mercury deposits were not uniformly distributed in the brain and most mercury was located in the rhombenephalon. There was a clear tendency for those regions in the brain stem and cerebellum which are primarily associated with motor systems to contain the heaviest mercury accumulation. In fact, significantly more mercury was found within the motor nuclei than in sensory nuclei. Microscopic examination showed that within the regions of mercury deposition, large cells generally contained more mercury than smaller cells and that mercury was located intracellularly with neurons containing more mercury than glial cells (45).

Research with methylmercury has provided a slightly different distribution pattern (46,47). Vandewater and associates (48) investigated the distribution, metabolism, and neurotoxicity of methylmercury using Swiss albino mice. An oral dose of [^{203}Hg] methylmercury chloride (10mg/kg) was given to male mice for a period of 1 to 9 days. The methylmercury was evenly distributed among the posterior cerbral cortex, subcortex, brain stem, and cerebellum. The anterior cerebral cortex had a significantly higher methylmercury concentration than the rest of the brain. A general trend of decreasing structural damage was observed with the cerebral cortex greater than the cerebellum greater than the subcortex greater than the brain stem. This uneven pattern of structural damage did not correlate with the brain distribution of methylmercury. It was suggested that various areas of the brain differ in their sensitivity to methylmercury since all areas

have the same methylmercury concentration and yet display different degrees of structural damage (48).

Obviously, inorganic mercury and methylmercury are distributed differently in the brain and, at present, there is no satisfactory explanation for these differences. It has been suggested that inorganic mercury disrupts the barrier function of the plasma membrane first and then moves into the cell, inhibiting cellular functions. Methylmercury, on the other hand, penetrates the cells without any noticeable damage to the barrier. Therefore, the observed differences in the handling of mercuric chloride could be explained due to the differences between the plasma membranes of the motor and sensory neurons (48).

Behavioral and neurophysiological changes are the earliest, and sometimes the only, indicators of chronic effects of exposure to neurotoxic agents (49,50). Clinical studies on patients exposed to mercury have shown a disturbance of visual perception as one of the most consistent signs of neurological impairment in humans. A concentric narrowing of the visual fields and reduced visual acuity is typically exhibited. The neurologic impairment underlying these visual anomalies appears to be lesions of the primary visual cortex.

O'Kusky (51) examined the ultrastructural changes in neurons and synapses of the primary visual cortex in rats exposed to methylmercury in order to identify the morphologically distinct cell types undergoing degeneration at the onset of neurologic impairment. Subcutaneous injections of methylmercuric chloride in physiological saline were given to neonatal rats at a dose of 5 mg Hg/kg/day beginning on postnatal day

5. The injections were continued until the animal demonstrated a persistent weight loss for 48 hours (51).

Clinical signs of neurologic impairment were relatively consistent among the nine methylmercury- treated animals with all animals exhibiting some degree of visual impairment. The vibrissa-placing and forelimb-placing responses appeared normal, but the visual-placing response was weak or absent. The methylmercury-treated animals, unlike the controls, failed to avoid a visual cliff, and when placed in a novel environment demonstrated difficulty in negotiating obstacles (51).

In four of the methylmercury-treated animals convulsive activity was displayed and lesions of the primary visual cortex were more extensive than in the nonconvulsive animals. Examination by electron microscopy of the visual cortex in the nonconvulsive animals showed a selective degeneration of axon terminals forming symmetric synapses. It was suggested that a specific class of cortical neuron, the aspinous or sparsely-spinous stellate neuron, is particularly vulnerable to methylmercury toxicity. These neurons have been shown to form symmetric synapses in the visual cortex of the rat and contain glutamic acid decarboxylase which is the enzyme that synthesizes the neurotransmitter, γ-aminobutyric acid (GABA). The GABAergic inhibition of the neocortex is mediated by these intracortical neurons (51).

The relatively selective impairment of GABAergic neurons in the visual cortex that occurs with methylmercury poisoning is consistent with the visual abnormalities that have been reported. For example, with impairment of the GABAergic neurons, a loss of orientation specificity in neurons of the visual cortex would explain the decreased

visual acuity. In addition, a loss of directional specificity may contribute to the concentric narrowing of the visual field as reported in some cases of methylmercury poisoning. The convulsive behavior exhibited by some of the animals exposed to methylmercury was suggested to reflect a seizure-induced pathology secondary to methylmercury impairment of GABAergic interneurons of the cerebral cortex. The exact cellular mechanism underlying the preferential degeneration of stellate neurons and symmetric synapses in methylmercury-treated animals is unclear. However, it has been proposed that an inhibition of mitochondrial respiration is involved (51).

Slotkin and Bartolome (52) also examined alterations in nerve signal transmission with methylmercury toxicity. It was shown that postnatal exposure to methylmercury resulted in changes in neurotransmitter uptake and turnover in presynaptic terminals, as well as development of postsynaptic adrenergic receptor binding sites. These alterations resulted in aberrant signal transmission across the synapse. Therefore, the neurotoxicity observed with methylmercury may be due to degeneration of nerve synapses (52).

Several recent studies conducted by Aschner in 1986 (53,54) examined changes in axonal transport in the rat optic system with methylmercury exposure. In one study, alterations in axonally-transported proteins in the retinal ganglion cells of rats were examined following exposure to methylmercury. Several groups of rats were given injections of methylmercury (4 mgHg/kg/day) for durations of 4-6 days and 12 days. Scintillation, histological, and SDS

polyacrylamide electrophoresis studies were employed to determine axonal transport (53).

An increased rate and volume of transported protein-bound radioactivity was observed in the visual system of the mature rat treated with methylmercury. This is consistent with reports that synthesis and axonal transport increase when degenerative changes in the nerve create a demand for repair substances. Therefore, the increase in rate and volume of flow in the methylmercury-treated visual system of rats presumably reflects metabolic alterations designed to increase the supply of materials to the nerve terminal. It may represent a period of compensation during the early stages of methylmercury exposure (53).

The composition of the transported polypeptides was analyzed by gel electrophoresis and selective changes were evident. These changes occurred in a small subset of proteins known as GAPs (growth-associated proteins). This indicated that the rat's visual system has the capacity to attempt regeneration; however, it is not clear whether this process can be maintained for long periods of time (53).

It is well known that a latency period occurs between the time of administration of methylmercury and the onset of symptoms. It has not been determined whether increased protein synthesis and axonal transport are maintained throughout the symptomatic stage of methylmercury exposure. It needs to be determined if exhaustion of protein synthesis and axonal transport is correlated to the onset of functional deficits in these animals (53).

Aschner's study helps explain the great specificity of methylmercury-induced injury in certain animal models, since some

systems appear to have regenerative capabilities (e.g. visual) while other systems appear to fail in this aspect (e.g.-sciatic). It is important to understand these regenerative processes if future studies are to examine the effects of methylmercury on the visual system (53).

Additional research has examined the effect of methylmercury on visual-evoked potentials (55) and spatial vision (56). It was shown that methylmercury decreased latencies of visual-evoked potentials and impaired spatial vision. Therefore, it would appear that methylmercury treatment has various effects on the visual system (55,56).

One of the side effects of methylmercury poisoning is sensorineural hearing loss with subsequent impairment of speech discrimination. Morphological studies have shown damage to the cochlea and outer hair cells in the apex of the cochlea as well as the sensory epithelium and peripheral myelinated nerve fibres in the crista ampullaris with chronic inorganic mercury intoxication (57,58). However, the hearing loss due to methylmercury cannot be explained solely on the basis of receptor cell lesions (57).

Wu and coworkers (57) examined cutaneous and auditory function in rats following methylmercury poisoning. Rats were given various doses of methylmercury chloride subcutaneously over a course of 5 days. Then, at varying times after the toxic exposure, up to one year, their sensory functioning was assessed using the behavioral method of reflex modification (57).

The first signs of cutaneous deficits and hearing impairments were noted about 1 to 2 weeks after exposure to the higher dosages of methylmercury (40 and 50 mg/kg). These effects were revealed by

decrements in startle inhibition produced by both transient auditory stimuli and also by shocks to the tail which preceded the acoustic startle reflex (57).

These deficits in cutaneous sensitivity and changes in reactivity are consistent with neurological damage produced in the periphery of the nervous system by exposure to methylmercury. However, auditory deficits are more difficult to explain. In the auditory processes of the superior temporal gyrus, central lesions have been observed in the primate brain. It has been suggested that these lesions may be at least partially responsible for the impairments in auditory perception noted in exposed humans. Since these impairments have been observed to be temporary in the rat, it was hypothesized that methylmercury produces demyelination in the auditory nerve which is equivalent to that seen in the dorsal roots of the spinal cord. Damage to the large fibers in the auditory nerve would thus impair the detection of rapid transients and other high frequency stimuli which is normally observed in humans exposed to methylmercury. However, some recovery may be seen following neural repair (57).

Only a limited number of clinical methods exist for evaluating sensory, perceptual, and even motor systems in animals. However, it is known that the brain produces consistent and time-locked changes in electrical activity in response to auditory stimuli. Surface electrodes can be used to record these evoked potentials from auditory structures in the brainstem. These responses are referred to as auditory brainstem evoked responses (ABER) or auditory brainstem responses (ABR). Evoked response testing is a non-invasive technique which provides highly

objective information without subject cooperation. Thus, ABR evaluations are used by many investigators to detect neurologic, otologic or audiologic dysfunction in both human beings and animals (59,60).

Auditory brainstem responses are averaged recordings of brain activity which result from an externally applied acoustic signal. By averaging these recordings, the background electrical activity can be extracted from the low voltage evoked components. The recordings consist of numerous waves with positive and negative peaks. Each wave represents composite neuronal activity of 1 or more brain structures. The activity of neural generators at progressively higher levels in the neuroaxis are generally represented by waves of successive auditory evoked responses with the early latency components arising almost totally within the brainstem. Thus, auditory brainstem responses reflect electrical events in the VIIIth cranial nerve and within the brainstem components of the auditory pathway (59).

A number of studies have been conducted in an attempt to describe, from an anatomic perspective, the neural generators of the ABR waves. However, it has not been possible to identify a given wave with a single nucleus or tract due to the complexity of the sound pathway and perhaps the variability among animal species. Even so, a proposed scheme has been derived from a variety of experimental studies to relate each ABR wave as a reflection of a generator system. There is general agreement that Wave I of the ABR is produced by acoustic nerve activity. Wave II appears to be generated from the cochlear nucleus, however, there is less agreement concerning the origin of this wave. Research suggests

that the generator of wave III is the trapezoid body and/or superior olivary complex but attempts to differentiate discrete nuclei of the complex have been unsuccessful. Waves IV and V appear to originate from the area around and within the inferior colliculus. In certain animal species, waves VI and VII will be exhibited in ABR measurements but the generators for these waves remain unclear (59,60).

Latency measurements of wave I can be used to examine the time from the inner ear or cochlea to the cochlear nerve. Therefore, this information may be used to indicate peripheral nerve damage which would effect the signal transmission to the CNS. Auditory threshold data can also be used to indicate this type of alteration. Wave amplitude data may be used to indicate the strength of the stimulus as the action potential reaches the different auditory centers of the brainstem. Interpeak latency (IPL) for waves I and V is defined as central transmission time, and appears to be a good indication of impulse transmission through the brainstem, at least to the level of the inferior colliculus which is assumed to be the generator of wave V. Lesions involving the cochlear nerve and brainstem have been shown to result in a prolonged IPL.

Thus, auditory brainstem response testing can be used to assess neurologic diseases, hearing impairment, as a measure of CNS maturation, and in monitoring the treatment of CNS disease. These diagnostic procedures have also been used in veterinary medicine to examine the effect of mercury intoxication on the auditory system of animals.

A study was done by Wassick and Yonovitz (61) in which methylmercury ototoxicity was determined using auditory brainstem

responses. Two groups of mice were given subcutaneous injections of either 4 mg/kg for 17 consecutive days or 8 mg methylmercury/kg for 6 days. Auditory brainstem responses were obtained as a result of pure-tone stimulation (4 to 78 kHz). To assure normal audibility and provide a baseline measure, pretreatment responses were obtained from each animal. Recordings were then taken weekly for three weeks after the initial injection. Latencies and physiologic thresholds were obtained from all 18 mice to assess methylmercury toxicity in order to elucidate which areas of the lower auditory pathway were affected (61).

The 4 mg/kg group showed significant threshold deficits at all dose test periods. The decrement persisted up to the last test period which suggested that the deficits were permanent. The frequency having the greatest deficit was at 32 kHz, confirming a high-frequency loss (61).

Significant threshold decrements with similar patterns to the lower dose group were also found in the 8 mg/kg group. However, the 16 kHz was the most affected frequency suggesting a loss in the most sensitive audibility range for mice. In addition, the overall deficit was not as great in this group. The reasons for these differences between dose groups were obscure, but may be related to differences between effects caused by acute and subchronic levels of methylmercury in the auditory system. Electron microscopic examination of the vestibular hair cells has shown a greater hair cell loss in chronically dosed animals than in the acute case and this is supporting evidence for a dose-dependent effect on the auditory pathway (61).

Striking differences from pretreatment values were revealed in analysis of the latency test parameter. Loss in the high-frequency

range was suggested to be due to frequency-specific deficits to the lower auditory pathway. Absolute latencies also tended to be shortened for the majority of frequencies and time periods with methylmercury exposure. Thus, these authors proposed that this evidence indicated that methylmercury affected nerve processes which regulated nerve conduction velocity in the CNS. They suggested the possibility that methylmercury causes impairment of slow conduction. Damage of the small diameter fibers has been offered as one explanation for this hypersensitivity in nerve tissue. This would result in the majority of transmission being derived from fast conducting, large diameter fibers. If the small diameter fibers are more sensitive to methylmercury, this makes the small diameter nerve fibers in the brainstem a possible target for methylmercury (61). However, this is contradictory to the mechanism proposed by Wu et al. (57); therefore, further research is necessary to determine if it is the large or small diameter nerve fibers which are affected by methylmercury.

In summary, there appears to be a difference in distribution patterns in the brain with exposure to inorganic mercury as compared to methylmercury (43,44,45,46,47,48). Various areas of the brain tend to be more susceptible to neurological damage depending of the type of mercury exposure (48). Synaptic degeneration has been shown in the rat visual cortex after administration of methylmercury. Also, methylmercury appears to preferentially attack certain neurons of the visual system (51,52). Changes in protein synthesis and axonal transport in the visual system have been determined, thus promoting the concept of regenerative processes occurring in the rat (53,54).

Research done using ABR methods to investigate the effect of methylmercury on auditory function has shown that this metal causes high-frequency auditory deficits in mice (61). Methylmercury may also alter the conduction velocity of auditory nerve fibers and, therefore, cause hypersensitivity in the brainstem, but it is unclear which fibers are most affected (61). In addition, methylmercury does appear to have a differential effect on cochlear and the lower auditory pathway depending on the dosage (61). It is clear that future research is needed to elucidate the mechanism of action by which methylmercury affects the auditory systems.

Methylation Process

Although poisoning by inorganic mercurials has been known since ancient times, only recently has there been greater attention to the organic mercury compounds. Approximately 80% of our daily intake of mercury is methylmercury, with the average intake varying from 20 to 80 μg/day or more in heavily contaminated foods (39). Methylmercury compounds pass easily through the blood-brain barrier and the placenta and are more likely to target the nervous system, testes, and the developing embryo/fetus (12,39). Additionally, the toxicity of short-chain alkylmercury compounds, such as methylmercury, is accentuated due to their persistence in mammals.

A number of mechanisms have been proposed for metabolism of inorganic mercury *in vivo*. Early research has shown that rats injected with mercuric salts will excrete a small portion as volatile mercury, thus, suggesting that these animals are able to convert inorganic

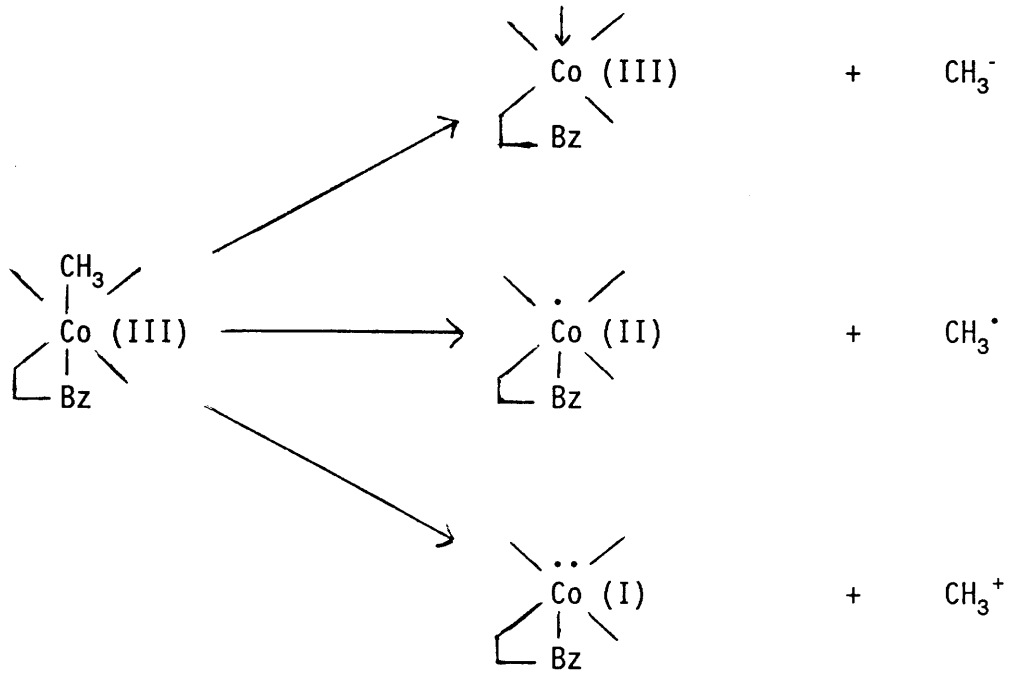
mercury either by a methylation reaction or reduction to the metallic form (62). Additional research done by Ogata and associates (63,64) has demonstrated that elemental mercury can be oxidized to produce the mercurous ion, Hg_2^{+2}, and the mercuric ion, Hg++. The mercuric ion can then be reduced to metallic mercury due to the activity of the xanthine oxidase system. This can be reversed with catalase activity (64). Another metabolic fate of inorganic mercury compounds is the biomethylation of mercuric ions via methyl B_{12} which has been shown to occur in certain bacteria (65,66,67).

Methylmercury is broken down slowly to inorganic mercury in the mammalian organism, but the site of demethylation has been difficult to determine (68). Suda and Takahashi (66) recently suggested that the spleen is an important site for the formation of inorganic mercury, and that macrophages participate in this biotransformation. It has been shown that the gut microflora also participate in the splitting of the carbon-mercury bond of methylmercury to produce inorganic mercury (66,67).

Therefore, it is important to examine the process by which inorganic mercury is converted to the potentially more toxic form of methylmercury. It has been established that bacteria not only in the soil and lakes, but also the intestinal tract can convert metallic and inorganic mercury forms to methylmercury (39,69,70,71,72). Pan-Hou and Imura (73) have shown that methylation of mercury which is mediated by methycobalamin may play an important role in the detoxication of the mercury by certain vitamin B_{12}-requiring strains of microorganisms.

On the basis of oxidation-reduction chemistry, the conditions for biomethylation can be formulated. Methylating agents which are available for methyl transfer in biological systems are known to involve three major coenzymes: 1) S-adenosylmethionine; 2) N^5-methyl-tetrahydrofolate derivatives; and 3) vitamin B_{12} derivatives. Research has indicated that vitamin B_{12} derivatives are the methylating agents for inorganic mercury salts since they are capable of transferring carbanion methyl groups (CH_3^-). S-adenosylmethionine can donate carbonium methyl groups (CH_3^+), while N^5-methyltetrahydrofolate can yield free radical methyl groups ($CH_3^\bullet$). Since vitamin B_{12} is capable of transferring methyl groups as free radicals ($CH_3^\bullet$), carbonium ions, or carbanions (Figure 1), theoretically there are three mechanisms which may be involved in the methylation of a number of metals, such as gold, tin, and platinum (8).

To date, two general mechanisms have been found for the methylation of a metal or metalloid by methylcobalamin: reactions in which the metal or metalloid acts as an electrophile (type I) and reactions in which the metal or metalloid acts to abstract a methyl radical (type II). The methylation of mercury is an example of type I which involves heterolytic cleavage of the Co-C bond of methylcobalamin with the transfer of a carbanion methyl group to the more oxidized state of the element, i.e. a carbanion methyl group is transferred to mercury to form methylmercury and a water molecule coordinates in the fifth ligand site of the cobalamin to give aquocobalamin [Co(III)] as a final product (Figure 2) (8).



Bz = 5,6-dimethylbenzimidazole

Figure 1. Examples of methylation reactions involving vitamin B_{12} (8).

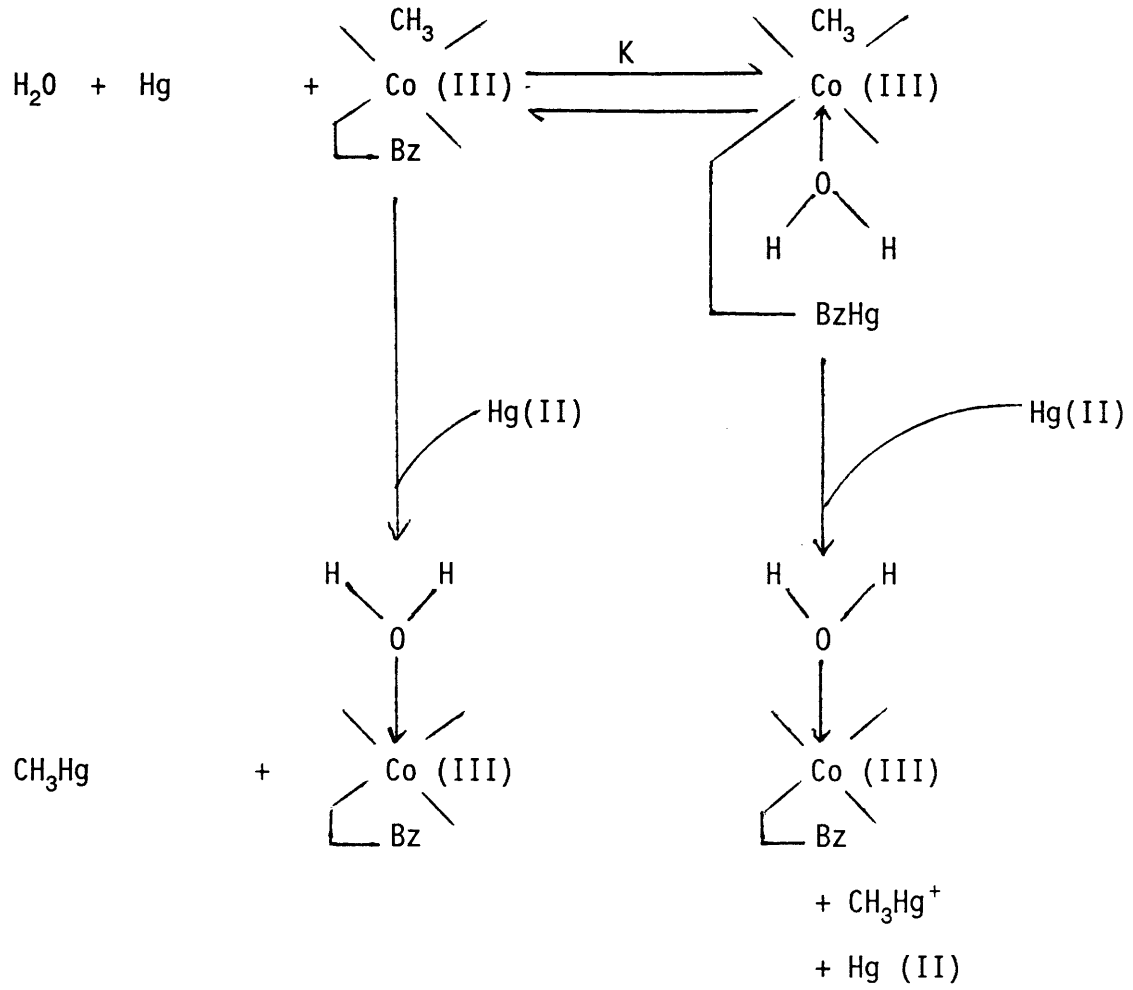


Figure 2. Mechanism for vitamin B_{12}-dependent methyl transfer to mercuric ion (type I) (8).

An example of a type II reaction is the methylation of thiols by methylcobalamin and involves reductive hololytic cleavage of the Co-C bond of methylcobalamin with the transfer of $CH_3^{\bullet}$ and the production of Co(II) cobalamin. The $CH_3^{\bullet}$ couples with the attacking metal or metalloid which results in a one-electron oxidation of the attacking element (8).

There is a metabolic interrelationship between folate, B_{12}, and methionine, but several important points remain unclear (9,74). The reaction which involves the enzyme, N^5-methyltetrahydrofolate methyltransferase, is an example of this interrelationship. As shown in Figure 3, this enzyme transfers a free radical methyl group from N^5-methyl tetrahydrofolate to vitamin B_{12} (Co+2). The B_{12} can then donate the free radical methyl group to homocysteine to form methionine and regenerate the Co+2 form of B_{12}. Methionine can be used to form S-adenosyl-methionine with the hydrolysis of ATP. Occasionally, for reasons not fully understood, the Co+2 form of B_{12} becomes oxidized to Co+3. When this occurs, riboflavin ($FADH_2$) can be used to reduce the B_{12} to the Co+1 form. S-adenosylmethionine will donate a carbonium (CH_3^+) methyl group to regenerate the Co+2 form of B_{12}. Thus, the cycle can begin again.

Use of Megavitamins

Since vitamin B_{12}, and possibly folate, appear to be involved in the methylation process of inorganic mercury, it is possible that individuals consuming megadoses of these vitamins may exhibit elevated

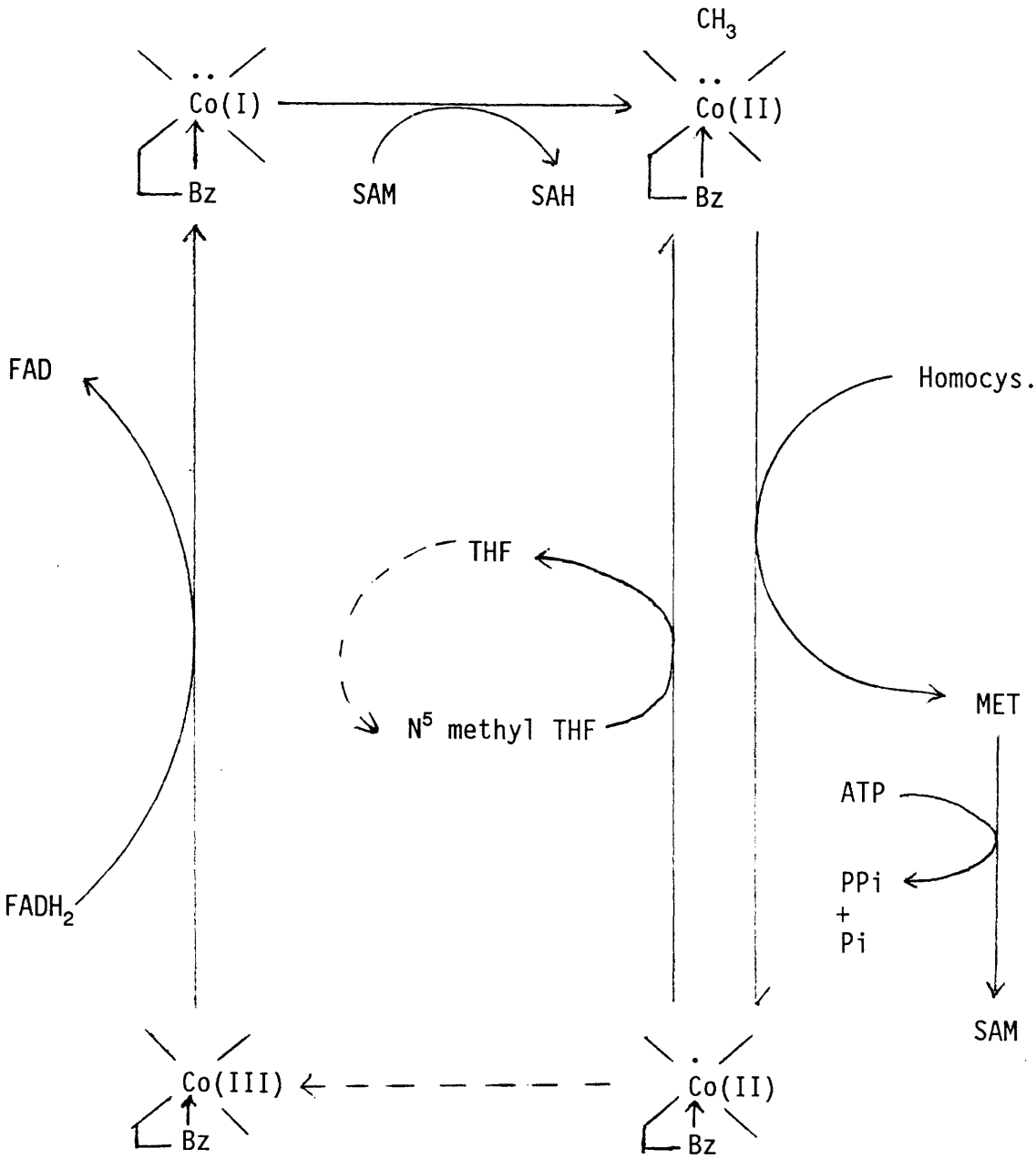


Figure 3. Interrelationship of folate, vitamin B_{12}, and methionine.

levels of methylmercury. Thus, it becomes important to examine the use of megavitamins in our society.

Vitamin advocates have in recent years argued that supplements, often in amounts ten or more times higher than recommended, will guard against a profusion of physical and psychological ills. Consumption is often encouraged by the philosphy that if a little is good, a lot is better (75). Consequently, the supplement business has blossomed intoa multibillion dollar industry due to the large-scale consumption of these products. It has been estimated that 40% to 50% of the adult population, both active and sedentary, rely on self-prescribed vitamin supplements (75,76,77). Athletes, coaches, and athletic trainers appear to be choice targets for the supplement sales force (76). Nutrition articles and advertisements in popular sports magazines have convinced some naive consumers that large doses of nutritional supplements are required for the active individual (76).

The vitamin B complex is one popular supplement that athletes consume. It has long been assumed that the water-soluble vitamins are nontoxic since excesses are excreted in the urine (76,78). These 8 vitamins, thiamin, riboflavin, niacin, pyridoxine, folacin, panthothenic acid, biotin and B_{12}, have been promoted to enhance athletic performance, but research has not supported this claim (76). Additionally, megadoses of the B vitamins have been reported to cause a number of side effects (78).

Probably the two most popular vitamin supplements are vitamin C and E (76,78,79). The sale of ascorbic acid supplements has skyrocketed due to Linus Pauling's claim that massive doses of vitamin C can cure colds.

Although the RDA for ascorbic acid is 60 mg a day, megadose advocates say we need as much as 10,000 mg (79). Like the B complex, vitamin C is soluble in water and excesses are excreted. No studies support the efficacy of vitamin C supplements as an ergogenic aid, and undesirable side effects have been demonstrated with large doses (76,80,81).

Potential Relationship of Megavitamins and Mercury Metabolism

Thus, as the research indicates, mercury presents human health hazards due to contaminated fishing waters and dental amalgams. Mercury apparently can exhibit toxic effects at various sites throughout the mammalian system and of significant importance is the possible neurotoxicological effects as a result of methylation processes which occur *in vivo*. This biomethylation of inorganic mercury via vitamin B_{12} suggests that higher levels of this vitamin may increase the amount of methylmercury found in the body. With the increased consumption of vitamin supplements in the United States, it is conceivable that mercury toxicity could rise significantly. Therefore, this project investigated the effect of megadoses of vitamins on the metabolism of mercury in various mammalian tissues. In order to examine possible neurological effects, auditory brainstem responses (ABR) were measured.

CHAPTER III

EXPERIMENTAL PROCEDURE

General Outline of Research

In order to study the effect of dietary intake on the *in vivo* methylation processes of inorganic mercury as well as the neurotoxic effect of methylmercury, three phases of investigation were conducted. Initially, the diets of guinea pigs were altered to include high levels of vitamin B_{12}, folate, and/or vitamin C. The effect of megavitamin ingestion on the methylation of mercury in various tissues of these animals was then examined. Next, the effect of dietary sulfur amino acid content on mercury metabolism was studied in rats by diet supplementation with methionine or cysteine. Finally, auditory brainstem evoked responses were used to study the neurotoxic effects of mercury metabolism and how these effects could be possibly altered by various dietary manipulations.

Preparative and Analytical Instrumentation

All reagents were analytical grade and specially selected for their low mercury content. Glassware was cleaned with nitric acid (4M) and rinsed with demineralized water. All solutions were prepared using demineralized water only.

Tissue homogenization was done on ice using a Brinkmann polytron homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.). All mercury assays were performed using 300 ml Wheaton Biological Oxygen Demand (B.O.D.) bottles. A controlled-temperature, water bath shaker

(Precision Scientific Company, Chicago, IL.) was employed for tissue digestion.

Subsequent mercury determinations were carried out by cold-vapor atomic absorption spectrometry. Absorption was measured with a Perkin-Elmer Model 3030 atomic absorption spectrophotometer equipped with a mercury, hollow cathode lamp. A cold-vapor apparatus was laboratory assembled according to directions provided by a Perkin-Elmer representative (personal communication) with an absorption cell (100 mm X 20 mm i.d.) used for the AAS measurements. The gas was first passed through an absorption cell (50 mm X 20 mm) containing potassium persulfate (Baker Chemical Company, Phillipsburg, N.J., Analytical Grade) which dried the material. After each measurement, the mercury was adsorbed in a carbon absorption tube (65 mm X 35 mm i.d.) which was connected to the apparatus with a bypass arrangement. The system was closed by means of a peristalic pump.

Animal Model

A pilot study was initially conducted to determine whether rats or guinea pigs would be best suited for the megavitamin portion of this study. It was determined that folacin and vitamin B_{12} play a more active role in the guinea pig than in the rat. This is possibly due to the fact that the guinea pig requires these vitamins for regeneration of methionine while the rat has a more active choline oxidase system (82). Consequently, the guinea pig accumulates more folacin and vitamin B_{12} than the rat with a dietary excess (83). A total of 55 male guinea pigs weighing approximately 250 grams were purchased from Hilltop Laboratory

Animals, Inc. (Scottdale, PA) and maintained on a 12-hour light-dark cycle in a controlled temperature environment.

Vitamin Preparation

Vitamin B_{12} and folic acid were purchased from ICN Biomedicals, Inc., Cleveland, OH, while L-ascorbic acid was obtained from Sigma Chemical Company, St. Louis, MO. Each vitamin was weighed and subsequently triturated with sucrose to a total weight of 500 g, which gave a final concentration of 4 mg folic acid, 50 μg B_{12}, and 1.8 g ascorbic acid per 4 g of triturate. This was then added to ground guinea pig chow at a concentration of 4 g/100 kg. The various vitamin combination mixtures and individual vitamin supplements were derived by adjusting the amount of sucrose so that vitamin concentrations remained constant. The control diet was prepared by adding sucrose at a concentration of 4 g/100 kg ground chow.

Dietary Regime

Vitamin treatment. Eight groups of five guinea pigs each were fed Purina Guinea Pig Chow 5025 and megadoses of vitamin B_{12}, folate, and/or vitamin C. The control diet consisted of animals fed ad libitum chow which contained all vitamins at levels required for normal guinea pig nutriture. One group of animals was fed a diet supplemented with megadoses of vitamin B_{12}, folate, and vitamin C. This group was referred to as the all vitamin treatment animals. Three groups of animals were provided diets which included megadoses of either B_{12} and folate, B_{12} and vitamin C, or folate and vitamin C. These groups served

as the combination vitamin treated animals and were referred to according to their respective vitamin blends. Finally, three groups of guinea pigs were given diets supplemented with the individual vitamins. These groups were called by the respective vitamin treatment, i.e. B_{12} only, folate only, vitamin C only. All diets were fed ad libitum for a total of eight weeks. Distilled water was freely available to all animals. Two groups of guinea pigs were weighed weekly to assure proper weight gain and feed consumption with vitamin treatment. Otherwise, all animals were housed in groups in polycarbonate cages with stainless steel wire lids and a raised wire floor with hardwood chip bedding.

Methionine and cysteine supplementation. Three groups of five each weaning-age (45-60 g bw.), male Sprague-Dawley rats (Taconic Farms, Germantown, N.Y.) were fed their respective diets for a 17 day dietary period. Animals were fed ad libitum one of three different 15% casein-based diets: 1) unsupplemented; 2) supplemented with 0.625% methionine; or 3) supplemented with 0.505% cysteine as shown in Table 1 (84). Distilled water was readily available and the animals were housed individually in galvanized wire-bottom cages.

Mercury Treatment

At the end of the dietary period, each animal was given 0.6 mg $HgCl_2$/kg body weight as a subcutaneous injection in isotonic saline every other day for nine doses (85). Animals were observed throughout this treatment period for overt signs of mercury toxicity. Twenty-four hours after the last injection, the animals were sacrificed and their

Table 1. Composition of Methionine or Cysteine Supplemented Diets

Dietary Constituent	Unsupplemented	Methionine	Cysteine
	g/100g	g/100g	g/100g
Casein	15	15	15
Sucrose	30	30	30
Cornstarch	30	30	30
Vegetable Oil[1]	2	2	2
Vegetable Shortening[2]	6	6	6
Vitamin Mixture[3]	2	2	2
Basal Salt Mix[4]	1.34	1.34	1.34
$CaCO_3$	1.32	1.32	1.32
$CaSO_4$-$2H_2O$[5]	0.04	0.04	0.04
Methionine	--	0.625	--
Cysteine	--	--	0.505
Non-nutritive bulk[6]	12.3	11.7	11.8

[1]Wesson Oil -- Hunt-Wesson Foods, Inc.

[2]Crisco -- Proctor and Gamble.

[3]Nutrition Biochemicals Corporation, Cleveland, Ohio. Vitamix diet fortification mixture was formulated to supply the following amounts of vitamins (g/kg vitamin premix): thiamin hydrochloride, 1.0; riboflavin, 1.0; niacin, 4.5; p-aminobenzoic acid, 5.0; calcium pantothenate, 3.0; pyridoxine hydrochloride, 1.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; biotin, 0.020; folic acid, 0.090; vitamin B_{12}, 0.00135; α-tocopherol, 22.0; vitamin A, 1.8; vitamin D, .125; and sufficient glucose to make 1 kg.

[4]Formulated to contain the following amounts of minerals (g/500g salt mixture): magnesium carbonate, 33.7; sodium chloride, 77.4; potassium chloride, 125.4; potassium monobasic phosphate, 237.8; ferric phosphate, 23; potassium iodide, 0.090; sodium fluoride, 0.01; manganese chloride, 0.45; aluminum potassium sulfate, 0.019; cupric acetate, 0.810 and zinc carbonate, 4.8.

[5]$CaSO_4$-$2H_2O$ provided 0.02% $SO_4^=$.

[6]Alphacel -- ICN Biochemical.

liver, spleen, heart, kidneys, lungs, and brain were removed and frozen at -80°C for subsequent mercury determinations. Muscle tissue was also removed from the thigh of the hind leg. In addition, hair samples were taken from the guinea pigs and blood samples were taken from several treatment groups. However, blood, muscle, and hair tissues were not analyzed in rats.

Animal Sacrifice and Tissue Preparation

Guinea pigs were anesthetized with a 10 ml interperitoneal injection of 1% Brevital Sodium (Eli Lilly and Co., Indianapolis, IN) and decapitated with shears. Blood was immediately collected in test tubes and stoppered. Rats were stunned by a blow to the back of the head and decapitated with shears. Selected tissues were rapidly excised from all animals, placed in storage containers on ice, and subsequently stored at -80°C. Hair samples were also taken from the back of the guinea pigs' necks and stored.

Upon thawing, a 25% (w/v) homogenate was prepared for each tissue, except blood and hair, using 1.5 M potassium hydroxide (w/v). Mercury has been shown to be stable in alkaline media over extended periods and alkali is more effective than acid in the hydrolyzation of protein (86).

The majority of methylmercury (more than 90%) and approximately 50% of metallic mercury in the blood has been found within the erythrocytes (87). Additionally, it has been reported that determination of mercury in blood samples can be difficult due to low concentrations (88). Therefore, blood samples were thawed and homogenized without dilution until a uniform solution was obtained. This was the form used for

subsequent mercury analysis and reflected all blood sample constituents.

Hair has been shown to accumulate and retain mercury over the life of the hair fiber. Thus, it is an excellent retrospective indicator of mercury in cases of acute poisoning as well as prolonged uptake of low concentrations of mercury compounds (14,89). In the present study, a weighed sample of 0.25 g hair was used for mercury analysis.

Selective Determination of Inorganic Mercury and Methylmercury by Cold-Vapor Atomic Absorption

One of the major problems in mercury analysis is the need to destroy the organic material and release the mercury from the sample (4,86). A number of methods exist for the destruction of organic materials (4,86,90,91). However, techniques requiring the digestion of samples do not enable inorganic mercury and methylmercury to be distinquished (86,90-97). Consequently, many methods have been proposed which involve the extraction of methylmercury using benzene and/or toluene (24,98-101). However, experimentation in this lab resulted in the conclusion that these methods could not be used by atomic absorption, as benzene and toluene gave falsely high readings. Experiments in which labelled mercury compounds were used have shown that inorganic mercury can be selectively determined in the presence of organomercurials by its selective reduction with tin (II) chloride (102). Magos et al. (88,103) discovered that the rate of methylmercury reduction and other organomercurials by tin (II) chloride can be made identical to that of inorganic mercury. This can be done if the amount of tin (II) chloride is above a certain level and a cadmium salt is

added to the reaction mixture. Thus, a method was developed so that inorganic mercury can be released first, followed by the release of methylmercury from the same sample (88,103).

The method used in this research was adapted from several sources (14,104-108) and allows for selective reduction of inorganic mercury and methylmercury using the same sample. These two measurements were then added together to determine total mercury concentration.

Mercury standard solutions. A mercury (II) stock solution was prepared by dissolving 0.6767 g $HgCl_2$ (Fisher Scientific Company, Memphis, TN., Analytical Grade) in 0.05 M sulfuric acid and diluting to 100 ml volume with the same acid solution. This yielded a concentration of 1.000 mg Hg/ml. From this solution, a working standard was prepared by diluting with acid solution to provide a total concentration of 0.4 μg Hg/ml.

The methylmercury stock solution (500 μg/L) was prepared by dissolving 62.58 mg CH_3HgCl (Pfaltz & Bauer, Inc., Waterbury, CT) in 100 ml of 95% ethanol. The working standard was prepared by diluting with demineralized water and yielded a final concentration of 0.5 μg CH_3Hg/ml.

Reagents. Solutions of 1% (w/v) L-cysteine, 1% sodium chloride, and 45% sodium hydroxide were prepared by dissolving these compounds in appropriate amounts of demineralized water. An 8 M sulfuric acid solution was also prepared using demineralized water. These were all analytical grade reagents which had been tested for mercury content by the manufacturer.

A 10% (w/v) tin (II) chloride solution was prepared by dissolving $SnCl_2 \cdot 2H_2O$ (Baker Chemical Company, Phillipsburg, N.J., Hg content < 0.5 ppm) in dilute HCl solution (100 ml concentrated HCl/L). This was prepared fresh daily.

The tin (II) chloride - cadmium (II) chloride solution was prepared by dissolving 25 g of $SnCl_2 \cdot 2H_2O$ and 5 g $CdCl_2 \cdot 2.5H_2O$ (American Scientific Company, Stone Mt., GA) in 50 ml of dilute HCl. The solution was then heated and mixed until it was clear. This solution was also prepared fresh daily.

Procedure. Representative samples of each tissue homogenate were pipetted to B.O.D. bottles: 5 ml of liver and muscle; 2.5 ml of brain, lungs, and heart plus 2.5 ml of 1.5 M KOH; 1.0 ml of kidney and spleen plus 4.0 ml of 1.5 M KOH; 1 ml of blood plus 5 ml of 1.5 M KOH; and 0.25 g weighed hair sample plus 5.0 ml of 1.5 M KOH. Then, 1 ml of 1% cysteine and 1.24 ml of 12 N sodium hydroxide were added to each reaction vessel. At all times, a total of 22.4 meq of hydrogen ions was maintained in each sample solution. The samples were digested in a water-bath at 85°C for five minutes. After digestion, the samples were immediately placed in an ice bath and 12.76 ml of 1% sodium chloride were added to each bottle. Total reaction volume was 20 ml and all samples were maintained on ice until AAS was performed.

Approximately 30 minutes before the start of a run, the atomic absorption spectrophotometer was switched on and the absorption reading verified at 253.7 nm. At the beginning of each run, inorganic mercury standards were run by adding 1 ml of 10% stannous chloride solution and

absorption values read. Also, absorption was determined for the methylmercury standard.

Each B.O.D. bottle containing the digested sample was placed in a ice water bath under the aerator tube assembly. The following were added in sequence; 1-2 drops Antifoam A Emulsion (Sigma Chemical Company, St. Louis, MO), 10 ml of 8 M sulfuric acid, 1 ml of 10% tin (II) chloride solution, 20 ml of 45% sodium hydroxide, and 50 ml of demineralized water. The aerator was immediately attached, the pump turned on, and the air flow was continued through the reaction vessel for exactly one minute. At this time the absorption peak for inorganic mercury was read. The aerator tube was immediately removed from the sample and the air flow switched to remove the mercury through the activated charcoal chamber. The pump was allowed to run for a maximum of one minute or until absorption was zeroed, at which time the air flow was stopped.

Exactly two minutes after the first reduction process was begun, 10 ml of 8 M sulfuric acid, 2 ml of tin (II) chloride - cadmium chloride reagent, and 20 ml of 45% sodium hydroxide were added sequentially to the sample. The aerator tube was attached immediately and the pump was switched on. The reaction was allowed to occur until a maximum absorption peak for methylmercury was obtained and recorded. At the end of the run, the air flow was again diverted through the charcoal absorption tube to remove all mercury.

The concentrations of inorganic mercury and methylmercury were subsequently calculated using the standard solution concentrations and absorption values. All samples were run in duplicate and extreme

variations (over 5%) in measurements resulted in repeated assay of the sample.

Auditory Brainstem Response (ABR) Testing

Preyor's reflex was performed on all animals midway through mercury treatment and immediately before ABR testing was conducted. This consisted of visual observation of ear twitching in response to a loud noise.

All ABR recordings were obtained in a quiet room. Animals were anesthetized with isoflurane. Recording electrodes were placed subcutaneously at the vertex and over the mastoid of the right ear and a ground electrode was inserted into the nap of the neck. Active electrode impedence was controlled below 10 kΩ and normally was within 1 kΩ of a reference electrode. The right ear was tested with pure tones while the left ear received white noise which was 30 dB SPL (sound pressure level) less than the testing signal. Potentials were evoked using pure tones of alternating polarity at a rate of 11.4/sec. The potential difference between the vertex and the mastoid electrode ipsilateral to the stimulated ear was amplified and band-passed at 150-3000 Hz. Responses (10 msec) to 256 stimuli were averaged to produce each ABR and a repetition of each stimulus paradigm condition was made to assess reproducibility. A total of four stimulus frequencies (8k, 6k, 4k, and 2k) were used beginning at an intensity of 105 SPL and decreasing in 10 dB steps until close to threshold and then, 5 dB steps to threshold. Following each experiment, the animals were

given an otoscopic examination to assure that the ear canal was free of cerumen.

After all tests were completed, hard copies of each recording were made. Four to 5 peaks of the averaged signal were identified and labeled with Roman numerals. In most cases, only one wave occurred in the III and IV latency range. Therefore, no value was recorded for wave IV and the single wave (III and IV complex) was labeled as wave III. Wave latencies were measured for waves I, II, and V with latency defined as the time from stimulus onset to the positive peak of each wave. These measurements were recorded to the nearest 0.01 msec. The amplitude of each wave was determined by measuring from its peak positivity to the following negative trough. These measurements were recorded to the nearest 0.01 μV. Interpeak latency (IPL) was derived by subtracting the wave I latency from the wave V latency. In addition, thresholds were determined by the appearance of peak I as an indicator.

Statistical Analysis

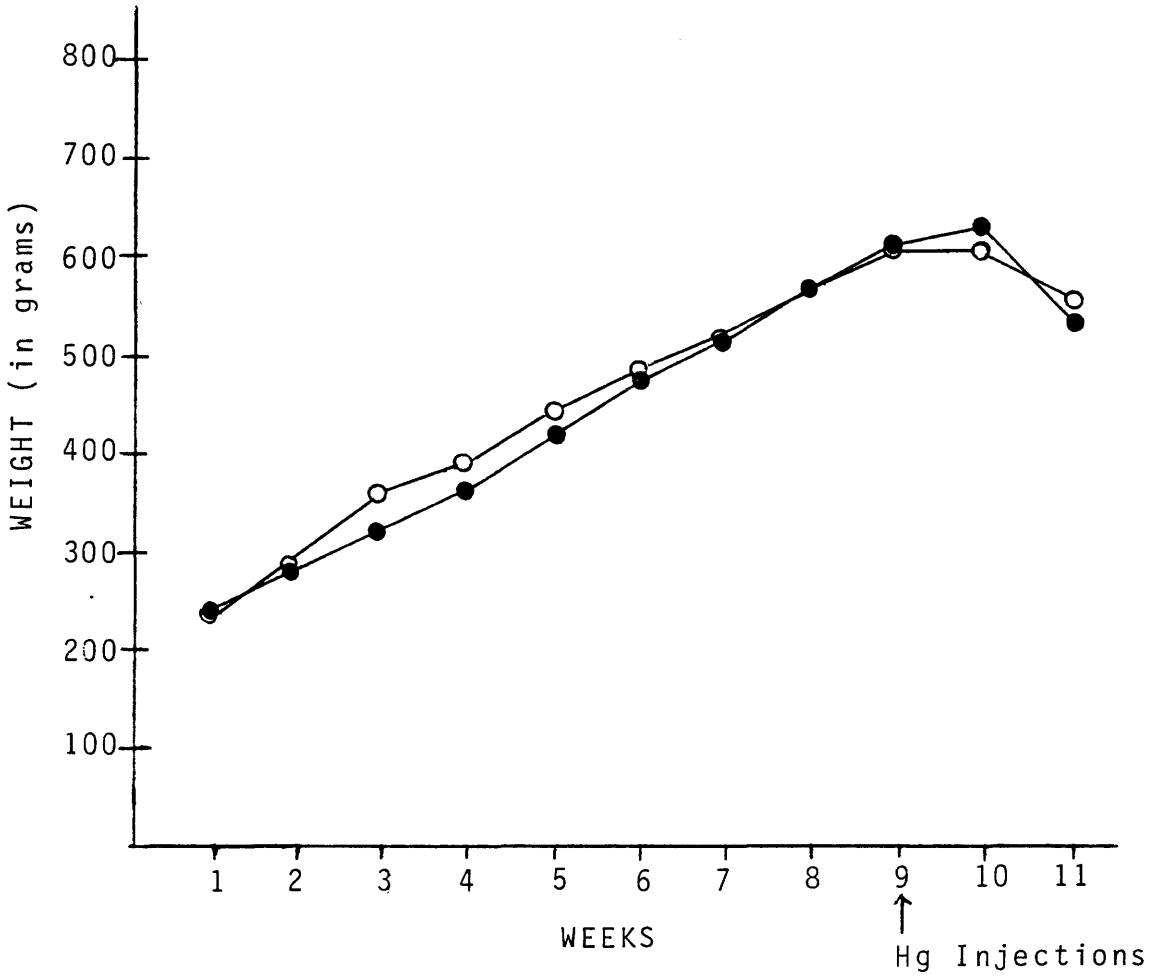
All data were evaluated by Analysis of Variance (ANOVA) performed with SAS (SAS Program, University of Tennessee) using the General Linear Model (GLM) procedures. Differences between groups for each tissue were determined by Duncan's multiple-range test (109).

CHAPTER IV

RESULTS

This research project consisted of three separate investigations. First, several groups of guinea pigs were fed either a control diet or a diet which included megadoses of vitamin B_{12}, folate, and/or vitamin C in various combinations. After eight weeks on the diet regime, these animals were given an injection of mercuric chloride every other day for a total of nine injections, sacrificed, and tissues removed. Each tissue was then analyzed for inorganic and methyl mercury. Total mercury and the percent inorganic and methyl mercury were subsequently determined. Weight/growth data were obtained for one group of guinea pigs fed either a control or B_{12}/folate diet. This information is charted on Figure 4 and the measurements are shown in Appendix 1. These data indicated that both groups had consistent weight gain, therefore; difference in growth was not a factor in the mercury data analysis. Also after mercury injections were begun, a slight weight loss was observed in both groups which is a phenomenon reported by a number of researchers (32,51,54,57).

Since methionine is also a potential methyl donor in mammals, a second aspect of this research was to investigate the effect of methionine or cysteine on mercury metabolism in rats. These animals were fed either a control diet, or methionine or cysteine supplemented diet for seventeen days, then given mercury injections every other day for a total of nine injections. After sacrifice, the tissues from these



Values represent the average for 5 guinea pigs in either the control (—●—) or B_{12}/folate (—○—) animal groups.

Figure 4. Weight gain of guinea pigs fed either a control or B_{12}/folate diet for 11 weeks.

animals were also analyzed for inorganic and methyl mercury with subsequent determination of total and percent mercury.

Finally, several groups of guinea pigs treated with megavitamins and mercury were tested for auditory brainstem responses (ABR). Following ABR evaluation the animals were sacrificed and tissue mercury analysis performed. The ABR data were used to examine the neurotoxicity of mercury.

The results reported here are divided into guinea pig data, rat cysteine/methionine data, and ABR analysis. Additionally, the guinea pig data are further divided into controls versus all vitamin dietary regime, controls compared to various vitamin combinations, and controls versus animals treated with megadoses of the individual vitamins.

Controls vs All Vitamins

A total of eight tissues were analyzed for inorganic and methylmercury. These values were added together to derive total mercury levels. Percent values were also calculated. The results are shown on Table 2. There were several trends observed between the two groups.

Inorganic mercury was higher in the heart (1.29 μg vs .72 μg) and lungs (.82 μg vs .74 μg) in the vitamin treated group compared to controls. However, inorganic mercury was lower in the liver (.52 μg vs .78 μg), muscle (.10 μg vs .21 μg), brain (.19 μg vs .30 μg), spleen (.52 μg vs .89 μg), kidney (16.96 μg vs 19.17 μg) and hair (.73 μg vs .80 μg) in the vitamin group compared to controls.

Methylmercury tended to follow the same trends as the inorganic form. It was lower in the vitamin group than controls in the liver

Table 2. Mercury Levels in Various Tissues of Guinea Pigs with the All-Vitamin Dietary Regime

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Liver					
Controls (n=18)	.78 ± .28	.37 ± .20	1.15 ± .46	69	31
Vitamins (n=8)	.52 ± .12	.25 ± .03	.77 ± .14	67	33
Muscle					
Controls	.21 ± .27	.18 ± .14	.39 ± .38	51	49
Vitamins	.10 ± .02	.13 ± .09	.23 ± .10	47	53
Brain					
Controls	.30 ± .17	.24 ± .15	.54 ± .31	56	44
Vitamins	.19 ± .06	.18 ± .07	.37 ± .11	51	49
Heart					
Controls	.72 ± .61	.35 ± .19	1.07 ± .76	63	37
Vitamins	1.29 ± .91	.60 ± .33	1.90 ± 1.24	66	34
Lungs					
Controls (n=13)	.74 ± .18	.40 ± .07	1.14 ± .22	65	35
Vitamins	.82 ± .16	.41 ± .07	1.23 ± .21	67	33
Spleen					
Controls	.89 ± .51	.59 ± .20	1.48 ± .69	58	42
Vitamins	.52 ± .10	.41 ± .10	.93 ± .18	56	44
Kidney					
Controls	19.17 ± 2.49	14.72 ± 2.11	33.89 ± 4.49	57	43
Vitamins	16.96 ± .75	13.46 ± .80	30.42 ± 1.44	56	44

Table 2. (Continued)

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Hair					
Controls (n=13)	.80 ± .26	.65 ± .23	1.44 ± .47	55	45
Vitamins	.73 ± .33	.70 ± .25	1.43 ± .48	51	49

Values represent the average ± S.D. The data for the all-vitamin treated group was not statistically different from controls.

(.25 μg vs .37 μg), muscle (.13 μg vs .18 μg), brain (.18 μg vs .24 μg), spleen (.41 μg vs .59 μg) and kidney (13.46 μg vs 14.72 μg), while it was higher in the heart (.60 μg vs .35 μg) and the lungs (.41 μg vs .40 μg). The only exception was found in hair tissue, which contained a higher methylmercury content in the vitamin groups (.70 μg) compared to controls (.65 μg).

The vitamin group exhibited lower total mercury concentrations in the liver (.77 μg vs 1.15 μg), muscle (.23 μg vs .39 μg), brain (.37 μg vs .54 μg), spleen (.93 μg vs 1.48 μg), kidney (30.42 μg vs 33.89 μg), and hair (1.43 μg vs 1.44 μg) compared to controls. Total mercury tended to be higher in the vitamin group than controls in the heart (1.90 μg vs 1.07 μg) and the lungs (1.23 μg vs 1.14 μg).

Interestingly, tissues from the vitamin group that had lower mercury concentrations had a higher percent of mercury as methylmercury. For example, the percent methylmercury in the liver (33% vs 31%), muscle (53% vs 49%), brain (49% vs 44%), spleen (44% vs 42%), kidney (44% vs 43%), and hair (49% vs 45%) was higher in the vitamin treated group compared to controls. Both the heart and lungs had lower percent methylmercury for vitamins versus controls (34% vs 37%, and 33% vs 35%, respectively).

As noted, there were a number of trends exhibited between the vitamin treated group and control animals. However, these differences were not statistically significant in any of the tissues analyzed.

Controls vs Vitamin Combinations

The results from five tissues are shown in Table 3. In the liver there was no statistical difference found between controls, B_{12}/vitamin C and folate/vitamin C for inorganic, methyl, and total mercury, as well as percent inorganic and methylmercury. The B_{12}/folate group was significantly higher than controls for inorganic mercury (1.05 μg vs .78 μg), methylmercury (.67 μg vs 37 μg) and total mercury (1.72 μg vs 1.15 μg). Also the percent methylmercury was higher ($p < 0.05$) in the B_{12}/folate group (40%) compared to controls (31%). In addition, the B_{12}/folate group had values which were significantly greater than the B_{12}/vitamin C group for inorganic, methyl, total, percent inorganic, and percent methylmercury, but had statistically different values from the folate/vitamin C group for only methylmercury concentration.

There were no differences between the control, B_{12}/folate, and B_{12}/vitamin C groups for the various mercury concentrations and percent measurements in muscle. The folate/vitamin C group was statistically different from the controls and the other combination groups for inorganic, methyl and total mercury concentrations, but was not different in percent inorganic or percent methylmercury for this tissue. Interestingly, the two B_{12} groups (B_{12}/folate and B_{12}/vitamin C) showed a higher percent methylmercury (61% and 57%, respectively) compared to the control and folate/vitamin C groups (49% and 50% respectively). However, this difference was not statistically significant.

In the brain, no differences were observed between controls and the vitamin combinations for inorganic, methyl or total mercury. The B_{12}/vitamin C group had a higher ($p < 0.05$) percent of mercury as

Table 3. Mercury Levels in Various Tissues of Guinea Pigs with the Vitamin Combination Dietary Regime

Treatment	μg Hg/g wet tissue			% Inorganic	% Methyl
	Inorganic Hg	Methyl Hg	Total Hg		
Liver					
Controls	.78 ± .28[a,b]	.37 ± .20[a]	1.15 ± .46[a,b]	69[a]	31[a]
B_{12}/Folate	1.05 ± .25[a]	.67 ± .09[b]	1.72 ± .33[a]	60[b,c]	40[b,c]
B_{12}/Vitamin C	.61 ± .09[b]	.27 ± .04[a]	.88 ± .13[b]	69[a]	31[a]
Folate/Vitamin C	.78 ± .10[a,b]	.38 ± .07[a]	1.15 ± .10[a,b]	67[a,b]	33[a,b]
Muscle					
Controls	.21 ± .27[a]	.18 ± .14[a]	.39 ± .38[a]	51[a,b,c]	49[a,b,c]
B_{12}/Folate	.12 ± .03[a]	.19 ± .04[a]	.31 ± .04[a]	39[b]	61[b]
B_{12}/Vitamin C	.27 ± .29[a]	.31 ± .19[a]	.58 ± .45[a]	43[a,b]	57[b,c]
Folate/Vitamin C	1.33 ± 1.60[b]	.83 ± .83[b]	2.16 ± 2.40[b]	50[a,b,c]	50[a,b,c]
Brain					
Controls	.30 ± .17[a]	.24 ± .15[a]	.54 ± .31[a]	56[a,b]	44[a,b]
B_{12}/Folate	.16 ± .02[a]	.15 ± .03[a]	.31 ± .04[a]	51[b]	49[a]
B_{12}/Vitamin C	.20 ± .08[a]	.29 ± .14[a]	.49 ± .22[a]	41[c]	59[c]
Folate/Vitamin C	.25 ± .09[a]	.18 ± .09[a]	.43 ± .14[a]	58[a,b]	42[a,b]
Heart					
Controls	.72 ± .61[a,b]	.35 ± .19[a,b]	1.07 ± .76[a,b]	63[a]	37[a]
B_{12}/Folate	.26 ± .03[b]	.33 ± .19[b]	.59 ± .20[b]	48[b]	52[b]
B_{12}/Vitamin C	.44 ± .31[b]	.23 ± .12[b]	.67 ± .43[b]	63[a]	37[a]
Folate/Vitamin C	.84 ± .73[a,b]	.39 ± .26[a,b]	1.23 ± .99[a,b]	64[a]	36[a]

Table 3. (Continued)

Treatment	μg Hg/g wet tissue			% Inorganic	% Methyl
	Inorganic Hg	Methyl Hg	Total Hg		
Lungs					
Controls	.74 ± .18[a]	.40 ± .07[a]	1.14 ± .22[a,b]	65[a,b]	35[a,b]
B_{12}/Folate	.69 ± .18[a]	.40 ± .07[a]	1.10 ± .19[b]	63[b]	37[a]
Spleen					
Controls	.89 ± .51[a,b]	.59 ± .20[a,b]	1.48 ± .69[a,b,c]	58[a,b]	42[a,b]
B_{12}/Folate	.70 ± .13[b]	.54 ± .05[a,b]	1.24 ± .17[a,b]	56[b]	44[a]
B_{12}/Vitamin C	1.42 ± 1.00[a,b]	.76 ± .46[b,c]	2.18 ± 1.46[b,c]	64[a]	36[b]
Folate/Vitamin C	1.39 ± 1.08[a,b]	.67 ± .37[a,b,c]	2.06 ± 1.45[a,b,c]	65[a]	35[b]
Kidney					
Controls	19.17 ± 2.49[a,b]	14.72 ± 2.11[a,b]	33.89 ± 4.49[a,b,c]	57[a]	43[a]
B_{12}/Folate	20.46 ± 2.43[a]	16.92 ± 1.61[c]	37.38 ± 4.01[c]	55[a,b]	45[a,b]
B_{12}/Vitamin C	17.91 ± .56[a,b]	14.11 ± .82[a,b]	32.02 ± 1.33[a,b]	56[a,b]	44[a,b]
Folate/Vitamin C	18.30 ± 2.00[a,b]	14.77 ± 1.42[a,b]	33.07 ± 3.30[a,b,c]	55[a,b]	45[a,b]
Hair					
Controls	.80 ± .26[a]	.65 ± .23[a]	1.44 ± .46[a]	55[a]	45[a]
B_{12}/Folate	.87 ± .20[a]	.78 ± .08[a,b]	.165 ± .25[a,b]	53[a]	47[a]
Blood (μg Hg/ml)					
Controls	.19 ± .04[a,b]	.18 ± .05[a]	.37 ± .09[a,b]	53[a]	47[a]
B_{12}/Folate	.18 ± .03[a]	.17 ± .02[a,b]	.35 ± .03[b]	51[a]	49[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

methylmercury compared to controls and the other vitamin combinations. The percent inorganic mercury also reflected this difference.

Controls were not statistically different from all the vitamin combinations for inorganic, methyl, and total mercury concentrations in the heart. However, the B_{12}/folate group had a significantly higher percent methyl (52%) measurement compared to controls (37%), the B_{12}/vitamin C group (37%) and the folate/vitamin C group (36%). A trend was noted in this tissue in which the two B_{12} groups exhibited the lowest mercury concentrations (inorganic, methyl, and total), while the folate/vitamin C group tended to have the highest concentrations.

Only one vitamin combination was studied in lung tissue. There was no difference in mercury concentrations or percent mercury with B_{12}/folate compared to controls.

In the spleen, no difference was observed between the controls and the various vitamin combinations for mercury concentration and percent mercury. The B_{12}/folate group had significantly higher percent methylmercury (44%) compared to the B_{12}/vitamin C (36%) and folate/vitamin C groups (35%). Interestingly, the two vitamin C groups exhibited a trend of higher mercury concentrations and percent inorganic mercury as compared to controls and the B_{12}/folate group.

In the kidney, no difference in inorganic mercury and percent mercury was shown for the vitamin combinations and controls. The B_{12}/folate group had a significantly higher methylmercury concentration (16.92 μg) than control (14.72 μg), B_{12}/vitamin C (14.11 μg), and folate/vitamin C (14.77 μg) groups. In addition, the B_{12}/folate group had significantly more total mercury (37.38 μg) compared to the

B_{12}/vitamin C group (32.02 μg). While this group exhibited an apparent increase in total mercury as compared to controls (33.89 μg) and the folate/vitamin C group (33.07 μg), this difference was not statistically significant.

Hair and blood were evaluated for the control and B_{12}/folate groups. Mercury concentrations and percent mercury were not significantly different in either of these tissues for the two groups.

Controls vs Individual Vitamin Treatment

The results from the experiments done to examine the effect of megadoses of B_{12}, folate, or vitamin C on mercury metabolism are shown in Table 4. In animals treated with the vitamins separately, the concentrations of all forms of mercury in the liver were significantly higher than the controls. Additionally, the percent methylmercury was greater ($p < 0.05$) in the B_{12} only, folate only, and vitamin C only treated groups compared to controls. The percent inorganic mercury was lower in the individual vitamin groups compared to controls and this difference was statistically significant.

In muscle there was no statistical difference in mercury concentrations (inorganic, methyl, and total) between the vitamin treated groups and controls. However, in the vitamin C group, the percent inorganic mercury was higher and percent methyl lower than control animals. These differences were statistically significant. There was also a tendency for the vitamin treated animals to have lower concentrations of all forms of mercury.

Table 4. Mercury Levels in Various Tissues of Guinea Pigs with the Individual Vitamin Dietary Regime

Treatment	μg Hg/g wet tissue			% Inorganic	% Methyl
	Inorganic Hg	Methyl Hg	Total Hg		
Liver					
Controls	.78 ± .28[a,b]	.37 ± .20[a]	1.15 ± .46[a,b]	69[a]	31[a]
B_{12}	1.58 ± .20[c]	1.16 ± .40[c]	2.74 ± .57[c]	59[c]	41[c]
Folate	1.60 ± .71[c]	1.22 ± .27[c]	2.83 ± .90[c]	56[c]	44[c]
Vitamin C	1.72 ± .54[c]	1.08 ± .19[c]	2.79 ± .54[c]	61[b,c]	39[b,c]
Muscle					
Controls	.21 ± .27[a]	.18 ± .14[a]	.39 ± .38[a]	51[a,b,c]	49[a,b,c]
B_{12}	.18 ± .04[a]	.14 ± .02[a]	.32 ± .04[a]	55[a,c,d]	45[a,c,d]
Folate	.15 ± .04[a]	.10 ± .04[a]	.25 ± .07[a]	60[c,d]	40[a,d]
Vitamin C	.16 ± .03[a]	.07 ± .02[a]	.24 ± .03[a]	69[d]	31[d]
Brain					
Controls	.30 ± .17[a]	.24 ± .15[a]	.54 ± .31[a]	56[a,b]	44[a,b]
B_{12}	.27 ± .04[a]	.19 ± .03[a]	.46 ± .03[a]	58[a,b]	42[a,b]
Folate	.30 ± .05[a]	.18 ± .02[a]	.48 ± .04[a]	63[a]	37[b]
Vitamin C	.23 ± .03[a]	.19 ± .02[a]	.42 ± .04[a]	56[a,b]	44[a,b]
Heart					
Controls	.72 ± .61[a,b]	.35 ± .19[a,b]	1.07 ± .76[a,b]	63[a]	37[a]
B_{12}	.32 ± .04[b]	.17 ± .04[b]	.50 ± .06[b]	65[a]	35[a]
Folate	.31 ± .06[b]	.13 ± .01[b]	.45 ± .05[b]	70[a]	30[a]
Vitamin C	.28 ± .06[b]	.14 ± .04[b]	.42 ± .06[b]	66[a]	34[a]
Lungs					
Controls	.74 ± .18[a]	.40 ± .07[a]	1.14 ± .22[a,b]	65[a,b]	35[a,b]
B_{12}	1.01 ± .30[b]	.42 ± .10[a]	1.43 ± .38[a]	70[c,d]	30[c,d]
Folate	.86 ± .10[a,b]	.32 ± .03[a]	1.18 ± .12[a,b]	73[d]	27[d]
Vitamin C	.90 ± .25[a,b]	.40 ± .11[a]	1.29 ± .35[a,b]	69[a,b,c]	31[b,c,d]

Table 4. (Continued)

Treatment	μg Hg/g wet tissue			% Inorganic	% Methyl
	Inorganic Hg	Methyl Hg	Total Hg		
Spleen					
Controls	.89 ± .51[a,b]	.59 ± .20[a,b]	1.48 ± .69[a,b,c]	58[a,b]	42[a,b]
B_{12}	1.73 ± .53[a]	.91 ± .18[c]	2.64 ± .70[c]	65[a]	35[b]
Folate	1.39 ± .98[a,b]	.81 ± .24[b,c]	2.21 ± 1.22[b,c]	60[a,b]	40[a,b]
Vitamin C	1.75 ± 1.16[a]	.93 ± .35[c]	2.68 ± 1.51[c]	63[a,b]	37[a,b]
Kidney					
Controls	19.17 ± 2.49[a,b]	14.72 ± 2.11[a,b]	33.89 ± 4.49[a,b,c]	57[a]	43[a]
B_{12}	19.08 ± 1.65[a,b]	15.84 ± .34[b,c]	34.91 ± 1.90[a,b,c]	54[b]	46[b]
Folate	18.60 ± 1.69[a,b]	15.57 ± .88[b,c]	34.16 ± 2.54[a,b,c]	55[b]	45[b]
Vitamin C	20.12 ± .88[a]	15.94 ± .67[b,c]	36.07 ± 1.17[b,c]	56[a,b]	44[a,b]
Hair					
Controls	.80 ± .26[a]	.65 ± .23[a]	1.44 ± .47[a]	55[a]	45[a]
B_{12}	.95 ± .32[a]	1.27 ± .63[a,b,c]	2.22 ± .87[a,b,c]	44[a,b]	56[a,b]
Folate	.95 ± .20[a]	1.84 ± 1.38[c]	2.79 ± 1.36[c]	40[b]	60[b]
Vitamin C	.95 ± .34[a]	1.47 ± .88[b,c]	2.43 ± .93[b,c]	43[a,b]	57[a,b]
Blood (μg Hg/ml)					
Controls	.19 ± .04[a,b]	.18 ± .05[a]	.37 ± .09[a,b]	53[a]	47[a]
B_{12}	.24 ± .05[a,b]	.15 ± .02[a,b]	.38 ± .07[a,b]	61[b]	39[b]
Folate	.20 ± .02[a,b]	.13 ± .01[b]	.33 ± .02[b]	60[b]	40[b]
Vitamin C	.28 ± .04[b]	.16 ± .01[a,b]	.44 ± .05[a]	64[a]	36[b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

No difference in mercury concentrations and percent mercury was observed with vitamin treatment in the brain tissue compared to controls. However, there was a tendency for the vitamin groups to have lower concentrations of mercury.

The heart also did not exhibit a statistically significant difference in mercury concentration or percent mercury between the vitamin treated groups and controls. However, the concentration of inorganic, methyl, and total mercury tended to be quite a bit lower in the vitamin treated animals than controls.

The vitamin B_{12} group had significantly higher inorganic mercury levels than controls (1.01 μg vs .74 μg respectively) in lung tissue. This was reflected also in a higher percent inorganic mercury (70%) and lower percent methyl (30%) for B_{12} animals versus controls (65% inorganic, 35% methyl). These differences were statistically significant. Additionally, when expressed as percent the folate group had significantly more inorganic (73%) and significantly less methyl (27%).

While the individual vitamin treated animals tended to have higher inorganic, methyl, and total mercury concentrations than controls in the spleen, these measurements were not statistically different. Also, the percent inorganic mercury tended to be elevated in vitamin treated animals, but was not statistically different from controls.

In the kidney there was no difference in mercury concentrations with vitamins given separately. These animals tended to show increased methylmercury and total mercury concentrations when compared to controls. The B_{12} group and folate group had significantly larger

percents of mercury present as methylmercury (46% and 45% respectively) compared to controls (43%).

The concentration of inorganic mercury was not statistically different for the vitamin group compared to controls for hair samples. Although these groups tended to have higher methylmercury concentrations than controls, only the folate and vitamin C groups had significantly higher. Additionally, only the folate group had significantly more mercury as percent methyl (60%) compared to controls (45%). However, the B_{12} (56%) and vitamin C groups (57%) appeared higher than controls. These differences were inversely reflected in the percent inorganic mercury.

In the blood there was no difference in concentration for all forms of mercury, but the vitamin treated animals exhibited an apparent increase in inorganic mercury concentrations and lower methylmercury concentrations compared to controls. When expressed as percent, the vitamin treated groups had significantly more inorganic mercury (B_{12} = 61%, folate = 60%, and vitamin C = 64%) than controls (53%) and less methylmercury.

Accumulated Guinea Pig Data

The data from all the various vitamin treatments compared to controls are displayed by tissue in Tables 5 - 13. In the liver the individual vitamin treated groups had significantly more inorganic, methyl, total and percent methylmercury than controls and all other treated groups. The B_{12}/folate group had significantly more inorganic mercury than the all vitamin and B_{12}/vitamin C groups. Of importance,

Table 5. Mercury Levels in the Liver Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	.78 ± .28[a,b]	.37 ± .20[a]	1.15 ± .46[a,b]	69[a]	31[a]
All Vitamins n=8	.52 ± .12[b]	.25 ± .03[a]	.77 ± .14[b]	67[a,b]	33[a,b]
B_{12}/Folate n=5	1.05 ± .25[a]	.67 ± .09[b]	1.72 ± .33[a]	60[b,c]	40[b,c]
B_{12}/Vitamin C n=4	.61 ± .09[b]	.27 ± .04[a]	.88 ± .13[b]	69[a]	31[a]
Folate/Vitamin C n=5	.78 ± .10[a,b]	.38 ± .07[a]	1.15 ± .10[a,b]	67[a,b]	33[a,b]
B_{12} Only n=5	1.58 ± .20[c]	1.16 ± .40[c]	2.74 ± .57[c]	59[c]	41[c]
Folate Only n=5	1.60 ± .71[c]	1.22 ± .27[c]	2.83 ± .90[c]	56[c]	44[c]
Vitamin C Only n=5	1.72 ± .54[c]	1.08 ± .19[c]	2.79 ± .54[c]	61[b,c]	39[b,c]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 6. Mercury Levels in the Muscle Tissue of Guinea Pigs with Different Dietary Vitamin Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	.21 ± .27[a]	.18 ± .14[a]	.39 ± .38[a]	51[a,b,c]	49[a,b,c]
All Vitamins n=8	.10 ± .02[a]	.13 ± .09[a]	.23 ± .10[a]	47[a,b,c]	53[a,b,c]
B_{12}/Folate n=5	.12 ± .03[a]	.19 ± .04[a]	.31 ± .04[a]	39[b]	61[b]
B_{12}/Vitamin C n=4	.27 ± .29[a]	.31 ± .19[a]	.58 ± .45[a]	43[a,b]	57[b,c]
Folate/Vitamin C n=5	1.33 ± 1.60[b]	.83 ± .83[b]	2.16 ± 2.40[b]	50[a,b,c]	50[a,b,c]
B_{12} Only n=5	.18 ± .04[a]	.14 ± .02[a]	.32 ± .04[a]	55[a,b,d]	45[a,b,d]
Folate Only n=5	.15 ± .04[a]	.10 ± .04[a]	.25 ± .07[a]	60[c,d]	40[a,d]
Vitamin C Only	.16 ± .03[a]	.07 ± .02[a]	.24 ± .03[a]	69[d]	31[d]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 7. Mercury Levels in the Brain Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	.30 ± .17[a]	.24 ± .15[a]	.54 ± .31[a]	56[a,b]	44[a,b]
All Vitamins n=8	.19 ± .06[a]	.18 ± .07[a]	.37 ± .11[a]	51[b]	49[a]
B_{12}/Folate n=5	.16 ± .02[a]	.15 ± .03[a]	.31 ± .04[a]	51[b]	49[a]
B_{12}/Vitamin C n=4	.20 ± .08[a]	.29 ± .14[a]	.49 ± .22[a]	41[c]	59[c]
Folate/Vitamin C n=5	.25 ± .09[a]	.18 ± .09[a]	.43 ± .14[a]	58[a,b]	42[a,b]
B_{12} Only n=5	.27 ± .04[a]	.19 ± .03[a]	.46 ± .03[a]	58[a,b]	42[a,b]
Folate Only n=5	.30 ± .05[a]	.18 ± .02[a]	.48 ± .04[a]	63[a]	37[b]
Vitamin C Only n=5	.23 ± .03[a]	.19 ± .02[a]	.42 ± .04[a]	56[a,b]	44[a,b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 8. Mercury Levels in the Heart Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	.72 ± .61[a,b]	.35 ± .19[a,b]	1.07 ± .76[a,b]	63[a]	37[a]
All Vitamins n=8	1.29 ± .91[a]	.60 ± .33[a]	1.90 ± 1.24[a]	66[a]	34[a]
B_{12}/Folate n=5	.26 ± .03[b]	.33 ± .19[b]	.59 ± .20[b]	48[b]	52[b]
B_{12}/Vitamin C n=4	.44 ± .31[b]	.23 ± .12[b]	.67 ± .43[b]	63[a]	37[a]
Folate/Vitamin C n=5	.84 ± .74[a,b]	.39 ± .26[a,b]	1.23 ± .99[a,b]	64[a]	36[a]
B_{12} Only n=5	.32 ± .04[b]	.17 ± .04[b]	.50 ± .06[b]	65[a]	35[a]
Folate Only n=5	.31 ± .06[b]	.13 ± .01[b]	.45 ± .05[b]	70[a]	30[a]
Vitamin C Only n=5	.28 ± .06[b]	.14 ± .04[b]	.42 ± .06[b]	66[a]	34[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 9. Mercury Levels in the Lung Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=13	.74 ± .18[a]	.40 ± .07[a]	1.14 ± .22[a,b]	65[a,b]	35[a,b]
All Vitamins n=8	.82 ± .16[a,b]	.41 ± .07[a]	1.23 ± .21[a,b]	67[a,b,c]	33[a,b,c]
B_{12}/Folate n=5	.69 ± .12[a]	.40 ± .09[a]	1.10 ± .19[b]	63[b]	37[a]
B_{12} Only n=5	1.01 ± .30[b]	.42 ± .10[a]	1.43 ± .38[a]	70[c,d]	30[c,d]
Folate Only n=5	.86 ± .10[a,b]	.32 ± .03[a]	1.18 ± .12[a,b]	73[d]	27[d]
Vitamin C Only n=5	.90 ± .25[a,b]	.40 ± .11[a]	1.29 ± .35[a,b]	69[a,c,d]	31[b,c,d]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 10. Mercury Levels in the Spleen Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	.89 ± .51[a,b]	.59 ± .20[a,b]	1.48 ± .69[a,b,c]	58[a,b]	42[a,b]
All Vitamins n=8	.52 ± .10[b]	.41 ± .10[a]	.93 ± .18[a]	56[b]	44[a]
B_{12}/Folate n=5	.70 ± .13[b]	.54 ± .05[a,b]	1.24 ± .17[a,b]	56[b]	44[a]
B_{12}/Vitamin C n=4	1.42 ± 1.00[a,b]	.76 ± .46[b,c]	2.18 ± 1.46[b,c]	64[a]	36[b]
Folate/Vitamin C n=5	1.39 ± 1.08[a,b]	.67 ± .37[a,b,c]	2.06 ± 1.45[a,b,c]	65[a]	35[b]
B_{12} Only n=5	1.73 ± .53[a]	.91 ± .18[c]	2.64 ± .70[c]	65[a]	35[b]
Folate Only n=5	1.39 ± .98[a,b]	.81 ± .24[b,c]	2.21 ± 1.22[b,c]	60[a,b]	40[a,b]
Vitamin C Only n=5	1.75 ± 1.16[a]	.93 ± .35[c]	2.68 ± 1.51[c]	63[a,b]	37[a,b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 11. Mercury Levels in the Kidney Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=18	19.17±2.49[a,b]	14.72 ± 2.11[a,b]	33.89 ± 4.49[a,b,c]	57[a]	43[a]
All Vitamins n=8	16.96 ± .75[b]	13.46 ± .80[a]	30.42 ± 1.44[a]	56[a,b]	44[a,b]
B_{12}/Folate n=5	20.46 ± 2.43[a]	16.92 ± 1.61[c]	37.38 ± 4.01[c]	55[a,b]	45[a,b]
B_{12}/Vitamin C n=4	17.91 ± .56[a,b]	14.11 ± .82[a,b]	32.02 ± 1.33[a,b]	56[a,b]	44[a,b]
Folate/Vitamin C n=5	18.30 ± 2.00[a,b]	14.77 ± 1.42[a,b]	33.07 ± 3.30[a,b,c]	55[a,b]	45[a,b]
B_{12} Only n=5	19.08 ± 1.65[a,b]	15.84 ± .34[b,c]	34.91 ± 1.90[a,b,c]	54[b]	46[b]
Folate Only n=5	18.60 ± 1.69[a,b]	15.57 ± .88[b,c]	34.16 ± 2.54[a,b,c]	55[b]	45[b]
Vitamin C Only n=5	20.12 ± .88[a]	15.94 ± .67[b,c]	36.07 ± 1.17[b,c]	56[a,b]	44[a,b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 12. Mercury Levels in the Hair Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/g wet tissue				
	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=13	.80 ± .26[a]	.65 ± .23[a]	1.44 ± .47[a]	55[a]	45[a]
All Vitamins n=8	.73 ± .33[a]	.70 ± .25[a,b]	1.43 ± .48[a]	51[a,b]	49[a,b]
B_{12}/Folate n=5	.87 ± .20[a]	.78 ± .08[a,b]	1.65 ± .25[a,b]	53[a]	47[a]
B_{12} Only n=5	.95 ± .32[a]	1.27 ± .63[a,b,c]	2.22 ± .87[a,b,c]	44[a,b]	56[a,b]
Folate Only n=5	.95 ± .20[a]	1.84 ± 1.38[c]	2.79 ± 1.36[c]	40[b]	60[b]
Vitamin C Only n=5	.95 ± .34[a]	1.47 ± .88[b,c]	2.43 ± .93[b,c]	43[a,b]	57[a,b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 13. Mercury Levels in the Blood Tissue of Guinea Pigs with Different Vitamin Dietary Regimes

Treatment	μg Hg/ml			% Inorganic	% Methyl
	Inorganic Hg	Methyl Hg	Total Hg		
Controls n=5	.19 ± .04[a,b]	.18 ± .05[a]	.37 ± .09[a,b]	53[a]	47[a]
B_{12}/Folate n=5	.18 ± .03[a]	.17 ± 02[a,b]	.35 ± .03[b]	51[a]	49[a]
B_{12} Only n=5	.24 ± .05[a,b]	.15 ± .02[a,b]	.38 ± .07[a,b]	61[b]	39[b]
Folate Only n=5	.20 ± .02[a,b]	.13 ± .01[b]	.33 ± .02[b]	60[b]	40[b]
Vitamin C Only n=5	.28 ± .04[b]	.16 ± .01[a,b]	.44 ± .05[a]	64[b]	36[b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

are the data showing that the B_{12}/folate group (.67 μg) had a significantly higher methylmercury concentration than the control (.37 μg), all vitamin (.25 μg), B_{12}/vitamin C (.27 μg), and folate/vitamin C (.38 μg) groups. This difference is portrayed in the percent methylmercury data with the B_{12}/folate group having significantly more as percent methylmercury compared to controls and the B_{12}/vitamin C group.

Table 6 shows the data for muscle tissue. The folate/vitamin C group had higher ($p < 0.05$) concentrations of all three forms of mercury compared to all other groups. However, the greatest percent methylmercury level observed was 61% in the B_{12}/folate group, which was significantly different from the B_{12}, folate, and vitamin C groups. Also the vitamin C group had significantly less mercury as percent methylmercury (31%) compared to all the other groups, except the B_{12} and folate groups.

The data for the brain tissue are shown in Table 7. There were no differences in the various mercury concentrations for all treatments versus controls. However, the B_{12}/vitamin C group did have a significantly higher proportion of mercury as methylmercury (59%) compared to controls (44%) and other vitamin treatments. A trend was also evident in that the groups treated with B_{12} and some combination (all vitamin, B_{12}/folate, or B_{12}/vitamin C) had a higher proportion of methylmercury values than controls. The individual vitamins and folate/vitamin C groups all had lower percent methyl values than controls.

In the heart (Table 8) there were no differences between controls and the various vitamin treated groups. However, the all vitamin group did have mercury concentrations which were significantly higher than the other vitamin treated groups, except the folate/vitamin C group. Furthermore, the B_{12}/folate group had proportionately more methylmercury (52%) than controls (37%) and the other vitamin treated groups.

Data for lung tissue (Table 9) were fairly consistent between groups; however, the B_{12} group had an inorganic mercury level (1.01 μg) which was significantly greater than controls (.74 μg) and the B_{12}/folate group (.69 μg). Methylmercury concentrations were not significantly different between groups. Although the B_{12} group had significantly more (1.43 μg) total mercury than the B_{12}/folate group (1.10 μg), both the B_{12} and folate groups had proportionately less mercury as methylmercury (30% and 27% respectively) than controls (35%) and the B_{12}/folate group (37%).

Inorganic and total mercury concentrations for the vitamin treated groups were not statistically different from control animals in the spleen (Table 10). The B_{12} and vitamin C groups did have inorganic and total mercury concentrations which were greater ($p < 0.05$) than the all vitamin group and the B_{12}/folate group. Also, the B_{12} and vitamin C groups had significantly higher methylmercury levels than controls, the all vitamin, and B_{12}/folate group. Both the all vitamin and B_{12}/folate groups had 44% methylmercury levels which were statistically different from the B_{12}/vitamin C group (36%), folate/vitamin C group (35%) and the B_{12} only group (35%).

Table 11 contains data for kidney tissue. The vitamin treated groups were not statistically different in inorganic and total mercury concentrations compared to controls. The B_{12}/folate and vitamin C groups had significantly more inorganic and total mercury than the all vitamin group. Additionally, methylmercury concentration in the B_{12}/folate group (16.92 μg) was significantly higher than controls (14.72 μg) and the all vitamin (13.46 μg), B_{12}/vitamin C (14.11 μg) and folate/ vitamin C (14.77 μg) groups. The all vitamin group had the lowest mercury concentrations of all groups while the B_{12}/folate group had the highest concentrations. Percent methyl values in the B_{12} and folate groups (46% and 45% respectively) were significantly higher than controls (43%).

In hair tissue (Table 12), inorganic mercury concentration did not differ among the groups. Both the folate group and vitamin C group had methyl and total mercury concentrations which were significantly higher than controls. Of interest also was the fact that the folate group had significantly more methyl and total mercury than the all vitamin and B_{12}/folate groups. While the vitamin treated groups all tended to have higher percent methylmercury levels than controls, only the folate group (60%) was significantly higher than controls (45%) and the B_{12}/folate group (47%).

The data for the blood sample are shown in Table 13. Inorganic mercury data indicated that the vitamin C group (.28 μg) was significantly higher than the B_{12}/folate group (.18 μg) but these groups were not different from controls. Methylmercury concentration in the folate group (.13 μg) was significantly lower than controls (.18 μg).

For total mercury concentration, only the vitamin C group (.44 μg) was significantly different than the B_{12}/folate group (.35 μg) and folate group (.33 μg). The three groups treated with individual vitamins all had percent methylmercury values which were significantly lower than the controls and B_{12}/folate group.

Rats Treated With Cysteine or Methionine

Six tissues were analyzed in rats to examine the effect of cysteine or methionine supplemented diet regimes on mercury metabolism. These data were compared to control animals fed a normal dietary regime. The results of this experiment are shown in Tables 14 - 19.

The data from the liver tissue are shown in Table 14. There was a general trend for the methionine group to have the lowest concentrations of all three forms of mercury. For example, the methionine group had an inorganic mercury level of 1.39 μg compared to the controls (2.02 μg) and the cysteine group (1.89 μg). Methylmercury measurements were .79 μg for the methionine group, .92 μg for controls, and 1.07 μg for the cysteine group while total mercury levels were 2.18 μg, 2.93 μg, and 2.96 μg for the methionine, controls and cysteine groups, respectively. However, these differences were not statistically different from control animals. Both the methionine and cysteine groups exhibited percent methyl values which were significantly higher than controls (36% for both vs 32% for controls).

In brain tissue neither mercury concentration or percent mercury did not differ for the methionine and cysteine groups from controls. Inorganic mercury data were very similar while the methyl and total

Table 14. Mercury Levels in the Liver Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	2.02 ± .68[a]	.92 ± .21[a]	2.93 ± .89[a]	68[a]	32[a]
Methionine n=5	1.39 ± .15[a]	.79 ± .11[a]	2.18 ± .26[a]	64[b]	36[b]
Cysteine n=5	1.89 ± .71[a]	1.07 ± .33[a]	2.96 ± 1.03[a]	64[b]	36[b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 15. Mercury Levels in the Brain Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	.35 ± .06[a]	.27 ± .05[a]	.62 ± .09[a]	56[a]	44[a]
Methionine n=5	.36 ± .05[a]	.30 ± .06[a]	.67 ± .07[a]	55[a]	45[a]
Cysteine n=5	.36 .07[a]	.32 ± .05[a]	.67 ± .09[a]	53[a]	47[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 16. Mercury Levels in the Lung Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	.65 ± .18[a]	.39 ± .08[a]	1.04 ± .25[a]	62[a]	38[a]
Methionine n=5	.67 ± .11[a]	.43 ± .08[a]	1.10 ± .19[a]	61[a]	39[a]
Cysteine n=5	.64 ± .05[a]	.40 ± .03[a]	1.03 ± .06[a]	62[a]	38[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 17. Mercury Levels in the Heart Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	.52 ± .10[a]	.30 ± .07[a]	.82 ± .14[a]	64[a]	36[a]
Methionine n=5	.53 ± .10[a]	.34 ± .06[a]	.86 ± .15[a]	61[a]	39[a]
Cysteine n=5	.51 ± .12[a]	.34 ± .05[a]	.85 ± .17[a]	59[a]	41[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 18. Mercury Levels in the Spleen Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	1.74 ± .36[a]	.99 ± .18[a]	2.73 ± .51[a]	64[a]	36[a]
Methionine n=5	1.36 ± .29[a]	.86 ± .22[a]	2.22 ± .49[a]	61[a]	39[a]
Cysteine n=5	1.53 ± .15[a]	1.01 ± .07[a]	2.54 ± .19[a]	60[a]	40[a]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 19. Mercury Levels in the Kidney Tissue of Rats with Methionine or Cysteine Supplemented Diets

	μg Hg/g wet tissue				
Treatment	Inorganic Hg	Methyl Hg	Total Hg	% Inorganic	% Methyl
Controls n=5	18.70 ± 2.17[a]	14.84 ± 1.20[a]	33.54 ± 3.34[a]	56[a]	44[a]
Methionine n=5	17.31 ± 1.73[a]	14.38 ± 1.33[a]	31.69 ± 3.05[a]	55[b]	45[b]
Cysteine n=5	16.89 ± 1.70[a]	14.19 ± 1.42[a]	31.08 ± 3.11[a]	55[b]	45[b]

Values represent the average ± S.D. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

mercury measurements were slightly higher in the two treatment groups compared to controls. Also, percent methyl was highest in the cysteine group (47%) compared to the controls (44%) and methionine groups (45%).

There were no differences between controls and the two treatment groups in the lungs for mercury concentrations or percent mercury. The methionine group did have the highest concentration values of all three groups.

In heart tissue there were also no statistical differences in concentrations or percent mercury between groups. Again, the methionine group tended to exhibit the highest concentration values, yet the cysteine group had the highest percent methylmercury (41%) compared to controls (36%) and the methionine group (39%).

In spleen tissue mercury concentrations and percent mercury were not statistically different for any of the groups. In fact, the concentration data from this tissue were very similar to the liver. The methionine group had the lowest values with inorganic at 1.36 μg, methyl at .86 μg, and total mercury at 2.22 μg. This compared to control inorganic mercury of 1.74 μg, methyl .99 μg, and total 2.73 μg, with the cysteine group having 1.53 μg inorganic, 1.01 μg methyl, and 2.54 μg total mercury. Additionally, both the methionine and cysteine groups had a higher percent methylmercury (39% and 40%, respectively) compared to controls (36%).

The data from the kidney also showed no statistical differences in concentrations for the three groups. However, the methionine and cysteine groups both tended to have lower mercury concentrations. These

two groups did have percent methylmercury values which were statistically different from the control animals.

Auditory Brainstem Response Data

The auditory brainstem response (ABR) data for controls and the mercury treated groups by the different frequencies are shown in Tables 20-23. The individual frequency means for each group are provided in Appendix 2. Guinea pigs which had been fed a control diet and had no exposure to mercury served as the control or untreated group. Recordings were obtained from this group and provided an example of normal guinea pig ABR measurements. These animals were then given subcutaneous injections of mercuric chloride (0.6 mg/kg body weight) every other day for a total of nine injections. At the end of mercury treatment, ABR recordings were obtained. This group was designated as the mercury only group and provided information on ABR dysfunction as a result of inorganic mercury intoxication. All vitamin groups received mercury treatment and were designated according to their respective vitamin regimes. ABR measurements from the vitamin groups with mercury treatment were subsequently compared to the recordings from the mercury only group. These data served to determine if a vitamin(s) had altered the mercury effect as measured by ABR.

All animals exhibited a positive Preyer's reflex except for three animals in the B_{12} group. By visual inspection, the ABR measurements for these animals correlated to the Preyer's reflex observations with delayed latencies and increased thresholds exhibited in these animals.

Table 20. Auditory Brainstem Responses at 8K Frequency in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Treatment	I Latency (msec)	I Amplitude (μV)	II Latency (msec)	II Amplitude (μV)	V Latency (msec)	V Amplitude (μV)
Untreated Controls n=10	1.59 ± .20[a,b]	.96 ± .52[a,b]	2.43 ± .27[a]	.52 ± .55[a,b]	3.91 ± .37[a]	.67 ± .54[a,b]
Controls + Hg n=5	1.54 ± .20[b,c]	.87 ± .51[a,b]	2.42 ± .24[a]	.51 ± .43[a,b]	3.95 ± .31[a]	.46 ± .41[c]
B_{12}/Folate + Hg n=5	1.51 ± .20[c]	1.05 ± .47[a]	2.41 ± .23[a]	.57 ± .51[a,b]	3.90 ± .39[a]	.76 ± .60[a]
B_{12} + Hg n=5	1.66 ± .29[a]	.90 ± .46[a,b]	2.45 ± .26[a]	.45 ± .34[a,b]	3.89 ± .32[a]	.77 ± .61[a]
Folate + Hg n=5	1.52 ± .21[b,c]	.83 ± .44[b]	2.40 ± .22[a]	.67 ± .44[a]	3.86 ± .32[a]	.55 ± .43[b,c]
Vitamin C + Hg n=5	1.60 ± .20[a,b]	.96 ± .44[a,b]	2.38 ± .19[a]	.43 ± .38[b]	3.85 ± .37[a]	.76 ± .58[a]

Values represent the average ± S.D. for all intensities. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 21. Auditory Brainstem Responses at 6K Frequency in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Treatment	I Latency (msec)	I Amplitude (μV)	II Latency (msec)	II Amplitude (μV)	V Latency (msec)	V Amplitude (μV)
Untreated Controls n=10	1.68 ± .30[a,b]	.79 ± .55[a]	2.54 ± .30[a]	.45 ± .51[a]	4.03 .36[a,b,c]	.54 ± .43[a]
Controls + Hg n=5	1.67 ± .24[a,b]	.65 ± .32[a]	2.59 ± .27[a]	.36 ± .29[a]	4.12 ± .39[a,b]	.29 ± .34[b]
B_{12}/Folate + Hg n=5	1.68 ± .32[a,b]	.77 ± .48[a]	2.60 ± .31[a]	.52 ± .48[a]	4.15 ± .53[a]	.57 ± .45[a]
B_{12} + Hg n=5	1.73 ± .23[a]	.73 ± .42[a]	2.54 ± .27[a]	.47 ± .43[a]	3.96 ± .27[c]	.63 ± .54[a]
Folate + Hg n=5	1.61 ± .22[b]	.68 ± .40[a]	2.52 ± .26[a]	.54 ± .48[a]	3.98 ± .28[b,c]	.52 ± .36[a]
Vitamin C + Hg n=5	1.71 ± .23[a]	.66 ± .35[a]	2.50 ± .25[a]	.35 ± .32[a]	3.92 ± .32[c]	.57 ± .45[a]

Values represent the average ± S.D. for all intensities. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 22. Auditory Brainstem Responses at 4K Frequency in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Treatment	I Latency (msec)	I Amplitude (μV)	II Latency (msec)	II Amplitude (μV)	V Latency (msec)	V Amplitude (μV)
Untreated Controls n=10	1.79 ± .31[a]	.92 ± .78[a,b]	2.64 ± .27[a,b,c]	.61 ± .68[a,b,c]	4.18 ± .36[a]	.56 ± .45[a]
Controls + Hg n=5	1.75 ± .24[a]	.72 ± .66[b,c]	2.67 ± .32[a,b,c]	.59 ± .69[a,b,c]	4.20 ± .33[a]	.35 ± .38[b]
B_{12}/Folate + Hg n=5	1.76 ± .28[a]	1.05 ± .78[a]	2.67 ± .29[a,b]	.74 ± .86[a,b]	4.12 ± .27[a]	.64 ± .50[a]
B_{12} + Hg n=5	1.90 ± .27[b]	.72 ± .51[b,c]	2.73 ± .31[a]	.43 ± .53[c]	4.12 ± .26[a]	.53 ± .46[a,b]
Folate + Hg n=5	1.77 ± .27[a]	.86 ± .69[a,b,c]	2.61 ± .25[b,c]	.82 ± .83[a]	4.10 ± .20[a]	.51 ± .41[a,b]
Vitamin C +Hg n=5	1.84 ± .29[a,b]	.65 ± .51[c]	2.56 ± .24[c]	.50 ± .55[b,c]	4.07 ± .31[a]	.52 ± .37[a,b]

Values represent the average ± S.D. for all intensities. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 23. Auditory Brainstem Responses at 2K Frequency in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Treatment	I Latency (msec)	I Amplitude (μV)	II Latency (msec)	II Amplitude (μV)	V Latency (msec)	V Amplitude (μV)
Untreated Controls n=10	2.17 ± .31[a]	.64 ± .48[a,b]	3.06 ± .28[a]	.42 ± .53[a,b]	4.52 ± .36[a,b]	.36 ± .28[a]
Controls + Hg n=5	2.16 ± .27[a]	.48 ± .42[b]	3.07 ± .31[a]	.41 ± .56[a,b]	4.59 ± .27[a]	.18 ± .13[b]
B_{12}/Folate + Hg N=5	2.08 ± .29[a]	.70 ± .48[a]	3.03 ± .29[a]	.52 ± .44[a,b]	4.41 ± .30[a,b]	.39 ± .28[a]
B_{12} + Hg n=5	2.30 ± .35[b]	.52 ± .38[a,b]	2.95 ± .30[a]	.49 ± .58[a,b]	4.43 ± .30[a,b]	.31 ± .12[a]
Folate + Hg n=5	2.11 ± .27[a]	.60 ± .41[a,b]	2.93 ± .26[a]	.63 ± .66[a]	4.46 ± .26[a,b]	.39 ± .26[a]
Vitamin C + Hg n=5	2.20 ± .29[a,b]	.47 ± .26[b]	3.30 ± .33[a]	.25 ± .25[b]	4.37 ± .29[b]	.32 ± .18[a]

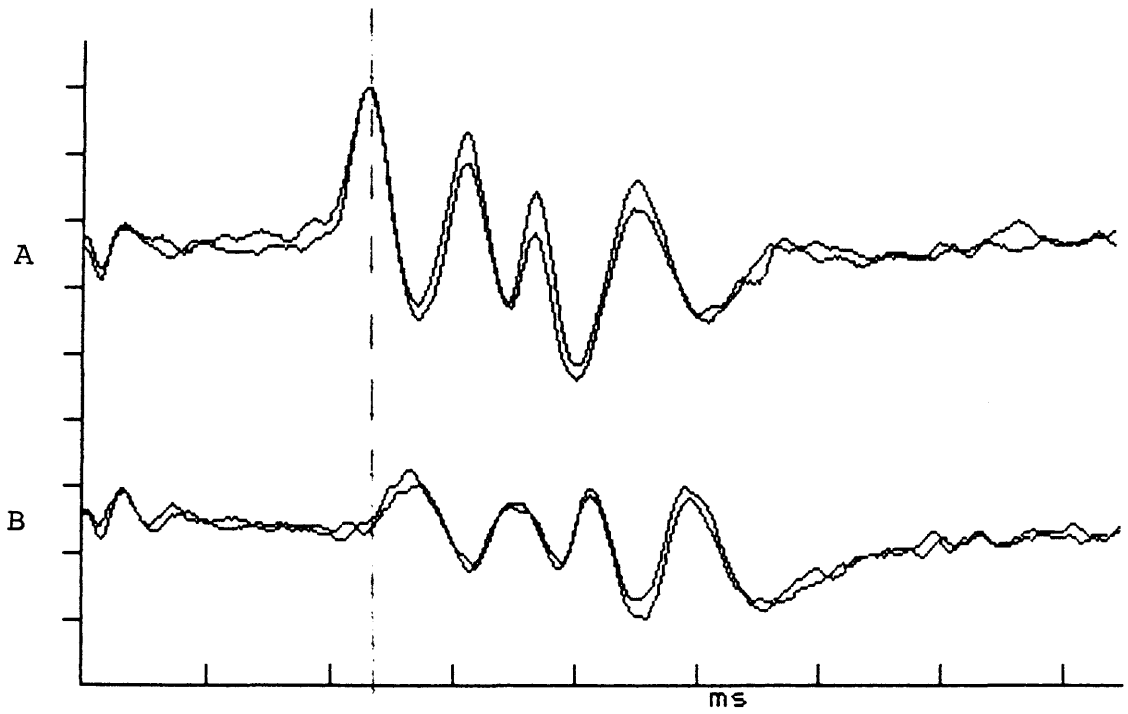
Values represent the average ± S.D. for all intensities. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

At a frequency of 8k, several differences were observed. The B_{12} group had a wave I latency of 1.66 msec. which was longer ($p < 0.05$) than the mercury only treated group (1.54 msec.). This increase in latency of wave I by the B_{12} group as compared to the mercury only group is depicted in Figure 5. Waves II and V latencies showed no differences between groups.

The amplitude of wave I was lowest for the folate group which was different ($p < 0.05$) from the B_{12}/folate group, but not the untreated control and mercury only groups. However, for wave II amplitude, the measurements were opposite from wave I. Although the folate group had the highest wave II amplitude (.67 μV) of all the groups, it was only statistically different from the vitamin C group (.43 μV). The wave V amplitude data indicated that the mercury only group had a lower ($p < 0.05$) amplitude than untreated controls and all the vitamin groups except the folate group.

At a frequency of 6k, wave I latencies were not significantly different between groups. However, the means for the B_{12} group and vitamin C group were longer than the mercury only treated group. Wave II latencies were not statistically different among the groups. Wave V latency data indicated that the B_{12} and vitamin C groups had latencies (3.96 msec. and 3.92 msec., respectively) which were different ($p < 0.05$) than the mercury only group (4.12 msec.).

The amplitudes for waves I and II were not different ($p < 0.05$) for all groups at the 6k frequency. The mercury only group generally had the lowest amplitude for these two waves and in wave V, this group had a amplitude (.29 μV) which was lower ($p < 0.05$) than the untreated



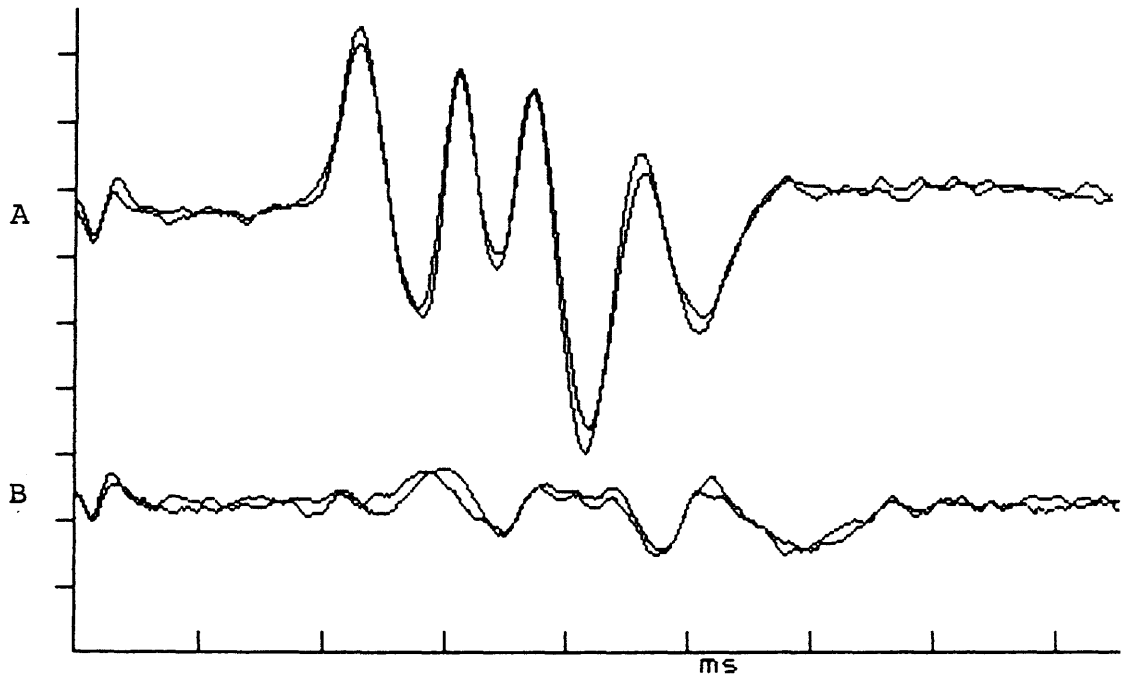
Tracings represent responses to 256 stimuli. Each trace was performed in duplicate. Vertical scale equal to .61 μV and horizontal to 1 msec.

Figure 5. Latency alterations of Wave I at 8K frequency and 105 dB intensity for mercury only (A) and B_{12} animal (B).

control (.54 μV) and the vitamin treated groups. There was a trend for the vitamin groups treated with B_{12} and/or folate to exhibit higher amplitudes compared to the mercury only group.

The B_{12} group had a wave I latency (1.90 msec.) that was longer ($p < 0.05$) than untreated controls and the mercury only group (1.79 msec. and 1.75 msec., respectively) as well as the B_{12}/folate group (1.76 msec.) and folate group (1.77 msec.) at a frequency of 4k. While this group also had the longest latency (2.73 msec.) for wave II, it was not different ($p < 0.05$) from the mercury only group (2.67 msec.). The wave V latencies were not different ($p < 0.05$) among the groups; however, the means for the vitamin treated groups were shorter for wave V latencies than the untreated and mercury only groups. This was similar to the 8k frequency data for wave V latencies.

The wave I amplitude measurements for the 4k frequency indicated that the B_{12}/folate group had an amplitude (1.05 μV) which was higher ($p < 0.05$) than the mercury only and B_{12} groups (.72 μV). The wave II amplitude data showed no differences among groups. However, the B_{12} group had the lowest mean for wave II amplitude, which was different ($p < 0.05$) than the B_{12}/folate and folate groups, although not different from the mercury only group. It was observed that the two folate treated groups generally had higher amplitudes than the other groups. For wave V amplitudes, the mercury only group (.35 μV) again was lower ($p < 0.05$) than the untreated control (.56 μV) as well as the B_{12}/folate groups (.64 μV). The alterations of wave amplitudes with mercury treatment are depicted in Figure 6 which compares a untreated control animal with a B_{12} treated animals.



Tracings represent responses to 256 stimuli. Each trace was performed in duplicate. Vertical scale is equal to .61 μV and horizontal scale equal to 1 msec.

Figure 6. ABR wave amplitude alterations with mercury treatment in untreated controls (A) and vitamin B_{12} treated (B) animal at 4K frequency and 105 dB intensity.

The B_{12} group had a wave I latency which was longer ($p < 0.05$) than all other groups except the vitamin C group at the 2k frequency. However, the wave II latencies were not different ($p < 0.05$) between the various groups. The vitamin C group had a wave V latency (4.37 msec.) which was shorter ($p < 0.05$) than the mercury only group (4.59 msec.). Again, the means of the vitamin groups for wave V latencies were shorter than untreated controls and mercury only animals.

The B_{12}/folate group had a wave I amplitude (.70 μV) which was significantly higher than the mercury only group (.48 μV). The wave II amplitude data yielded no differences between the mercury only group and remaining groups. Once again, the wave V amplitude was most affected in the mercury only group, which was significantly lower (.18 μV) than the control and other treated groups.

The data for the interpeak latencies (IPL) are shown in Table 24. The B_{12} group had IPL's which were shorter ($p < 0.05$) than controls and mercury only treated animals at 8k, 4k, and 2k frequencies. This group's IPL were also significantly shorter than the mercury only group at the 6k frequency. In addition, the B_{12} group exhibited IPL's which were shorter ($p < 0.05$) than the B_{12}/folate group at all frequencies. Interpeak latencies in the vitamin C group were significantly shorter than all the other groups except the B_{12} group for all frequencies. In the 6k frequency, IPL's for the mercury only group (2.47 msec.) were longer ($p < 0.05$) than the untreated controls (2.35 msec.).

In Table 25 the threshold data are presented. The B_{12} group had thresholds which were higher ($p < 0.05$) at 8k (70.0 dB) compared to the control (51.5 dB), mercury only (56.0 dB), and B_{12}/folate (56.0 dB)

Table 24. I-V Interpeak Latencies for ABR's at Various Frequencies in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Treatment	8K (msec)	6K (msec)	4K (msec)	2K (msec)
Untreated Controls n=10	2.34[a,b]	2.35[a,b]	2.40[a,b]	2.36[a]
Controls + Hg n=5	2.42[a]	2.47[c,d]	2.46[a]	2.43[a]
B_{12}/Folate + Hg n=5	2.40[a]	2.49[d]	2.37[a,b]	2.33[a]
B_{12} + Hg n=5	2.24[c]	2.26[b,e]	2.24[c]	2.09[b]
Folate + Hg n=5	2.37[a]	2.37[a,c]	2.35[b]	2.37[a]
Vitamin C + Hg n=5	2.26[b,c]	2.22[e]	2.25[c]	2.17[b]

Values represent the average for each group. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

Table 25. Threshold Values for ABR's at Various Frequencies in Guinea Pigs with Mercury Treatment and Various Vitamin Regimes

Groups	8K (dB)	6K (dB)	4K (dB)	2K (dB)
Untreated Controls n=10	51.5 ± 3.4[a]	58.5 ± 6.7[a]	57.0 ± 5.9[a]	69.0 ± 4.6[a,b]
Controls + Hg n=5	56.0 ± 2.2[a]	61.0 ± 4.2[a,b]	60.0 ± 3.5[a,b]	75.0 ± 7.1[a,b]
B_{12}/Folate + Hg n=5	55.0 ± 0.0[a]	60.0 ± 3.5[a,b]	53.0 ± 2.7[a]	66.0 ± 2.2[b]
B_{12} + Hg n=5	70.0 ± 16.2[b]	73.0 ± 17.9[b]	69.0 ± 17.1[b]	77.0 ± 12.5[a]
Folate + Hg n=5	62.0 ± 5.7[a,b]	66.0 ± 13.9[a,b]	60.0 ± 11.7[a,b]	74.0 ± 9.6[a,b]
Vitamin C + Hg n=5	60.0 ± 3.5[a,b]	62.0 ± 5.7[a,b]	59.0 ± 4.2[a,b]	75.0 ± 7.1[a,b]

Values represent the average ± S.D. for all intensities. Data sharing a common subscript letter in a column are not significantly different ($p > 0.05$).

groups. Additionally, the B_{12} group had the highest thresholds at all frequencies compared to the remaining groups. Of particular interest is the fact that the B_{12} group had significantly higher thresholds than the B_{12}/folate group at the 8k, 4k and 2k frequencies. It also should be noted that at the 4k and 2k frequencies all the mercury treated groups had higher thresholds than the untreated control animals except the B_{12}/folate group.

CHAPTER V

DISCUSSION

Mercury concentrations appeared to be lowered with the all vitamin treatment compared to controls in all tissues except for the heart and lungs while percent methyl mercury was increased. It is possible that with all three vitamins present at megadoses, these animals were able to detoxicate and excrete mercury more efficiently. It has been shown that bacteria and yeasts can detoxicate metallic mercury by first methylating it and then, demethylating it to elemental mercury, possibly by going through the mercurous form (110). It has been suggested that this biotransformation may also occur in humans, but the exact mechanism remains unclear (87). Therefore, since methylation possibly is the first step in the detoxication process, the animals with the all vitamin treatment may have been able to quickly methylate the mercuric chloride which is reflected in the increase in percent methylmercury values. The methylmercury was then further detoxicated and removed from the tissues, thus, leaving decreased tissue concentrations. The higher mercury concentrations in the lungs and heart with the all vitamin treatment may represent blood concentrations i.e. transport through the body for excretion.

The next step in this investigation was to determine which vitamin(s) were playing a role in mercury metabolism. The B_{12}/folate and B_{12}/vitamin C groups seemed to exhibit the most activity in terms of methylmercury concentration and percent methylmercury. When compared to control animals, the B_{12}/folate group had a higher methylmercury

concentration in the liver, kidney and hair and this difference was statistically significant in the liver and kidney. Additionally, an increased percent methylmercury content in all the tissues analyzed was observed in the B_{12}/folate group when compared to controls, but this difference was only significant in the liver and heart. The B_{12}/folate and B_{12}/vitamin C groups both had the highest percent methylmercury values of all the groups in the muscle and brain. In fact, the B_{12}/vitamin C group was significantly higher than controls in the brain. It would appear that the two B_{12} combination groups (i.e. B_{12}/folate, B_{12}/vitamin C) were similar to the all vitamin treated group in explaining the percent methylmercury data for the various tissues. Therefore, B_{12} apparently is the most important (the common denominator) vitamin in terms of increasing the percent methylmercury levels.

Another interesting concept is evident from these data. The folate/vitamin C group exhibited an increase in mercury concentration (both inorganic and methyl) in the muscle and heart. The folate and vitamin C only groups had increased concentrations in the lungs. Therefore, as observed in the all vitamin treatment group, folate and/or vitamin C appear to be the vitamins involved in increasing mercury concentrations in the heart and lung tissues. Also of interest with the vitamin combination groups, was the tendency for methylmercury to decrease proportionately even though the concentrations increased. This was also similar to the all vitamin group data.

In order to isolate the specific vitamin activities in the various tissues, the vitamins were given separately. An increase in mercury concentrations was observed in the liver and spleen for all the

individual vitamins, but vitamin C exhibited the highest inorganic mercury values in these tissues compared to all the vitamin treated groups. Of particular interest was an increase in methylmercury concentration for the individual vitamin groups in the spleen, kidney, and hair. Also, with the individual vitamins, the percent methylmercury increased in the liver, kidney, and hair, but decreased in the muscle, brain, heart, lung, spleen, and blood. Of those tissues which exhibited an increase in percent methylmercury, the folate group showed the highest elevation in the liver and hair while the B_{12} group showed the highest elevation in the kidney. Furthermore, in those tissues with a decrease in percent methylmercury, the vitamin C group was lower ($p < 0.05$) than controls in percent methylmercury in the muscle and blood. Therefore, it would again appear that B_{12} and/or folate play a role in methylation of mercury, while vitamin C may affect the increase in mercury concentrations, particularly inorganic mercury, and thus, result in a decrease in percent methylmercury.

As these data began to accumulate, there were several tissues which presented unique information. First of all, the liver may be the most significant tissue in terms of the methylation process and eventual excretion. It possibly is the organ where the inorganic mercury is methylated and then "shipped out" for excretion. It has been proposed (64,87,110) that the methylation process is enzymatic and certainly the liver would be rich in enzyme systems. Also, as reported by Alexander and Aaseth (34), methylmercury apparently is transported from the liver to the kidney attached to glutathione. Since the liver is able to synthesize glutathione, it would support this concept of methylation and

excretion. When all the vitamins were given together, the liver's ability to methylate mercury was enhanced with subsequent export to the kidneys for excretion, thus, the mercury concentrations declined in the various tissues. Additionally, an increase in percent methylmercury was observed in most of the vitamin groups as compared to controls. This phenemenon may indicate that the liver is a site of methylation, but without all the vitamins present the process is slowed.

The three groups treated with the individual vitamins had increased mercury concentrations. A possible explanation for this is that mercury accumulated due to the liver's inability to methylate it quickly and thus, get rid of it. This inability possibly is because all the necessary vitamins were not available at appropriate levels.

The brain was an important tissue to investigate in this study since only the methylated form of mercury is able to cross the blood-brain barrier. Vitamin combinations which included B_{12} appeared to be the most effective in altering the percent methylmercury content in this tissue. Particularily significant is the B_{12}/vitamin C group activity, which displayed the greatest increase in methylmercury concentration and percent methylmercury. This gives rise to an intriguing concept. Vitamin B_{12} and C may be acting synergistically in mercury metabolism. Hypothetically, vitamin C may in some way reduce the Co+3 to Co+2 so that folate is able to re-methylate the B_{12} by donating a free radical methyl ($CH_3^{\bullet}$). The additional electron enables a carbanion (CH_3^-) to be released from B_{12} in order to methylate mercury. The B_{12} only group did not exhibit the same trends as the B_{12} combination

groups which may be due to a lack of contributing vitamins to re-methylate B_{12}.

Hair tissue has long been recognized as an excellent indicator of methylmercury levels in the body. This is due to the sulfur containing protein, keratin, found in hair which readily binds to methylated mercury (13). Accordingly, the hair data from all the animals treated with vitamins seemed to support this concept. In each case the concentration and percent methylmercury increased with dietary intake of vitamins. The greatest increase occurred in those animals given folate only. This is similar to the data in the liver for the folate only group. Consequently, folate must be playing a role in the methylation of mercury either in combination with vitamin B_{12} or alone.

As reported previously, folate (N^5-methyltetrahydrofolate) can contribute a free radical methyl group to B_{12} (Co+2). However, this form of B_{12} theoretically cannot methylate mercury which requires a carbanion ion. As suggested earlier, vitamin C may act synergistically with B_{12} and folate resulting in the release of a carbanion from B_{12} and subsequent methylation of mercury. None of the diets in this study were deficient in any vitamin. Therefore, the folate group may have had sufficient levels of B_{12} and/or vitamin C to methylate the mercury and rapidly regenerate the proper form of B_{12}. This resulted in the high level of methylation exhibited in hair.

Another hypothesis is proposed to explain the unexpected results displayed by the folate treated groups in this investigation. It may be possible that folate (N^5-methyltetrahydrofolate) can contribute a carbanion (CH_3^-) and methylate mercury. For example, the N^5-methyl THF

becomes desaturated at the 5,6 bond which results in the release of a carbanion group and the formation of dihydrofolate. This reaction is similar to that which occurs in thymidylate synthesis due to the activity of thymidylate synthetase. This would certainly explain the high level of methylated mercury which resulted in folate treated animals in the liver and hair. It may also be the reason for the high mercury concentrations observed in the muscle with folate/vitamin C treatment.

Animals treated with vitamin C and/or some combination displayed conflicting data. Treatment with vitamin C by itself resulted in increased mercury concentrations, particularily inorganic, in the liver, spleen, kidney, hair and blood compared to controls. In muscle, the folate/vitamin C group had statistically significant increased mercury concentrations, while in the brain the B_{12}/vitamin C group exhibited increased levels of methylmercury. A recent report (111) showed that the cupric (Cu++) form of copper was an efficient catalyst of ascorbate oxidation. It was suggested that in the presence of any transition metal, the oxidation of ascorbate can occur. Therefore, the mercuric ion may effectively oxidize ascorbate resulting in the reduction of mercury to the mercurous (Hg+) form. In this form, a free radical methyl group could bind to the mercury. This implies that N^5-methyl THF could methylate the metal. Also, the mercurous form is known to be less toxic to mammals. In fact, mercurous has been used in the past by humans as a laxative and diuretic (112). This may explain the increased levels of inorganic mercury in certain tissues with vitamin C treatment or some combination thereof.

The muscle tissue provided data which indicated that the all vitamin or vitamin combination treatments resulted in an increase in percent methylmercury. With the exception of the folate/vitamin C group, the mercury concentrations were low and consistent among the various groups. Yet, there was an obvious increase in the percent methylmercury with vitamin treatment except in the individual vitamin groups. Methylmercury is lipid soluble and therefore, is more likely to be associated with lipid substances in the body. Since the muscle samples were not devoid of lipid, these data may be a reflection of lipid storage of mercury in the guinea pig.

The heart, lungs, and spleen tissues generally provided data which may be influenced by the high degree of blood perfusion through these organs. The levels of mercury varied according to treatment and tissue but may reflect the ability of the tissue to concentrate mercury. The percent mercury data for the vitamin treated groups were consistent with or slightly lower than controls, except in the heart where the B_{12}/folate group was higher. However, this group had increased methylmercury in the liver, muscle, kidney, and hair. So the heart data may simply reflect the blood transport from or to these tissues in the treated animals.

Since the kidney is an excretory organ, the data from this tissue would indicate the accumulation of mercury for excretion. The mercury concentration in the kidney was approximately 10-20 times higher than in any other tissue. It would be informative to measure urine excretion of mercury in order to determine the maximum clearance rate of mercury in these animals. However, in this investigation, it appears that the

kidneys did not lose their ability to concentrate mercury and thus, it is assumed the dose level of mercury given in this study was appropriate.

ABR Testing

The auditory brainstem response testing was performed in two groups of vitamin and mercury treated guinea pigs. Prior to mercury exposure, the control animals were tested to establish normal data.

The ABR testing served two functions in this study. Thus far, no research has been done to investigate the effect of inorganic mercury treatment on guinea pig ABR. Therefore, this study was the first to examine the effect of inorganic mercury intoxication on the auditory system as evaluated by ABR data. Mercury treatment may result in peripheral damage and/or CNS alterations. Evidence has been presented to indicate damage by mercuric chloride to the sensory epithelium in the apical area (low frequency) of the guinea pig cochlea (113) and it has been shown to damage the mitochondria in hair cells and nerve terminals, which in turn results in impaired mitochondrial function, i.e. oxidative phosphorylation, calcium transport and phosphate accumulation was suppressed while ATP hydrolysis, potassium and magnesium transport were induced (114). Therefore, the mercuric chloride interferes with cell respiration and ATP synthesis which would delay signal transmission. However, in the present study, wave latencies across frequencies were not different for the mercury only treated animals compared to untreated controls. Thus, a large number of fast conducting, large diameter fibers have not been affected by mercury

treatment. In addition, IPL's were not significantly different in these two groups. This would suggest that impulse conduction time in the lower auditory centers and CNS has not been altered by inorganic mercury treatment regardless of frequency.

However, treatment with inorganic mercury (mercury only group) resulted in a decrease in all wave amplitudes compared to untreated controls. This group exhibited a significantly lower wave V amplitude at all frequencies compared to untreated controls. Wave amplitude data are difficult to interpret due to the extreme variability among animal species. However, alterations in wave amplitudes can be due to damage of the peripheral or CNS auditory systems. If the damage occurs within the brainstem areas of the auditory system, it would be due to methylmercury toxicity since this is the only form of mercury which can cross the blood brain barrier. These animals were not deficient in any vitamin and thus, these data may simply represent a normal methylation process of inorganic mercury and subsequent transport into the CNS.

In order to determine if the decrease in wave amplitude was due to methylmercury damage of the auditory centers within the brainstem, the IPL data were examined. Research has demonstrated that methylmercury exposure results in shorter interpeak latencies (61). Yet, in the study reported here, the mercury only group exhibited IPL means which were longer than untreated controls. Furthermore, the wave V latency means for the mercury only group were longer than untreated controls while wave I latencies means were uneffected or slightly shorter than untreated controls. While these differences were not statistically significant, damage within the brainstem is indicated which supports the

concept that the inorganic mercury has been converted to methylmercury. Damage of the larger diameter or faster conducting fibers would result in longer interpeak latency values. Therefore, the decrease in wave amplitudes observed in mercury only treated animals would be due to signal transmission through the slower, small diameter fibers resulting in a decrease in nerve signal strength.

Wave amplitudes can also be reduced when peripheral damage has occurred. Again, damage to the larger fibers would result in signal transmission through the smaller diameter fibers, and thus, a decrease in the strength of the action potential. Both inorganic mercury and methylmercury have been shown to cause damage to the cochlea hair cells and/or cochlear nerve. Thus, the data generated from the present investigation requires further research to determine the exact structures which are effected by the different forms of mercury.

Second, this research appears to support the theory that vitamin B_{12} treatment may potentiate the mercury effect, particularily in the peripheral auditory system. The B_{12} only group had the greatest increase in wave I latency at every frequency for all groups tested. This group exhibited a significantly longer wave I latency at 8k, 4k, and 2k frequencies than the mercury only treated group. Since the other mercury treated groups were not statistically different from the mercury only group at each frequency, B_{12} appears to potentiate the mercury effects in the cochlea and/or cochlear nerve possibly due to its role in the methylation of mercury.

According to the original hypothesis of this investigation, B_{12} treatment should result in increased methylation of inorganic mercury.

With increased amounts of methylmercury available, the increased latencies may represent degeneration of hair cells in the base of the cochlea which has been demonstrated with methylmercury exposure (115). Methylmercury has also been shown to result in loss of cochlear microphonics and whole nerve action potentials (116) and this would be demonstrated by prolonged latencies in ABR testing. It has also been hypothesized that methylmercury exposure results in demyelination in the auditory nerve (57). Damage to the large fibers in the auditory nerve would impair rapid transients and result in longer wave I latencies. Therefore, the delayed latencies reported here indicates damage to the cochlea and/or cochlear nerve but the exact nature of the damage remains elusive.

The idea that peripheral damage occurred due to increased methylmercury levels with B_{12} is supported by the threshold data. While all the mercury treated groups tended to exhibit an increase in threshold values compared to untreated controls, the B_{12} group produced the greatest increase in threshold intensity at all frequencies. The B_{12} group had significantly higher threshold values than the untreated controls and mercury only group at the 8k frequency. These data indicate a high-frequency loss which would result from damage to the basilar membrane at the base of the cochlea.

As noted earlier, mercuric chloride has been shown to result in damage to the apex area of the basilar membrane which would be demonstrated by a low frequency loss (57,58). Furthermore, research using methylmercury has shown a high frequency loss in mice (61). This suggests frequency-specific deficits to the lower auditory pathway

possibly due to the type of mercury. In this investigation, the high frequency loss would indicate damage due to methylmercury exposure. This possible nerve damage and subsequent increase in threshold value observed at the 8k frequency also supports research on high-frequency sensorineural deafness reported in 80% of the patients with methylmercury poisoning at Minamata Bay in the 1950's (117).

B_{12}/folate treatment did not appear to effect the wave I latencies, which is not consistent with the B_{12} group data. However, the B_{12}/folate group did increase the amplitude of wave I at the 8k, 4k, and 2k frequencies. Also this group and the folate group exhibited the greatest increase in amplitudes of wave II at all frequencies but these differences were not significant compared to untreated controls and the mercury only group. This alteration in wave amplitude certainly suggests that CNS function has been effected by these treatments. However, further analysis is necessary to establish if the wave latency (i.e. area under the specific wave) remains constant with each treatment. But, a decrease in wave amplitude would normally be expected if damage to the higher auditory centers within the brainstem has occurred. These data demonstrated an increase in amplitude which is difficult to explain.

The possibility exists that with B_{12}/folate treatment, an increased amount of methylmercury is produced. Methylmercury may cause impairment of slow conducting, small diameter fibers. This would result in a majority of transmission occurring from fast conducting, large diameter fibers, thus, the reduced dispersement of these fibers may produce a stronger impulse which may be demonstrated by elevated wave amplitudes.

Inspection of interpeak latencies values revealed shortened latencies for the B_{12} and vitamin C groups compared to the mercury only group. A probable explanation for these data can be obtained by examining latencies for waves I and V. The wave I latency means for these two groups were longer than the mercury only treated group at all frequencies. In addition, these groups exhibited wave V latency means which were shorter than the mercury only group. However, upon further inspection of the wave V latencies, it becomes apparent that the decrease in IPL's with vitamin treatment compared to the mercury only group is due to an increase in latency with mercury treatment. The vitamin treated groups exhibited wave V latency values which were very similar to the untreated controls. Therefore, this evidence clearly indicates that the increase in IPL with B_{12} and vitamin C treatment is due to a shift in wave I latency rather than an affect on nerve processes which regulate nerve conduction velocity in the CNS. The data from the B_{12} group can again be explained due to its role in methylation of mercury; however, an explanation for the vitamin C data remains unclear. It would appear that vitamin C potentiates the mercury affect in the auditory nerve possibly due to its oxidation/reduction potential.

Methionine and Cysteine Supplementation in Rats

Since investigation of methylation processes of inorganic mercury was the intent of this research, a study was conducted in rats to examine the possible role of methionine as a methyl donor via S-adenosylmethionine (SAM) transfer. Several points require clarification. First, SAM donates a carbonium (CH3+) group which

theoretically would not be the methyl form accepted by mercury (Hg++). However, SAM is involved in the regeneration of cobalt (Co+2) in B_{12} in those instances when the B_{12} becomes oxidized. Due to its role in B_{12} metabolism, it would seem important to investigate SAM's effect on mercury methylation. Second, rats are able to synthesize vitamin C (118) and as demonstrated in the guinea pig data, vitamin C may play a role in mercury metabolism. In addition, these animals were given a diet sufficient in all vitamins including folate and B_{12}. Thus, rats may be able to alter the methylation of mercury due to their ability to make vitamin C available, as well as having sufficient dietary folate and B_{12}. Third, cysteine has been shown to result in decreased SAM concentrations in the rat liver (119). Also, a methionine-sparing effect has been demonstrated with cysteine supplementation (120). Therefore, a cysteine supplemented dietary regime was included in this study.

The methionine treated group exhibited the lowest mercury concentrations of all three groups in the liver and spleen while having the highest concentrations in the lungs and heart. While there was an apparent increase in percent methylmercury in all tissues with methionine treatment compared to controls, this was only statistically different in the liver and kidneys. These data are very similar to the guinea pigs in the all vitamin group. It, again, raises the question that these animals can detoxicate the mercury more readily and excrete it from the body. Thus, the lowered concentrations are seen. Also, these data suggest that methionine is altering the mercury methylation

process since an increase in percent methyl is observed. This possibly is due to methionine's role in regenerating B_{12}.

Those animals given the cysteine supplemented diets presented data which were slightly more complex. Assuming that cysteine can lower the liver concentration of SAM, it seems that these animals would exhibit a decrease in methylation potential. Yet, this group demonstrated the highest methylmercury concentrations in the liver, brain, and spleen as well as the highest percent methylmercury levels in the brain, heart, and spleen as compared to both the control and methionine treated groups. However, the cysteine group exhibited percent methylmercury values similar to the methionine group in the liver and kidneys which were the only measurements statistically different from controls. These data suggest that cysteine may also play a role in mercury methylation.

A recent report (121) has shown that brain uptake of methylmercury is enhanced by chronic L-cysteine infusion. It was suggested that the translocation of methylmercury across the blood-brain barrier may be mediated by a neutral amino acid carrier transport system (121). In the present study, the cysteine treated group did exhibit a higher concentration of methylmercury in the brain tissue which may lend support to this theory. Another possible explanation for the results shown in the cysteine treated group is the oxidation/reduction theory suggested for the vitamin C treated groups. Cysteine is also easily oxidized and, in fact, prefers the oxidized state. Consequently, mercury exposure may result in the oxidation of cysteine and the reduction of mercuric to mercurous. As suggested earlier, the mercurous form is not as toxic to mammals or it may be methylated by

N^5-methyltetrahydrofolate since it can accept a free radical methyl group. Certainly, this requires further research to elucidate the role of cysteine in the methylation of mercury.

In conclusion, the results presented here generally support the concept that dietary intake of megadoses of vitamin B_{12}, folate, and/or vitamin C can alter the methylation, i.e. metabolism of inorganic mercury. While ingestion of megadoses of all three vitamins resulted in decreased mercury concentrations in all tissues except heart and lung, individual vitamins or a combination of vitamins consumed in megadoses resulted in increased methylmercury concentrations. Furthermore, the ABR testing provided additional evidence that mercury can result in neurological alterations and these can be influenced by a dietary vitamin intake. In addition, dietary supplementation with the sulfur containing amino acids, methionine and cysteine, may effect this methylation process.

Factors which can alter the methylation process of mercury in vivo may have practical significance. For instance, in those geographical areas where there is a relatively high consumption of foods contaminated by mercury, the toxic effects of mercury ingestion may be significantly enhanced in those individuals who supplement their diets with vitamins. In addition, those individuals in the dental profession who may be frequently exposed to mercury compounds used in dental amalgams should become aware of the possibility that megavitamin consumption may potentiate the methylation process of mercury. Thus, further research is needed in this area and dissemination of this information via educational programs is necessary.

CHAPTER VI

SUMMARY

Ingestion of megadoses of certain vitamins appears to influence the methylation of mercuric chloride *in vivo*. The increased concentration of methylmercury and percent methylmercury values exhibited in the brain tissue with dietary consumption of vitamin B_{12} supports the hypothesis that this vitamin is involved in methylating mercury and, thus, allowing the transport of methylmercury across the blood-brain barrier. In addition, auditory brainstem response measurements showed that B_{12} treated animals displayed delayed wave I latencies and higher threshold values compared to untreated controls and mercury only animals. This again suggests that this vitamin effects the methylation process.

The incorporation of high levels of folate into the diet also influences mercury methylation. Folate treated animals tended to have increased concentrations of both inorganic and methylmercury, particularly in the muscle tissue. Animals given B_{12}/folate and mercuric chloride also exhibited increased ABR wave amplitudes at the lower frequencies compared to the mercury only group. Further research is needed to determine the exact mechanism involved by which folate is able to methylate mercury.

The addition of vitamin C in the diet, particularly in combination with B_{12} and/or folate, resulted in increased methylmercury levels and percent methylmercury values in the muscle, brain, and hair. An apparent synergistic effect was observed with vitamin C and the other vitamins. It has been suggested that vitamin C may play a role in the

re-methylation process of B_{12} by donating an electron to the methyl group contributed by folate. Alternately, the mercuric form of mercury may oxidize vitamin C and be subsequently reduced to the mercurous ion. This form is less toxic but may also be methylated by folate eliminating the involvement of B_{12}. The vitamin C group provided interesting ABR information. This group exhibited higher threshold measurements compared to controls and interpeak latencies were significantly shorter with vitamin C treatment. Certainly, further work is necessary to examine these possible interactions.

And finally, diets supplemented with the amino acids cysteine and methionine indicated that these nutrients may also be involved in the methylation process of mercury. The increase in percent methylmercury levels found in the hepatic and renal tissues of rats is possibly due to the role of methionine in various methylation activities via S-adenosylmethionine. Contrary to the research which has shown that cysteine inhibits certain reactions involving SAM, the animals treated with cysteine exhibited increased percent methyl levels in the liver, brain, heart, spleen and kidney tissues. Therefore, it would appear that cysteine effects mercury metabolism possibly via oxidation/reduction reactions. Again, this is an area requiring additional investigation.

In conclusion, the methylation of mercuric chloride <u>in vivo</u> can be increased with the ingestion of megadoses of vitamin B_{12}, folate, or vitamin C. Therefore, individuals who take large amounts of these nutritional supplements and who ingest fish which are possibly contaminated with industrial spills containing mercury, could have

increased methylmercury exposure with subsequent neurological damage. Additionally, dental professionals should become aware of this potential. Further research is required to elucidate these effects and the possible mechanisms which are involved in the methylation of mercury *in vivo*.

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APPENDIXES

APPENDIX 1

Table 1-1. Weight Data

Week	Controls (g)	B_{12}/Folate (g)
1	243.79 ± 17.84	242.52 ± 2.74
2	279.31 ± 33.11	290.81 ± 14.01
3	326.01 ± 32.82	356.49 ± 22.76
4	364.02 ± 41.48	393.70 ± 38.19
5	424.69 ± 49.52	441.33 ± 52.10
6	476.85 ± 54.96	481.03 ± 73.64
7	518.68 ± 50.79	520.61 ± 79.50
8	570.87 ± 56.03	567.09 ± 83.74
*9	610.92 ± 56.45	605.36 ± 84.93
10	633.61 ± 57.49	606.80 ± 93.90
11	540.95 ± 54.55	560.55 ± 91.14

Measurements represent the average ± S.D.

*Mercury injections were begun on week #9.

APPENDIX 2

Table 2-1. Frequency Means for Untreated Controls by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.31	1.54	2.13	1.11	3.59	1.40	2.28
95	1.43	1.23	2.25	.76	3.64	1.10	2.21
85	1.58	1.09	2.37	.47	3.79	.69	2.21
75	1.62	.90	2.51	.33	3.95	.34	2.33
65	1.72	.72	2.59	.28	4.13	.20	2.41
55	1.85	.28	2.77	.13	4.57	.12	2.70
6K							
105	1.36	1.56	2.22	1.08	3.79	1.16	2.43
95	1.51	.88	2.37	.59	3.81	.82	2.29
85	1.65	.68	2.51	.25	3.91	.50	2.26
75	1.74	.66	2.59	.23	4.06	.25	2.32
65	1.95	.42	2.83	.18	4.33	.15	2.38
55	2.13	.20	3.12	.09	4.62	.10	2.53
4K							
105	1.48	2.01	2.34	1.48	4.05	1.03	2.57
95	1.61	1.24	2.51	.83	4.03	.77	2.42
85	1.75	.70	2.60	.41	4.05	.61	2.30
75	1.87	.53	2.72	.19	4.17	.37	2.30
65	2.04	.46	2.89	.20	4.42	.19	2.39
55	2.20	.33	3.12	.12	4.61	.14	2.51
2K							
105	1.87	1.25	2.80	.92	4.41	.70	2.55
95	2.01	.76	2.88	.39	4.34	.31	2.37
85	2.15	.49	3.08	.24	4.39	.28	2.21
75	2.37	.33	3.26	.26	4.66	.25	2.27
65	2.51	.23	3.52	.13	4.93	.20	2.42

Table 2-2. Frequency Means for Mercury Only Animals by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.28	1.08	2.12	.88	3.62	.83	2.34
95	1.39	1.11	2.23	.82	3.85	.86	2.46
85	1.47	1.05	2.36	.57	3.76	.54	2.29
75	1.59	.91	2.47	.39	3.97	.17	2.38
65	1.72	.65	2.62	.19	4.29	.11	2.56
55	1.83	.32	2.79	.15	4.35	.09	2.53
6K							
105	1.34	.97	2.18	.85	3.80	.53	2.47
95	1.50	.64	2.48	.38	3.82	.38	2.32
85	1.62	.71	2.58	.25	3.98	.14	2.36
75	1.73	.64	2.71	.24	4.10	.27	2.36
65	1.85	.67	2.82	.19	4.52	.20	2.68
55	2.03	.20	2.88	.16	4.77	.19	2.71
4K							
105	1.41	1.80	2.29	1.79	3.93	.64	2.52
95	1.59	.76	2.43	.69	4.12	.39	2.53
85	1.76	.51	2.75	.28	3.96	.47	2.23
75	1.83	.42	2.73	.18	4.26	.22	2.43
65	1.98	.31	2.90	.24	4.52	.13	2.55
55	2.22	.20	3.18	.05	4.74	.08	2.52
2K							
105	1.84	.96	2.75	1.01	4.52	.27	2.68
95	1.97	.55	2.94	.34	4.59	.11	2.61
85	2.16	.37	3.09	.24	4.49	.15	2.28
75	2.42	.24	3.29	.12	4.60	.19	2.18
65	2.55	.17	3.61	.15	4.96	.12	2.40

Table 2-3. Frequency Means for B_{12} Treated Animals by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.43	1.29	2.23	.80	3.62	1.45	2.20
95	1.56	1.08	2.37	.56	3.78	1.02	2.22
85	1.68	.85	2.55	.33	3.98	.53	2.30
75	1.90	.75	2.50	.30	4.02	.37	2.12
65	1.80	.52	2.75	.07	4.24	.12	2.44
55	1.86	.17	-	-	-	-	-
6K							
105	1.54	1.21	2.37	.88	3.85	1.06	2.31
95	1.67	.66	2.49	.44	3.76	.92	2.17
85	1.75	.60	2.61	.17	4.00	.43	2.25
75	1.81	.76	2.57	.39	4.02	.29	2.21
65	1.95	.41	2.90	.18	4.29	.15	2.34
55	2.12	.12	-	-	-	-	-
4K							
105	1.64	1.27	2.51	.78	4.00	.86	2.36
95	1.79	.83	2.65	.49	4.02	.53	2.23
85	1.99	.48	2.88	.24	4.07	.63	2.17
75	1.88	.63	2.75	.28	4.01	.56	2.13
65	2.07	.49	2.91	.15	4.18	.22	2.11
55	2.18	.29	3.08	.26	4.57	.08	2.39
2K							
105	2.07	.76	2.87	.69	4.27	.39	2.14
95	2.30	.58	3.03	.38	4.47	.34	2.06
85	2.33	.48	2.93	.36	4.42	.31	2.09
75	2.38	.31	-	-	4.47	.27	2.09
65	2.70	.19	-	-	4.69	.18	2.08

Table 2-4. Frequency Means for Folate Treated Animals by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.28	1.17	2.18	1.01	3.57	1.01	2.29
95	1.38	1.05	2.28	.97	3.67	.86	2.29
85	1.48	.93	2.39	.68	3.80	.45	2.32
75	1.61	.73	2.52	.39	4.02	.18	2.41
65	1.79	.44	2.64	.26	4.37	.10	2.59
55	1.87	.19	2.78	.12	-	-	-
6K							
105	1.34	1.28	2.25	1.20	3.75	.93	2.40
95	1.49	.73	2.37	.69	3.79	.70	2.30
85	1.61	.50	2.50	.29	3.88	.49	2.23
75	1.76	.48	2.70	.23	4.20	.21	2.45
65	1.88	.40	2.86	.14	4.35	.09	2.45
55	1.90	.24	-	-	4.54	.12	2.64
4K							
105	1.45	2.02	2.35	1.93	3.98	.96	2.54
95	1.58	1.13	2.48	.90	3.95	.72	2.37
85	1.70	.55	2.59	.41	4.05	.47	2.35
75	1.83	.52	2.73	.33	4.06	.35	2.23
65	1.99	.42	2.96	.25	4.23	.19	2.25
55	2.15	.24	3.00	.14	4.47	.12	2.32
2K							
105	1.84	1.02	2.75	1.10	4.30	.71	2.46
95	1.98	.79	2.80	.89	4.42	.36	2.44
85	2.12	.41	3.07	.19	4.45	.26	2.32
75	2.41	.29	3.22	.13	4.68	.24	2.27
65	2.40	.19	-	-	4.70	.14	2.30

Table 2-5. Frequency Means for Vitamin C Treated Animals by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.33	1.41	2.18	.79	3.50	1.48	2.17
95	1.46	1.22	2.29	.43	3.60	1.22	2.14
85	1.57	1.12	2.43	.36	3.75	.73	2.18
75	1.68	.89	2.52	.27	3.93	.34	2.25
65	1.82	.48	2.60	.12	4.30	.14	2.48
55	1.91	.15	-	-	4.72	.12	2.74
6K							
105	1.42	1.05	2.27	.59	3.66	1.20	2.24
95	1.59	.82	2.31	.59	3.72	.89	2.13
85	1.72	.64	2.54	.18	3.83	.41	2.11
75	1.88	.47	2.63	.23	4.16	.16	2.27
65	1.93	.37	2.77	.14	4.35	.11	2.39
55	2.00	.12	2.84	.09	4.20	.09	2.20
4K							
105	1.46	1.47	2.34	.85	3.82	.87	2.36
95	1.61	.99	2.48	.86	.89	.92	2.28
85	1.83	.52	2.52	.38	3.93	.70	2.10
75	1.94	.39	2.66	.16	4.08	.29	2.13
65	2.09	.28	2.87	.10	4.38	.12	2.32
55	2.16	.17	2.90	.02	4.49	.08	2.33
2K							
105	1.90	.79	2.69	.46	4.28	.47	2.37
95	2.04	.56	3.06	.25	4.23	.33	2.17
85	2.30	.33	3.20	.12	4.44	.25	2.14
75	2.45	.25	3.34	.08	4.52	.25	2.02
65	2.60	.14	-	-	4.52	.12	1.91

Table 2-6. Frequency Means for B_{12}/Folate Treated Animals by Individual Intensities

Intensity (dB)	I Lat (msec)	I Amp (μV)	II Lat (msec)	II Amp (μV)	V Lat (msec)	V Amp (μV)	I-V IPL (msec)
8K							
105	1.22	1.63	2.12	1.34	3.58	1.48	2.36
95	1.36	1.26	2.23	.73	3.61	1.17	2.24
85	1.44	1.18	2.30	.61	3.72	.95	2.28
75	1.56	1.10	2.44	.43	3.91	.45	2.35
65	1.66	.77	2.63	.19	4.09	.22	2.44
55	1.81	.33	2.74	.14	4.62	.14	2.81
6K							
105	1.32	1.45	2.23	1.31	3.81	1.07	2.49
95	1.48	.81	2.40	.60	3.81	.94	2.33
85	1.59	.79	2.50	.30	4.07	.48	2.48
75	1.72	.75	2.64	.31	4.23	.28	2.51
65	2.05	.41	2.92	.30	4.62	.24	2.57
55	2.05	.20	3.08	.15	4.62	.12	2.60
4K							
105	1.41	2.43	2.32	2.30	3.94	1.47	2.53
95	1.53	1.29	2.44	1.03	3.93	.65	2.40
85	1.64	.87	2.56	.49	3.95	.76	2.31
75	1.78	.74	2.71	.28	4.04	.52	2.26
65	1.96	.56	2.89	.16	4.36	.25	2.39
55	2.22	.40	3.14	.17	4.52	.17	2.30
2K							
105	1.76	1.32	2.76	1.07	4.36	.82	2.61
95	1.89	.93	2.84	.42	4.19	.38	2.30
85	2.05	.59	2.98	.40	4.31	.32	2.24
75	2.24	.42	3.20	.34	4.48	.27	2.23
65	2.48	.23	3.61	.27	4.86	.16	2.29

VITA

Nancy Elizabeth Zorn is a native of Florida and received a B.S. degree in Home Economics Education from Florida State University in 1976. After working as a 4-H Extension Agent in Lake City, Florida, she enrolled in the graduate program at Florida State University and received a M.S. degree in Nutrition and Food Science in 1982. She then worked as Director of Dietary Services for Heritage Healthcare in Tallahassee, Florida and also did private consulting in dietetics for a number of facilities. Furthermore, she has taught in both a secondary school system and community college.

In 1985 she reentered graduate school in Nutrition and Food Science at The University of Tennessee, Knoxville. As a doctoral student, she served as a graduate research assistant for 12 consecutive quarters. She is a member of Omicron Nu, Sigma Xi, American Chemical Society, and the American Dietetics Association. She has been recognized by the Department of Nutrition and Food Sciences for excellence in research as a graduate student. The Ph.D. degree was subsequently awarded in 1988, with a major in nutrition and a collateral minor in mammalian physiology.