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The Relationship of Toxic Effects of Mercury to Exacerbation of the Medical Condition classified as Alzheimer's Disease

THE RELATIONSHIP OF THE TOXIC EFFECTS OF MERCURY TO EXACERABATION OF THE MEDICAL CONDITION CLASSIFIED AS ALZHEIMER'S DISEASE

By

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Abstract: Mercury(II) or Hg^{2+}, is neurotoxic and when exposed to normal brain tissue homogenates, is capable of causing many of the same biochemical aberrancies found in Alzheimer's diseased (AD) brain. Also, rats exposed to mercury vapor show some of these same aberrancies in their brain tissue. Specifically, the rapid inactivation of the brain thiol-sensitive enzymes tubulin, creatine kinase and glutamine synthetase occurs on the addition of low micromolar levels of Hg^{2+} or exposure to mercury vapor, and these same enzymes are significantly inhibited in AD brain. Further, extended Hg^{2+} exposure to neurons in culture has been shown to produce three of the widely accepted pathological diagnostic hallmarks of AD. These are elevated amyloid protein, hyper-phosphorylation of Tau, and formation of neurofibillary tangles. The hypothesis is that mercury and other blood-brain permeable toxicants that have enhanced specificity for thiol-sensitive enzymes are the etiological source of AD. Included in this category are other heavy metals such as lead and cadmium that act synergistically to enhance to toxicity of mercury and organic-mercury compounds, like thimerosal that is found in vaccines and other medicines. This hypothesis is also able to explain the genetic susceptibility to AD that is expressed through the APO-E gene family. Specifically, a reduction of APO-E gene types carrying cysteines decreases the ability to remove mercury and other thiol-reactive toxicants from the cerebrospinal fluid. This increases brain exposure to thiol-reactive toxicants and the risk of AD.

RATIONALE FOR THE HYPOTHESIS:

AD is a disease of unknown etiology. However, it is widely accepted that most AD is not directly genetically inherited and that some external vector, such as a toxicant exposure or an infection, must be involved for the disease to progress into a clinically observable condition. In the USA the rate of AD is very similar for rural versus urban peoples and it does not vary appreciably from state to state. Therefore, if a toxicant is involved then this toxicant must be of a very personal nature, like what we eat or what is placed into our bodies through other sources such as dental fillings, vaccines, etc.

The involvement of infectious agents such as bacteria, virus or yeasts; while possible at this time, seems not to be directly involved. This is based on the huge amount of National Institutes of Health (USA) and other world-wide funds spent on AD to identify the causal factors and they have not detected a consistent microbial vector. If an infectious agent were involved (like in AIDS and polio) it seems as if it would have been identified by now. However, focal infections caused by microbes in the oral cavity must still be considered as these microbes are known to produce toxicants such as hydrogen

sulfide, methyl-mercaptan, gliatoxin and other compounds that inhibit thiol-sensitive enzymes.

For any toxicant, or class of toxicants, to be proposed as involved in the etiology of AD they must be available equally to individuals living in markedly different locations. The toxicant proposed must explain the genetic susceptibility concept of AD. Further, under experimental conditions the toxicants must be able to cause the exacerbation of the many biochemical aberrancies found in AD brain. Based on our research and a literature review, mercury and mercury containing compounds from dental amalgams, vaccines, other medicinals and preservatives used in paints, seed grains, etc. represent a class of compounds that fill this requirement.

Mercury and organic mercurials are neurotoxicants. Further, the enzyme inhibitory effects of mercury are synergistically enhanced by exposures to other toxicants such as lead and cadmium (smokers). Even the simultaneous presence of EDTA (ethylene-diamine-tetraacetic acid, a common food additive) or metal binding antibiotics such as tetracycline can enhance mercury toxicity. Therefore, any determination of a safe level of mercury exposure using rats in a cage being feed carefully monitored food and water is not reliable for determination of a "safe level of exposure to mercury" for humans. The fact is that science does not know what the combined toxic effects of many toxicants or enhancers of toxicity would be if present with mercury and therefore cannot identify a safe level of exposure.

Therefore, thiol-reactive toxicants such as mercury, cadmium, lead and certain organics are rational suggestions as being exacerbating factors for AD, or possibly even causal. However, mercury is the one toxicant that has been shown to reproduce many of the biochemical aberrancies and diagnostic hallmarks of AD. Also, mercury exposure is readily available to most humans. It is reasonable to propose that exposure to mercury is one of the major toxic factors involved in early onset AD. Further, that simultaneous exposures to other toxicants or factors enhance the toxicity of mercury and hasten the onset of AD, especially in those individuals who are genetically susceptible.

RESEARCH REVIEW AND RESULTS:

Enzyme Inhibition and Protein Partitioning Results.

Research regarding Alzheimer's disease (AD) done in our laboratory in the late 1980s was directed towards detecting aberrancies in the nucleotide binding proteins of AD post-mortem brain tissue versus age-matched, non-demented control brain samples. Basic to all of our findings was the following observation. Two very important brain nucleotide binding proteins, tubulin and creatine kinase (CK), showed greatly diminished activity and nucleotide binding ability. Further, they were abnormally partitioned into the particulate fraction versus the soluble fraction of AD brain tissue by simple centrifugation (1,2).

Both tubulin and CK are proteins that bind the nucleotides GTP (guanosine-5'-triphosphate) and ATP (adenosine-5'-triphosphate), respectively. We use a "photoaffinity labeling" technology to determine the availability of these binding sites before and after addition of mercury or other toxicants (21). This technology is explained in detail at www.altcorp.com for those interested in the detailed chemistry. Using this technology our laboratory has demonstrated that both tubulin and CK had diminished

biological activity in AD brain compared to age-matched controls. Since AD is not directly a genetically inherited disease we searched for possible toxicants that might mimic the specific findings observed in AD brain.

Our first finding was simple and straight-forward. After testing numerous heavy metals we observed that only Hg^{2+} could mimic the AD effect in homogenates of normal brain at concentrations that might be expected to be found in brain (3,4). The observation was that Hg^{2+} at very low micromolar levels ($\cong$ 1 micromolar) could rapidly and selectively abolish the GTP binding activity of tubulin (M_r = 55,000 daltons) without any noticeable effect on the other GTP binding proteins protein(s) observed at an M_r of about 42,000 daltons, that are present in both control and AD brain at approximately equal levels. Therefore, concerning heavy metals the addition of only mercury at low micromolar levels to control brain homogenates gave a GTP binding profile that was identical to that observed in AD brain and that chelation of Hg^{2+} by EDTA did not prevent but enhanced this effect (4,5,6). Further, additional results have shown that the addition of Hg^{2+} to control brain homogenates not only caused the decrease in nucleotide interaction but could also support the abnormal partitioning of tubulin into the particulate fraction as observed in AD brain (7). This was especially effective in the presence of other divalent metals, such as zinc, which is elevated in AD brain. The recent video demonstrating Hg^{2+} specific stripping the tubulin from the neurofibrils shows the tubulin abnormally aggregating at the base of the neuron, supporting the partitioning we observed in brain homogenates (http://movies.commons.ucalgary.ca/mercury).

It is critical to understand that both tubulin and CK in normal brain are found primarily in the soluble fraction of a homogenate. Yet, both proteins appear of normal size and unmodified on reducing polyacrylamide gel electrophoretic analysis (PAGE). This indicates that both intact tubulin and CK have formed crosslinks with other proteins that are insoluble under physiological conditions. Yet, these crosslinks are readily disrupted by the common dithiolthreitol (DTT) reduction procedure used before PAGE. What tubulin and CK have in common is that both have a very reactive sulfhydryl in their nucleotide binding sites that, if modified, inhibits their biological activity (14, 15).

Mercury has a very high affinity for sulfhydryls and has been proven to be a potent inhibitor of the biological activity of both of these proteins. Also, mercury is divalent and can form crosslinks between soluble proteins like tubulin and CK and is known to cause protein aggregation. A generalized single step reaction would be as given in reaction 1.

1: Protein-A-SH + Protein-B-SH + $Hg^{2+} \Rightarrow$ Protein-A-S-Hg-S-Protein-B + 2 H^+

This chemistry would allow the formation of aggregates that would abnormally appear in the particulate fraction. Due to its dithiol structure DTT is an excellent chelator of mercury. The massive amounts of DTT used in reducing gels could chelate and remove mercury from the proteins resulting in their becoming soluble again and migrating as unmodified on gel electrophoresis as observed as shown in reaction 2.

2. Protein-A-S-Hg-S-Protein-B + DTT $\Rightarrow$ Protein-A-SH + Protein-B-SH + DTT-Hg

The correct criticism of any homogenate test is that it may not occur in a living animal. Therefore, experiments were done to determine if mercury vapor, the primary form that escapes from dental amalgams, could mimic the effect in rats exposed to such vapor for various periods of time (5). Rats are different from humans in that they can synthesize vitamin C whereas humans have to ingest vitamin C. Vitamin C is thought to be somewhat protective against heavy metal toxicity and other oxidative stresses. However, we observed that the tubulin in the brains of rats exposed to mercury vapor lost between 41 and 75 percent of the nucleotide binding capability demonstrating a similarity to the aberrancy observed in AD brain and confirming the homogenate results (5).

There is also an "excito-toxic" amino acid hypothesis for the cause of AD wherein excito-toxic amino acid glutmate builds up in brain tissue causing neuronal death. This is a reasonable hypothesis and could co-exist with the thiol-sensitive enzyme/mercury hypothesis. The activity of Hg^{2+} sensitive glutamine synthetase (GS) was measured in AD brain and the amount of GS in the cerebrospinal fluid of AD versus control patients was determined. GS was found it to be inhibited in AD brain and copies of GS were elevated in the cerebrospinal fluid (12, 22). It has also been predicted by two groups that the elevation of GS in the cerebrospinal fluid of AD patients has potential as a diagnostic aid for AD (12,16). However, it is reasonable to conclude that brain GS would be rapidly inhibited by Hg^{2+} produced by oxidation of mercury vapor. This inhibition would cause a rise in glutamate based excito-toxicity and could cause neuron death. Further, glutamate is transported by molecular motors down the microtubules that are destroyed by Hg^{2+}. Therefore, both the metabolism and transport of glutamate would be immediately affected by exposure to mercury. The measurement of GS in cerebrospinal fluid is most likely a measure of glial cell toxicity and death as would be expected in several central nervous system diseases, including AD.

Illnesses that lower our metabolic energy levels also lower our ability to synthesize the reducing equivalents that allow our body to bind and dispose of excess mercury. Hg^{2+} is known to inhibit the metabolic processes in mitochondria that produce ATP and NADH by inhibiting the enzymes of the citric acid cycle and the electron transport system. These nucleotides are absolutely required for both the synthesis of reduced glutathione (GSH) and to reduce glutathione after it is oxidized. GSH in the reduced state is the major biomolecule involved in the natural removal of mercury from the body. Therefore, as mercury slowly accumulates in the body it weakens the body's natural defense against all forms of other heavy metal toxicities and increases the overall oxidative stress expressed by reactive oxygen species formation. It is well known that AD brain tissue suffers from greater oxidative stress in all cellular components versus similar tissues from control subjects. This would be expected and it is well documented that mercury increases oxidative stress in biological tissues. Further, Hg^{2+} is well known to inhibit numerous other enzymes important to neurological function, including the Na/K ATPase that is necessary for recovery from a nerve-action potential. Therefore, the many numerous aberrancies observed in AD brain would be expected within a hypothesis that proposes exposure to Hg^{2+} is a major contributor to this disease.

Relevant Mercury Exposures and Measurments.

Mercury from Dental Amalgams;

The fact that mercury has inhibitory effects on tubulin, CK and GS and that these proteins are proven to be aberrantly inhibited in AD does not alone conclusively prove that mercury exposure causes AD. However, it definitely proves that chronic, daily exposure to mercury would at least exacerbate the clinical conditions of AD. Is such an exposure to mercury likely? The answer is yes, and this makes mercury involvement in AD plausible.

First, the question must be addressed if there is enough mercury in an amalgam filling to continue a low chronic level exposure for years? The answer is yes. For example, if a single large amalgam filling contained 1 gram of mercury (1 million micrograms) and lost a significantly toxic 10 micrograms per day there would be enough mercury for 100,000 days or about 274 years of exposure. A small tenth of a gram mercury filling would last 27 years. So enough mercury is within amalgam fillings to provide a consistent chronic toxic exposure for the life of most fillings.

Second, does mercury emit from amalgams at a rate that should cause concern? The answer is yes. Dental amalgams, or "silver fillings" as organized dentistry calls them, are approximately 50% mercury by weight and it is quite easy to demonstrate that mercury vapors readily emit from these fillings. The actual amount can only be determined with the amalgam in a closed container and the amount of mercury released being determined using solid, time proven chemical techniques and instrumentation. The accurate level of mercury released cannot be accomplished on amalgams in the mouth. In a carefully designed study in a sealed container Chew et al. tested the "long term dissolution of mercury from a non-mercury-releasing amalgam (trade name Composil)" (9). Their results demonstrated "that the overall mean release of mercury was 43.5 +/-3.2 micrograms/cm^2/24hr, and the amount of mercury released remained fairly constant during the duration of the experiment (2 years)".

In my opinion, this 43.5 micrograms/cm^2/day is not an insignificant amount of mercury exposure if one considers the number of years a 70 year old individual living today may have been exposed to chronic mercury levels from his amalgams. Additionally, 43.5 micrograms/cm^2/day is the level released without galvanism, excess heat, or pressure from chewing, all factors that increase mercury release from amalgams in the mouth (26).

Some may disagree with the figure presented above and indeed, amalgams of different manufacture may release more or less. However, the pro-amalgam supporters have not published any carefully controlled study similar to the one above repudiating the finds of this research group. They definitely have all of the scientific laboratory expertise needed to do this. Instead, they utilize "estimates" of release based on urine and blood levels that are widely known to vary dramatically with time and not to be reliable. In judging science one looks for what is not published that obviously should have been.

Does the Presence of Amalgams Contribute Significantly to Mercury Body Burden?

There have been numerous published reports of increased tissue mercury levels in subjects and the relationship to increased number of amalgams fillings (see 10, 11, 25 and references therein). Also, the World Health Organization Scientific Panel found ranges

of mercury exposures from 3 to 70 micrograms/day with the bulk being from amalgam fillings (31). Data relevant to this question was addressed by a recent NIH study using 1,127 military personnel (20). Soldiers in this study had an average of 20 amalgam surfaces with ranges from 0 to 66 surfaces. Each 10 surfaces increased the urine mercury level 1microgram/liter or an average of 4.5 micrograms/day. This study indicated that individuals with an average number of amalgam fillings had about 4.5 times the urine mercury levels as controls without amalgams. Those soldiers with over 49 surfaces averaged over 8 times the urine level observed in the non-amalgam controls. Further, the blood and urine mercury levels corresponded well with the number of amalgam fillings (20). The results above are consistent with an earlier study where urinary mercury levels dropped by a factor of 5 after the removal of several amalgam fillings. The conclusion of the authors was that mercury from dental amalgams exceeds that from all forms of food, air and fluids (23). All of the data on urine or blood mercury levels must be considered with the knowledge that approximately 80% of inhaled mercury vapor is retained in the body. Mercury typifies a "retention" toxicity and much of the mercury taken into the body is absorbed by the solid tissues. The amount in urine represents mercury being excreted. However, the main question is how much is being retained in the different body tissues.

In contrast to other reports there was published in the J. American Dental Association research that measured mercury levels in brain and other neurological tissues and concluded "Our results do not support the hypothesis that dental amalgam is a major contributor to brain Hg levels. They also do not support the hypothesis that Hg is a pathogenetic factor in AD (25)." I can't explain how amalgams can increase blood mercury levels and not increase brain mercury levels. However, these researchers presented data showing no significant increase in Hg level in several brain regions between control and AD subjects. They surprisingly included data showing that the Hg levels in control olfactory region was more than double that of the corresponding AD olfactory tissue. This olfactory mercury increase in control subjects could have several explanations.

One explanation could be they were not precise in estimating the amount of mercury exposures of their subjects and the controls they selected were much more exposed to mercury than the AD subjects selected. The olfactory region is outside the blood-brain barrier and should be a consistent internal standard for mercury exposure in the air breathed in by the subjects.

Another explanation would be that the controls, even though exposed to more than double the mercury levels of the AD subjects, as evidenced by the olfactory region Hg levels, had a mechanism that protected their brain tissues from also having double the mercury levels. If this were true, then dividing the brain mercury levels by the olfactory mercury levels would give results that clearly show a significant ability of the controls to have a mechanism that protects brain tissue from mercury that is lacking in the AD subjects. This mechanism could be the presence of the protective APO-E protein genotypes (see below) and other predisposition factors not yet known.

The debate continues on whether or not human mercury exposures reach levels in the brain and other tissues that could be considered toxic or harmful (24,25). There are several reasons why the brain levels of mercury would not directly correlate to the

damage being done. The level of selenium in the diet, which could bind with mercury rendering it less toxic, is the most straight-forward example. Also, the determination of the levels of mercury toxicity that could cause neurological disease has been done using animals, such as rats and monkeys, under tightly controlled laboratory conditions where the diet is carefully monitored to exclude other toxicants. Further, any test animal that becomes ill or infected by microbial sources is removed from the study. However, humans do not live under such restricted conditions. For example, we are exposed to numerous infections and additional heavy metal imbalances in AD brains have been reported numerous times. Cigarette smokers are exposed to excess cadmium (Cd^{2+}) and lead (Pb^{2+}) toxicity is not that uncommon in the inter-city environment or for those exposed to leaded gasoline fumes for many years. This means that the synergistic toxicities of combined heavy metals must be considered for humans.

It is also questionable whether or not brain mercury levels should be expected to remain high in AD brain. A report by Hock et al. (27) stated that in early onset AD the blood levels of mercury were almost three fold higher than the control groups and that these increases were unrelated to the patients' dental status. The concluded that the explanation of increased mercury in AD would include yet unidentified environmental sources or release from the brain tissue with the advance in neuronal death. The AD brain loses 25% of its average weight by time of death making the latter explanation reasonable. It is a well-known biochemical event that cells or tissues rid themselves of denatured, unusable protein.

The inhibition and break down of neuronal tissue may also explain another observation related to AD. It is documented that AD patients have elevated olfactory thresholds and impaired odor identification. It is further suggested that in patients with mild cognitive impairment, olfactory problems may have clinical value as an early diagnostic predictor for diagnosis of AD(28, 29, 30). Mercury in the oral cavity must interact with the olfactory bulb. Due to the neurotoxicity of mercury, this could impair olfactory sensitivity. Also, based on our hypothesis impaired olfactory response would almost have to occur.

Our laboratory has shown that one can add various metals to human brain homogenates to levels that alone do not affect nucleotide binding to tubulin, yet the very presence of these metals synergistically increases the toxicity of Hg^{2+}. That is, the presence of Pb^{2+}, Zn^{2+} and Cd^{2+}, at non-toxic levels, decrease the amount of Hg^{2+} required for 50% inhibition of tubulin or creatine kinase viability. It is important to remember the "Periodic Chart of the Elements" which places Zn, Cd and Hg in the same IIB category and all have high affinity for thiol groups. In other words, mercury is much more toxic in the presence of other metals that compete with mercury for the binding sites on protective biomolecules (e.g., APO-E2 & E3, glutathione or GSH, metallo-thionine, etc.).

It is also important to note that the "test tube levels" of mercury are not representative of what would happen in a dynamic system where a constant level of mercury is being supplied by the amalgams. Since mercury toxicity is a "retention toxicity" all mercury pulled from the system, or retained by the tissue, is replaced by more mercury being constantly released from the amalgams and the Hg^{2+} level and toxicity in

solution remains constant. In the test tube as the mercury is pulled out of solution the free Hg^{2+} concentration in solution drops making the soluble aspect less toxic with time.

Are Amalgams Capable of Producing Toxic Solutions?

To propose deleterious effects of amalgams while in the mouth the amalgams must be able to produce toxic effects outside of the mouth. Wataha et al. reported that extracts of the amalgam material (trade name, Dispersalloy) "was severely cytotoxic when Zn release was greatest, but less toxic between 48 and 72 hours as Zn release decreased" (8). Zn is a trace material in dental amalgams and a needed supplement for living neurons. Therefore, it did not seem likely that the toxicity was due to Zn emitting from the amalgams. When we compare the toxicity of Hg^{2+} in brain homogenates as described above (refs. 3 & 4), the addition of 0, 10 and 20 micromolar Zn^{2+} increased the inhibition of GTP binding to tubulin from 4% to 50% and 76%, respectively (7,13). This supports the concept that the Zn correlation to increased toxicity was due to the synergistically enhanced toxicity of the mercury released from the amalgam. Further,other studies in our laboratory have shown that soaking of amalgams in distilled water for less than one hour created a solution that also caused rapid inhibition of brain tubulin and creatine kinase similar to that observed on adding Hg^{2+} solutions. Therefore, it appears that the toxicity of solutions in which amalgams were soaked is not caused by direct Zn^{2+} toxic effects. Rather, enhanced toxicity is due to the Zn^{2+} or other amalgam heavy metals stimulating the toxicity of mercury by occupying biomolecule chelation sites. This would result in a higher concentration of free Hg^{2+} capable of inhibiting the activity of critical nucleotide binding proteins such as tubulin and CK.

The observed synergistic toxicity of other heavy metals with Hg^{2+} has been supported in animal models. Combining an LD-1 solution of Pb^{2+} with an LD-1 solution of Hg^{2+} gave a solution with an LD of 100, instead of an LD-2, when injected into rats (19). The bottom line is that mercury toxicity is enhanced by the presence of other heavy metals. Therefore, when one considers the toxicity of a certain body level of mercury it is somewhat meaningless unless the body level of other heavy metals is also considered.

With the complexity of our environment and the confounding factors involving neurological diseases, and without major government supported epidemiological studies proving safety, it is impossible to state with assurance, as many amalgams supporters do, that this exposure does not place the individuals at greater health risk. The "lack of proof of damage" from mercury exposure seems unwarranted to be used as "proving the safety of any material" that unnecessarily exposes individuals daily to several micrograms of mercury.

Genetic Susceptibility Considerations.

Any hypothesis of the etiology of AD must consider information on genetic susceptibility. The best known genetic risk factor for AD is the correlation of APO-E genotypes to the age of onset of AD (24a,b). Individuals can inherit any combination of the alleles APO-E2, E3 or E4. Individuals inheriting APO-E2 or combinations of APO-E2 and E3 are much less likely to get early onset AD than are individuals who have inherited APO-E4 genes. Also, APO-E2 appears to be more protective than APO-E3 against early onset AD. Therefore, it is necessary that the mechanism of mercury toxicity contain an explainable relationship for the APO-E genetic susceptibility. This is

accomplished in a straight-forward manner by considering the basic structural difference between these three alleles. Simply put, the protective APO-E2 has two sulfhydryls (cysteines) that can bind mercury or other heavy metals that APO-E4 lacks. For example, in APO-E3, one of APO-E2 cysteines is replaced by an arginine and in APO-E4, both of the APO-E2 cysteines are replaced by arginines (32). Therefore, lack of protection against early onset AD was proposed to follow the loss of mercury binding sulfhydryls from APO-E proteins (6).

The protection provided by APO-E2 is reasonable when considering the nature and biochemical assignment of APO-E proteins. APO-E proteins are involved in cholesterol transport and all three alleles do this reasonably well. However, APO-E is classified as a "housekeeping protein". That is, in contrast to tubulin, GS and CK, which are meant to stay inside of cells where they are synthesized, APO-E is meant to leave the brain cells carrying damaged cholesterol through the cerebrol spinal fluid (CSF), across the blood-brain barrier into the blood where it is removed by the liver. It fits into the hypothesis that while APO-E2 or E3 are leaving the brain cells and traversing the CSF they likely bind and remove mercury, other heavy metals or other sulfhydryl reactive toxins that may have made it into the central nervous system thereby protecting the brain neurons (6). APO-E4 cannot as effectively bind mercury and therefore does not provide the protective parameters that APO-E2 and E3 have. It is interesting to note that the second highest level of APO-E protein in the body is in the CSF that bathes and protects the brain.

Oral Super-toxins Produced by Reaction With Dental Mercury.

Many recent literature and popular press reports state that the presence of periodontal disease raises the risk factor or exacerbates the condition of several other seemingly unrelated diseases such as stroke, low birth weight babies, cardiovascular disease (See October 1996 issue of Periodontology). The anerobic bacteria of periodontal disease produce hydrogen sulfide (H_2S) and methyl thiol (CH_3SH) from cysteine and methionine, respectively. This accounts for the "bad breath" many individuals have.

However, in a mouth that produces H_2S, CH_3SH (from periodontal disease) and Hg^0 (from amalgam fillings) the very likely production of their reaction products, HgS (mercury sulfide), CH_3S-Hg-Cl (methyl-thiol mercury chloride) and CH_3S-Hg-S-CH_3 (Dimethylthiol mercury) has to occur. This is simple, straight-forward chemistry whose occurrence is supported by easily observable "amalgam tattoos". These tattoos are purple gum tissue surrounding certain teeth where the gum and tooth meet and primarily caused by HgS as determined by elemental analysis of such tissue.

HgS is one of the most stable forms of mercury compounds and is the mineral form found in ore, called cinnabar, from which mercury is mined from the earth. All of these oral site produced compounds are classified as extremely toxic and the latter compound, dimethylthiol-mercury is very hydrophobic and its solubility would be similar to dimethyl-mercury (CH_3-Hg-CH_3). Dimethyl-mercury was the compound that was made famous in the press where only a small amount spilled on the latex gloves of a Dartmouth University chemistry professor caused severe neurological problems and finally death 10 months later. In my opinion, the extreme lethality of CH_3-Hg-CH_3 compared to other forms of mercury is due to its ability to collect in hydrophobic regions

of the body, like the central nervous system. CH_3-Hg-CH_3 is similar to CH_3-S-Hg-S-CH_3 in its hydrophobic characteristics.

Logic implies that anyone with periodontal disease, anaerobic bacterial infected teeth and mercury containing fillings would be exposed daily to these very toxic compounds. In our laboratory we synthesized the two methylthiol-mercury compounds and tested them. They are extremely cytotoxic at 1 micromolar or less levels and are potent, irreversible inhibitors of a number of important mammalian enzymes, including tubulin and CK.

A recent report stated that the tissues of individuals who died of Idiopathic Dilated Cardiomyopathy (IDCM) had mercury levels of 178,400 ng/g tissue or 22,000 times more than their controls who died of other forms of heart disease. IDCM is a disease where young athletes drop dead during strenuous exercise. It seems impossible for a tissue to bind this much mercury on protein without early notice of injury through pain and lack of bioenergy. However, if this mercury were to combine with H_2S produced by a local anerobic infection the mercury could precipitate out in the tissue as HgS as it does in "amalgam tattoos" causing a buildup without killing the tissue immediately. However, one has to ask where does this excess mercury come from. Many times this occurs to young intercity athletes who are not on a high seafood diet. My opinion is that dental amalgam is the source of this mercury. Also, if HgS is being made in the heart tissue the very cytotoxic CH_3-S-HgX and CH_3-S-Hg-S-CH_3 are also being made.

To determine if toxic teeth could have an effect on the enzymes/proteins of human brain we have done the following study. Several very toxic teeth were incubated for 1 hour in distilled water. Aliquots of these solutions were then added to control human brain homogenates and the resulting samples tested for tubulin viability and partitioning. The results showed that about 40% inhibited the viability of tubulin and caused partitioning. Therefore, depending on the type of anerobic microbial infection existing in avital teeth it is possible to have a toxicant production that would exacerbate the condition classified as AD. It is also probable that many of these teeth were extracted from mouths containing amalgam and the toxins in these teeth may also consist partially of extremely organic-mercury compounds as described above.

Based on the potential clearance represented by elevated blood levels of mercury in early onset AD patients, the synergistic effects of other heavy metals, the fluctuating GSH levels during illness and aging, and dietary factors (e.g. selenium levels) there is no reason to believe that the adverse effects of mercury from amalgams would be dose dependent in any straight-forward manner in post-mortem AD brain. To expect this would fly in the face of published data and scientific logic. Further, to eliminate mercury as a factor in AD based on statistically insignificant increases above normal in post-mortem brain samples is not warranted. Also, involvement of genetic factors likely plays a key role.

<u>Studies Involving Neuronal Cultures and Diagnostic Markers for AD.</u>

A recent publication supports our contention that mercury from dental amalgams poses a major threat to the exacerbation of AD. Olivieri et al. demonstrated that exposure of neuroblastoma cells to sub-lethal doses (36×10^{-9} molar) of Hg^{2+} caused a rapid drop in GSH, an increased secretion of β–amyloid protein and an increased phosphorylation of

the microtubulin protein Tau (17). The latter two of these biochemical changes are uniquely observed in AD brain tissues and are widely considered to be diagnostic, pathological markers of the disease. β-amyloid protein makes up the 'amyloid plaques' that was one of the first diagnostic markers reported for AD brain pathology. A very strong component of AD researchers believe that amyloid protein is the cause of AD. Therefore, mercury exposure at nanomolar levels causes neuroblastoma cells to produce a protein that is believed to be involved directly in AD. This lead the authors of this paper to conclude that mercury would have to be consider as causal for AD (17).

Further, the recent report of the response of neurons in culture rapidly forming neurofibillary tangles on exposure to extremely low levels of mercury, by a process involving loss of microtublin structure, completes the picture that mercury is capable of causing the formation of three widely accepted major pathological diagnostic hallmarks of AD in neuronal cultures (18). An impressive video accompanying this publication and available at http://movies.commons.ucalgary.ca/mercury shows the addition of 2 microliters of 10^{-7}M mercury to a 2 milliliter solution bathing neurons caused a rapid stripping of the tubulin from the neurofibrils leaving them bare. This would be predictable from our earlier data showing mercury interfering with normal tubulin-GTP interactions and the abnormal partitioning of tubulin into the particulate fraction of brain tissue(3,4,6). The bare neurofibrils then aggregate forming neurofibrillary tangles (NFTs) similar to those observed in AD brain. The final mercury concentration of 10^{-10}M in these experiments is roughly 100 to 1000 times lower than the 10^{-7}M levels normally found in human brain of individuals with amalgam fillings. The majority of the mercury in brain is likely bound by protective compounds like GSH or selenium and not free to cause neuronal damage. However, it is not unreasonable to consider that some of this mercury is present as free Hg^{2+} some fraction of the time, especially when illness or other toxicities lower the GSH levels.

However, these two recent publications supports the initial contention that mercury first rapidly inhibits thiol-sensitive enzymes like tubulin, creatine kinase and glutamine synthetase and dramatically affects metabolism and membrane structure. The stripping of tubulin leads to the formation of NFTs and the exposing Tau for hyper-phosphorylation. This is followed by elevated production of β–amyloid protein that can aggregate into senile plaques. all diagnostic markers for AD. It is consistent with the mercury toxicity hypothesis for AD that neurofibillary tangles, hyper-phosphorylated Tau, amyloid plaques and increased oxidative stress observations are the result of neuronal toxicity and death in AD, they are not the cause. The cause is exposure to environmental toxicants like mercury that attack enzymes with the most reactive thiol groups.

CONCLUSION:

The data on the effects of mercury on the nucleotide binding properties and the abnormal partitioning of two very important brain nucleotide binding proteins proven to be aberrant in AD brain first suggested that mercury must be considered as an exacerbating factor to the condition classified as AD. This has been strongly supported by the recent finds that nanomolar levels of mercury causes neuroblastoma cells to secrete β-amyloid protein and increase phosphorylation of the microtubulin associated protein Tau, both major biochemical observations related to AD. Also, neurons in culture

exposed to Hg^{2+} at the 10^{-7} to 10^{-10} M levels have conclusively been visually shown to rapidly produce abnormal tubulin aggregation, resulting in particulate partitioning as observed in AD brain. Also, this stripping of tubulin from the neurofibrils results in the formation of NFTs that are indistinguishable from those observed in AD brain. and used as a diagnostic marker of the disease(18). These facts alone warrant serious consideration of mercury as a certain exacerbating factor for AD, if not causal.

Consideration of mercury as a causal or exacerbating factor for AD is especially relevant when mercury is present in combination with other heavy metals such as zinc (Zn) cadmium (Cd) and lead (Pb). Synergistic toxicity is not an exception but is observed as a general rule (19). This obviates the argument that mercury must be significantly elevated in AD brains to be considered causal or contributing to the disease state. Further, the reaction of oral mercury from amalgams with toxic thiols produced by periodontal disease bacteria very likely enhances the toxicity of the mercury being released. Humans are likely the only mammals with amalgam fillings and periodontal disease. Bluntly, the determination of safe body levels of mercury by using animal data where the animals have not been exposed to other heavy metals is not scientifically justifiable. Mercury is much more toxic to individuals with other heavy metal exposures. It is my opinion that one of the major unanswered questions concerning the toxic effects of mercury is "does the combination of mercury with different heavy metals lead to different clinical observations of toxicity?"

Finally, mercury biochemically mimics numerous observations seen in AD brain tissues including inducing the formation of widely accepted diagnostic hallmarks of the disease. Further, the synergistically toxicity of mercury with other heavy metals, microbial produced oral toxins and certain metal chelators is obvious. It is also a scientific fact that amalgams contribute greatly to overall mercury body burden and are capable of producing cytotoxic solutions with properties like mercury solutions. Therefore, it seems very reasonable to consider a hypothesis that mercury would be the major contributor to early onset AD.

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Thimerosal-Containing Vaccines and Neurodevelopment Outcomes

FORWARD: Thimerosal or merthiolate is a derivative of thiolsalicylate where ethyl-mercury is attached though the sulfur. It is defined as a preservative or anti-microbial in medical use. This anti-microbial action is dependent on thimerosal breaking down releasing ethyl-mercury that can penetrate cell membranes and bind to intracellular enzymes, inhibiting them, and causing cell death. Further, in certain biological environments the ethyl-mercury can further break down releasing mercury cation (Hg2+). Hg2+ is also very reactive with enzymes and proteins inhibiting their biological functions and causing cell injury or death. Both ethyl-mercury and Hg2+ are very neuro-toxic compounds.

However, ethyl-mercury is more rapidly partitioned into the hydrophobic (fatty) tissues of the central nervous system and is a more potent neuro-toxin than Hg2+ based on this "partitioning factor". It is this partitioning factor that makes organic-mercurials such as dimethyl-mercury so neuro-toxically lethal (this is the compound that caused the death of a Dartmouth University chemistry professor after she was exposed to a drop or two on her gloved hand). The concern with organic-mercurials, such as thimerosal, is that such compounds can be perceived as "pro-toxicants" just as certain pharmaceuticals can be classified as "pro-drugs". This means that the original compound, e.g. thimerosal, is less reactive giving the compound time to partition into certain areas of the body before it breaks down releasing the ethyl-mercury and then further releasing Hg2+. However, while attaching ethyl-mercury to thiolsalicylate makes the ethyl-mercury less reactive it most likely allows increased partitioning into the central nervous system before the ethyl-mercury is released and thereby, increases the neuro-toxicity per unit ethyl-mercury involved.

Considerable caution must be taken when stating what is the "toxic level" of mercury and any mercury containing compound. Humans are not rats in a pristine cage where their environment can be controlled to ensure that other toxicities and infections are not occurring. The level of mercury that would cause toxicity in a healthy individual is much higher than what would be needed to cause a toxic effect in an individual that is ill or under oxidative stress. This is because additional stresses lower the amount of protective compounds that bind mercury and render it less harmful. If an individual is low on these protective compounds, then less mercury or thimerosal would be needed to cause a clinical effect. Below I will present my interpretation of our research and that from other laboratories that focus on the potential toxicity of injected thimerosal in the vaccine mixture.

BIOCHEMICAL TOXICITY STUDIES: In my laboratory we have recently done an evaluation of the potential *in vitro* toxicity of vaccines containing thimerosal as a "preservative" versus those vaccines not containing thimerosal. In these preliminary studies, vaccines with thimerosal added consistently demonstrated in vitro toxicity that was markedly greater than the non-thimerosal or low thimerosal containing vaccines. We also compared the toxicity of the vaccine solutions with solutions of pure thimerosal and

with solutions of mercury chloride. Mercury is a known neurotoxin and its mechanism of neurotoxicity has been studied in our laboratory for the past 10 years. To determine the relative toxicity we used two different biological testing systems: (i) brain homogenates and (ii) a mixture of four purified mammalian enzymes. In human brain homogenates we had earlier observed that mercuric ion rapidly inhibited tubulin viability at low micromolar levels, mimicking the situation in Alzheimer's diseased brain, but was less toxic to actin **(see Figures 1& 2)**. Both tubulin and actin are polymerizing proteins that are actively involved in neurite growth cone activity. In contrast to mercuric ion, vaccines containing thimerosal inhibited both tubulin and actin viability **(see Figure 3)**. This would indicate that thimerosal has the potential to be much more damaging to neurite development than equivalent levels of mercuric ion. It is my hypothesis that thimerosal releases ethyl-mercury which most certainly interferes with neurite growth and neuronal development in infants through rapid inhibition of several thiol-sensitive enzymes/proteins including actin, tubulin and creatine kinase. This supports the concept that thimerosal in biological solutions injected into the human body could cause a number of systemic problems identified as disease states.

CELL CULTURE WORK ON THIMEROSAL TOXICITY: The toxicity results obtained in our biochemical toxitity studies were not at all unexpected since thimerosal and other compounds containing a similar thiol-organic mercury group are widely known to be especially potent neurotoxic agents. Our biochemical toxicity results are very consistent with the reported toxicity of thimerosal containing vaccines versus non-thimerosal containing vaccines as observed in cell culture studies (*Kravchenko et al., Evaluation of the Toxic Action of Prophylatic and Therapeutic Preparations on Cell Cultures III. The Detection of Toxic Properties in Medical Biological Preparations by the Degree of Cell Damage in the L132 Continuous Cell Line. Zh. Mikrobiological Epidemiol. Immunobiol. (3):87-92, 1983*). The results of this research demonstrated the toxicity of thimerosal (merthiolate) by showing cell damage of the 1:10,000 concentration found in vaccines after dilution of this mixture to 1 part per 128. The conclusion was that thimerosal use for medical and biological preparation (i.e. vaccines) manufacturing is inadmissible, especially in pediatrics. Other studies on cytotoxicity of thimerosal compared it to another mercury containing preservative (phenylmercuric acetate) and thimerosal was 5 times more toxic with only a two minute exposure to the cells. The LD50 for thimerosal was 2.2 micrograms/ml for a 24 hour exposure to human conjunctival cells and the comment was made that "the longer the contact time of these preservatives, the severer the damage to the ocular tissue".

In collaboration with another professor in our department we have now included toxicity studies using human brain neurons in culture. Our initial studies have shown that thimerosal is quite toxic to these neurons in culture. Further, studies using vaccines with and without thimerosal present demonstrated that the presence of thimerosal greatly enhanced the toxicity. The neuron toxicity studies mirror the results we observed in the enzyme toxicity studies mentioned above with the thimerosal being more toxic than inorganic mercury. Further studies are underway at the present time.

CASE HISTORIES ON THE TOXICITY OF THIMEROSAL AND OTHER

ETHYL-MERCURY RELEASING COMPOUNDS: A recent review covers much of the case history literature on the little that is known about ethylmercury toxicity (*L. Magos, Review on the Toxicity of Ethylmercury, Including its Presence as a Preservative in Biological and Pharmaceutical Products, J. Applied Toxicology 21, 1-5, 2001*). The conclusions reached by the author of this review is that "ethylmercury may present a risk when blood mercury concentrations approaches or exceeds 1.0 microgram per ml and severe intoxication occurs when blood mercury concentration approaches or exceeds 2 micrograms per ml."
In the context of the literature reviewed the conclusions by Dr. Magos seems reasonable. However, this conclusion was based primarily on ethylmercury and methylmercury exposures from occupational exposures, dietary intake, externally applied tinctures along with vaccination data on adults. It should be noted that in considering deceased patients the one infant had a blood mercury (from an externally applied tincture) that was measured at 1.34 micrograms per ml, a young boy had a blood mercury of 5 micrograms per ml (from eating pork from a pig feed ethylmercury) and adults had 15 micrograms per ml (from eating bread made with seed treated with a compound that generated ethylmercury). Without the needed extensive data to make a conclusion, it appears as if the younger the patient the more deadly or toxic the ethylmercury is at a lower concentration. This is further supported by the other (Kostial, K., et al. Influence of Age on Metal Metabolism and Toxicity, Environmental Health Perspectives, v25, 81-86, 1978) who state "results obtained in sucklings show a very high intestinal absorption of all metals which is partly attributed to milk diet; a higher whole body retention, higher blood levels and a much higher accumulation in the brain". Certainly, no conclusion of safe levels of exposure to ethylmercury on infants could be made from the data reviewed by Dr. Magos.

The exposures reviewed were from different delivery modalities and there is a considerable difference in the toxicity of many materials when oral intake is compared to injections via the vaccine route. Total mercury in the blood stream does not distinguish between bound mercury (e.g. that coupled with glutathione and being removed from the body) and unreacted mercury (that available to cause further damage). Ratios of bound and free ethylmercury are likely to be different if ethylmercury is eaten or inhaled versus injected, bypassing the protective systems available in the intestines. It was also pointed out in the review that the blood/urine ratios varied from 3.4 to 18 indicating that urine mercury levels are inferior for monitoring ethylmercury exposures. However, since ethylmercury should partition between blood and urine at a consistent ratio this data could also be interpreted to indicate that the mercury in some of these patients is coming from more than just ethylmercury (e.g. dental amalgams that are the major source of human mercury body burden). In a report on mercury levels in squirrel monkeys treated intranasally with thimerosal (*Blair, A., Clark, B., Clarke, A and Wood, P., Tissue Concentrations of*
f Mercury After Chronic Dosing of Squirrel Monkeys with Thimersal, Toxicology,v3, 171-176, 1975) it was shown that exposure to 0.002% thimerosal daily for 6 months, with a total of 2,280 μg given, lead to a 174/29 or about 6.0 ratio of mercury in the brain/blood ratio indicating that thimerosal leads to a more rapid build up of brain versus blood mercury. However, it was pointed out that the highest brain total (250ng/g) was still

below the 3-9 μg/g where neurological symptoms appear, but this later value would depend on the oxidative stress of the patient and could be much lower.

The review states that "ethylmercury in medicinal preparations declines with time" and gave examples of 38%, 64% and 85% decreases in ethylmercury in plasma and immunoglobin G samples. This mercury did not disappear and the loss of ethylmercury has to be due to ethylmercury reacting covalently with the protein-thiols in the medicinal prepartions. In aged medicinal preparations, increased ethylmercury reaction with protein-thiols in the preparations would likely change the neurotoxity effects of the resulting mercury complexes compared to pure ethylmercury. How this pre-reacted ethylmercury would contribute to blood levels of mercury appears unknown, but it is likely to be quite different from pure ethylmercury. However, what is known is that ethylmercury retains its severe toxicity after prolonged exposure in living animals. This is supported by a case mentioned in the Magos review where ethylmercury obtained by "consumption of meat from a pig fed with ethyl-mercury" caused severe damage to adults and killed two young boys. It seems as if ethylmercury can retain its severe toxicity after a period of incubation time in a living pig, butchering and storage of meat, followed by cooking. Then the concept that the faster decomposition of ethylmercury, relative to methylmercury, decreases its toxicity compared to methylmercury seems to be such a small difference as to be insignificant. What is solidly observed is that ethylmercury (and other organic-mercurials) can withstand considerable exposure to a living system, storage in a biological environment, exposure to high heat in the presence of muscle tissue, and still produce a lethal toxicity when taken orally.

In a 1972 a (*National Geographic , Quicksilver and Slow Death, v142, #4, 507-527, 1972*) a similar report was presented where the pig was fed seed coated with Panogen, a methylmercury pesticide. The family ate the pig as above and the four children suffered severe neurological damage. But, in contrast to the ethylmercury poisoning above, they all lived. One of the children was *in utero* during the consumption of the pork, suffered the most and was born blind and mentally retarded. Again, this supports the concept that the younger the human the more detrimental the toxic effect the organic mercury compounds will have.

It appears certain that much of the blood level mercury in these patients presented in the Magos review could be from sources other than pure ethylmercury. In my opinion, I do not believe that a safe level of ethylmercury can be arrived at by only comparing blood levels of mercury if we do not know the chemical nature of all of the contributing mercury sources, the initial source of the mercury or if the presence of other compounds were involved (e.g. antibiotics that bind heavy metals such as tetracycline and enhance thimerosal toxicity:see below in Synergistic Toxicity).

It is of major concern that ethylmercury from thimerosal in vaccines is a special situation. It is injected with millimolar levels of aluminum and it is probable that thimerosal, a negatively charged molecule, has formed a salt compound with the positively charged aluminum cation that would change its partitioning, breakdown rate, and may have a synergistic effect on the toxicity of any mercuric ion produced from the

ethylmercury. Aluminum is a known neurotoxin and to be causally involved in macrophagic myofasciitis. The enhanced toxicity of ethylmercury in the presence of other toxic agents is to be expected. Few of the clinical cases included in the Magos review were from vaccine but the one that was discussed problems which occurred in a 44 year old adult with a blood mercury of 0.104 μg per ml, so low that Dr. Magos called the diagnosis "unconvincing". Perhaps co-administration of thimerosal with aluminum in the Hepatitis-B vaccine represents the "other aetiological factors than ethylmercury" that might have been responsible for his mercury like induced symptoms at such low concentrations. The authors of the report on this patient state "this patient had evidence of previous environmental exposure to mercury" and this data can imply that thimerosal is more toxic in patients previously exposed to materials that sensitize them.

DR. MAGOS REPORT TO THE IOM, SUMMER 2001: Dr. Magos makes several statements that reasonable individuals with scientific experience could disagree about. First, "The consequence of faster decomposition is that, compared with methylmercury, the neurotoxic potential of ethylmercury declines faster." This requires the assumption that ethylmercury breaks down to Hg2+ as a toxic factor. What if the breakdown product was a conjugate of cysteine known to enhance the toxicity of mercuric ion? What if the breakdown was caused by reactive oxygen species generated in response to an infection? It is known that ethylmercury breaks down 10 times faster in the presence of reactive oxygen species (*Suda, I, and Takahashi, H., Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical. Arch. Toxicol. 66, 34-39, 1992*) making the production of toxic Hg2+ occur more rapidly at sites of high level of reactive oxygen, and in the body this would be at sites of infection or inflammation or within mitochondria, the important energy producing organelle. In my opinion, the enhanced chemical ability to breakdown ethylmercury versus methyl mercury at sites of reactive oxygen production (usually sites of oxidative stress) makes ethylmercury a much more dangerous compound than methylmercury as it attacks chemically at a site of infectious damage.

In section 2.b.a Dr. Magos quotes his research as showing that methylmercury treated rats had 1.55 (males) and 2.4 (females) the mercury in their brains as did ethylmercury treated rats. In addition, the ethylmercury treated rats had 3.4 fold more inorganic mercury in their brains. He states that this "excludes the possibility that the cleavage itself or the formed inorganic mercury is responsible for the brain damage. If this were the case, the brain ethylmercury treated rats would be more affected than the brain of methylmercury treated rats (which didn't occur by his analysis)." The problem with this conclusion is that Dr. Magos expects the damage caused by methylmercury to be the same as that caused by a combination of ethylmercury and 3.4 fold extra Hg^{2+}. This is not likely as methyl and ethyl mercury would partition into the hydrophobic areas of the brain whereas Hg^{2+} would most likely react in the hydrophilic aspect of the brain. The inhibition of specific brain enzymes by thimerosal (ethylmercury) compared to Hg^{2+} are markedly different.

SYNERGISTIC TOXICITY WITH THIMEROSAL: Since about 1989 my laboratory has been actively involved in research regarding the toxic effects of elemental

mercury and the relationship of this toxicity to neurological diseases, primarily Alzheimer's disease. One fact that has become extremely obvious to me during this past 11 years is that it is impossible to determine the exact toxic level of mercury or mercury containing compounds that is safe for all humans. There are several reasons why mercury should not be considered safe for humans at the measurable levels currently reported as "safe" by current government monitoring agencies. One of these is the obvious effects of other metals on increasing the toxicity of identical levels of mercury. An example is that of zinc ion, an essential metal for normal cell function. Yet, in the presence of mercuric ion, the addition of zinc enhances the toxicity level significantly **(see Figure 4)**. Cadmium and lead are even more potent at enhancing the toxicity of mercuric ion. This concept of synergistic toxicity of mercury with other metals is supported by prior research that demonstrated that a mixture of mercury and lead at LD-1 levels of each metal produced a mixture with an LD-100 effect, at least 50 times the additive effect minimally expected (*Schubert, J., Riley, E.J. and Tyler, S.A., Combined Effects in Toxicology—A Rapid Systematic Testing Procedure: Cadmium, Mercury and Lead. J. of Toxicology and Environmental Health, 4:763-776, 1978*).

The synergistic effects of different compounds with thimerosal are not all known but some do exist. For example, the commonly used antibiotic, tetracycline, is known to enhance thimerosal toxicity. *Crook and Freeman, Reactions Induced by the Concurrent Use of Thimerosal and Tetracycline, American J. of Optometry & Physiological Optics v60,#9, pp759-7611983*, reported that the use of tetracycline in humans induced and increased the irritation and inflammation of the ocular tissues caused by thimerosal. These results were confirmed in studies using rabbits. Therefore, it is obvious that concurrent treatment of infants with other drugs and/or antibiotics has the possibility to enhance the toxic effects of thimerosal exposures. Further, it was postulated that the synergistic effects of tetracycline was due to the metal binding properties of this antibiotic that may have delivered the toxic metal more effectively to the site(s) inducing enhanced toxicity. This data clearly demonstrates that there is no know level of safety for the use of thimerosal, especially in infants being treated with other medicinals that would enhance the toxicity of the ethyl-mercury released such as occurred with tetracycline (a commonly used antibiotic).

Since each human would likely have a level of toxicity from other mercury and non-mercury containing sources it would be impossible to determine the exact level of mercury that would induce observable toxicity in each human. Many environmental toxicants could work synergistically with ethyl-mercury rendering the ethyl-mercury much more toxic than it would be in the absence of these other toxicants (e.g., elemental mercury from dental amalgams, cadmium from smoking, lead from paint and drinking water, aluminum, etc.). Humans are not rats in a pristine cage, eating rat chow carefully prepared to eliminate any toxicants. Humans smoke, drink alcohol, have numerous mercury emitting amalgam fillings, eat questionable food, and drink water known to contain other toxicants. Finally, it is impossible to state the toxic effect of any injection of thimerosal unless one knows the toxic exposure of the individual to other heavy metals or other environmental toxicants.

THE EFFECTS OF AGE AND HEALTH ON THIMEROSAL TOXITICY: The detrimental effect of any specific level of mercury or mercury containing compound would have on any one individual's metabolic system would be directly proportional to both the level of "protective bio-compounds" (e.g., glutathione, metallothioine) that exist within that person on the time of exposure and, the ability to physiologically clear such toxicants from the body. The level of the protective compounds would certainly be directly dependent on two factors, age and health. Infants, with their immature physiology and metabolism would not be expected to handle mercury as efficiently as mature adults. The elderly have been shown to have decreased "protective" glutathione levels compared to middle aged and young adults. Melatonin, a hormone, is known to be decreased in the aged and melatonin is known to increase the neuron and cellular concentration of glutathione. Glutathione is the natural compound that binds mercuric ion and aids in its removal from the body. This explains partly why the aged are also more susceptible to oxidative toxicants such as mercury.

The elderly also have weakened immune systems and are more susceptible to microbial infections are known to lower their chemical energy levels and, further, to reduce their ability to synthesize the proteins that protect them from heavy metals. Infants have their own weaknesses regarding toxic exposures. Infants do not make much bile in their early months of life and are less able to remove mercury through bilary transport, the major route for mercury removal. They also do not have a fully developed renal system that would remove other heavy metals (e.g. aluminum) as effectively as adults. The age factor must always be considered for response to heavy metal exposure as well as spurious microbial infections.

THE EFFECTS OF GENETIC SUSCEPTIBILITY ON MERCURY TOXICITY: Genetically susceptibility is of critical importance. For example, other researchers have shown that genetic carriers of the brain protein APO-E2 are protected against Alzheimer's disease (AD) whereas genetic carriers of the APO-E4 genotype are at enhanced risk factor for developing AD. APO-E proteins are synthesized in the brain with the assigned physiological task of carrying waste material from the brain to the cerebrospinal fluid, across the blood-brain barrier into the plasma where the material is cleared by the liver. The biochemical difference between APO-E2 and APO-E4 is that APO-E2 has two additional thiol groups, capable of binding and removing mercury (and ethyl-mercury) that APO-E4 does not have. The second highest concentration of APO-E proteins is in the cerebrospinal fluid. Therefore, it is my opinion that the protective effects of APO-E2 is due to its ability to protect the brain from exposure to oxidants like mercury and ethyl-mercury by binding these toxicants in the cerebrospinal fluid and keeping them from entering the brain. I strongly object to labeling those "genetically susceptible" as "having a genetic disease" because they are the first injured on exposure to modern toxicants. Humans did not evolve breathing mercury vapor or having organic-mercury compounds injected in them as infants.

SIMILARITY TO ACRODYNIA: The argument that the thimerosal containing vaccines could not deliver the amount of mercury to cause a systemic illness is somewhat refuted by the history of the disease classified as acrodynia. Perhaps autism will end up

like acrodynia, where the removal of the causative material (i.e. the mercury containing teething powders) lead to cessation of the disease and the identification of the cause. Due to the perceived low levels of mercury in the teething powders and the wide-spread use of mercury in medicine at that time it was 10 years after the removal of the mercury containing teething powders before medicine acknowledged that mercury exposure was the causal factor. It is significant to notice that many of the symptoms of acrodynia are similar to the clinical symptoms of children identified today as autistic, with attention deficit disorder, etc. that have no family history of such diseases or illness classifications.

SUMMARY: It is the inability to see the effects of chronic, low level toxicities on human health that has been, and remains, our greatest failing as intelligent beings. For example, within the past year two publications in refereed scientific journals have emerged from major foreign research universities demonstrating that mercury can induce the formation of three major pathological diagnostic hallmarks of Alzheimer's disease. The production of these diagnostic hallmarks occurred at non-lethal concentrations near or below the levels of mercury reportedly found in most human brains. First, mercury has been shown to induce an increase in amyloid protein secretion (the component of amyloid plaques) and to increase the phosphorylation of a protein called Tau {*see Oliveri et al., J. of Neurochemistry, V 74, p231, 2000*}, and to produce neurofibillary tangles {*Leong et al., NeuroReports V12(4), 733, 2001*}. All of this was done with neurons in culture and represent observations found and considered diagnostic of Alzheimer's disease. Further, in a very recent article by Dr. Ashley Bush in the journal Neuron it is implied that Alzheimer's disease may be caused by heavy metal buildup. This article focused on removal of zinc and copper by chelation decreasing amyloid plaque formation in rats---mercury was not studied. However, these metals, along with silver, are the components of dental amalgams. This work is in agreement with data published earlier from my laboratory in refereed articles and summarized in one single article {*Pendergrass and Haley, Metal Ions in Biological Systems V34, Cahpter 16, Mercury and Its Effects on Environment and Biology, Siegel and Sigel EDS., Marcel Dekker, Inc. 1996*}. This data basically demonstrated that addition of very low amounts of mercury to normal human brain homogenates inhibited critical thiol-sensitive enzymes (creatine kinase, glutamine synthetase and tubulin) that are also dramatically inhibited in Alzheimer's diseased brain. Research in our laboratory clearly demonstrates that thimerosal rapidly inhibits these enzymes as well as several other metabolically important enzymes.

Further, data presented in *Aschner et al. in Methylmercury Alters Glutamate Transport in Astrocytes, Neurochemistry International, v37, #2-3, pp 199-206, 2000* indicate that organic-mercury compounds dysregulate excitatory amino acid homeostasis and may cause glutamate-mediated excitotoxic mechanisms to be involved on exposures that cause neuron death or injury. Glutamate toxicity is one hypothesis proposed to explain the slow deterioration of AD as it was reported that the enzyme, glutamine synthetase, that removes toxic glutamate was elevated in AD cerebrol spinal fluid (*D. Gunnersen and B. Haley, PNAS, USA, v89, 11949, 1992*) and inhibited in AD brain (*Butterfield et al., J. Neurochemistry, v68, 2451, 1997*). Glutamine synthetase is rapidly inhibited by the divalent mercuric ion as it has two divalent metal ion (manganese)

binding sites required for activity. It is obvious that ethyl-mercury from thimerosal would have the same effect on glutamine synthetase as mercury and methyl-mercury and impair nervous system glutamate metabolism. Consistent with this concept is the reported ability of astrocytes (the brain cells that contain glutamine synthetase that converts toxic glutamate to non-toxic glutamine) to preferentially concentrate brain organic-mercury (*Ashner, Astrocytes as Modulators of Mercury-Induced Neurotoxicity, Neurotoxicology v17, #3-4, pp663-669, 1996*). The straight-forward conclusion is that any exposure to mercury or mercury containing compounds (e.g. thimerosal) would exacerbate any medical condition affected by the inability to metabolize glutamate.

The chemical rationale for the neurotoxicity of thimerosal is that this compound would release ethyl-mercury as one of its breakdown products. Ethyl-mercury is a well-known neurotoxin. Further, combining thimerosal with the millimolar levels of aluminum cation plus significant levels of formaldehyde, also found in these vaccines, would make the vaccine mixture of even greater risk as a neurotoxic solution. The synergistic effects of mercury toxicity with other heavy metal toxicities (Pb, Cd, Zn) has been established in the literature for many years. Further, using this vaccine mixture on infants who are ill and do not have fully developed bilary (liver) and renal (kidney) systems could greatly increase the toxic effects compared to that observed in healthy adults.

The toxic effects of exposure to thimerosal to adults and infants and always been reported to have dire consequences, including death. Similar exposures, even at lower level, in infants should have more severe consequenses compared to those observed in adults made toxic by exposure to similar ethyl-mercury containing compounds. Mercury is primarily removed through the bilary system and aluminum is removed by the renal system. Inability to rid the body of these toxicants would greatly increase the damage they are capable of doing.

While one can understand the necessity of using an anti-microbial "preservative" in vaccines to prevent contamination it represents poor judgement to use a "preservative" that breaks down into a well-known neurotoxin when safer "preservatives" were available. Further, it has come to my attention through several parents that a significant number of physicians encourage mothers to have their infants receive multiple vaccinations during one visit. In one report a 13 pound baby was given 4 vaccinations. This would result in the equivalent of a 130 pound adult receiving 40 vaccinations in one day. This is quite unreasonable in my opinion, but appears to happen with a great deal of regularity in practice. Physicians do this as they are not warned of the possible consequences and are regularly informed by vaccine providers that the vaccines are totally safe. No steps were taken to recommend against this procedure.

It is very difficult to prove that mercury or organic-mercury compounds cause any specific disease that is identified by its related symptoms. This is due to the fact that mercury toxicity from various types of mercury containing materials may be considerably different and the genetic susceptibility and age of the victim would alter the response. This difficulty is further compounded due to the high numbers of confounding factors

presented in the current human environment. However, since infants get autism and related disorders, and many of our aged are afflicted with AD, we know that they have crossed the thin-red line into the neurologically diseased state. There can be no doubt that the purposeful use of mercury in medicine and dentistry, especially if it was prolonged and excessive, would significantly contribute to the onset of their disease. In my opinion, this is especially true in the case of the injection of thimerosal via vaccines in day old infants and toddlers.

FIGURE 1: COMPARISON OF THE VIABILITY OF BRAIN TUBULIN IN CONTROL (NON-DEMENTED) VERSUS ALZHEIMER'S DISEASED BRAIN.

FIGURE 2: A COMPARISON OF THE EFFECTS OF MERCURIC ION ADDITION ON CONTROL (NON-DEMENTED) AND ALZHEIMER'S DISEASED BRAIN.

FIGURE 3: A COMPARISON OF THE EFFECTS OF THIMEROSAL ADDITION ON CONTROL (NON-DEMENTED) AND ALZHEIMER'S DISEASED BRAIN.