NEW RESEARCH CONNECTS MERCURY TO ALZHEIMER’S DISEASE!
A new research study connecting mercury to Alzheimer’s Disease (AD) has just been published in a peer-reviewed medical publication, the *Journal of Neurochemistry!* [see “Science” section in this issue.] This study determined that mercury increased the secretion of amyloid and tau phosphorylation in nerve cells!
Alzheimer’s Disease (or *Senile Dementia of the Alzheimer’s-Type [SDAT]*) is characterized by two features found on autopsy: 1) Amyloid plaques, and 2) Neurofibrillary tangles. The medical scientific community has been divided on the issue of which of these two features is the causative, or even the classic diagnostic feature of AD. In the search for the etiology of AD, that controversy now becomes moot. It has now been scientifically demonstrated that mercury causes BOTH features!
Although the etiology of AD must be considered multifactorial and the existence of genetic predisposition must be considered, the scientific EVIDENCE connecting mercury to AD has been steadily, and convincingly, accumulating. In 1986, scientists at the University of Kentucky Sanders-Brown Center on Aging began publishing the post-mortem results of metal concentrations in AD brains compared to controls, using Neutron Activation Analysis. [studies cited in this article are abstracted in the “Science” section of this issue.]
The first study determined the concentrations of 16 elements in gray and white matter of various regions of AD and control brain tissue (Ehmann, 1986). The largest significant differences were elevations of mercury and bromine and depletion of rubidium in AD brains. The Kentucky scientists next determined the metal concentrations in the brain areas most associated with AD - the amygdala, hippocampus
and nucleus basalis of Meynert (Thompson, 1988). The results were impressive. The scientists stated: "The elevation of mercury in AD nucleus basalis of Meynert, as compared to age-matched controls, is the largest trace-element imbalance observed to date in AD brain." Mercury levels in amygdala were also elevated. The authors also stated (p. 5): "The source of Hg in brain is not known, although environmental pollution and dental amalgams have been widely suggested."

Finally, the researchers determined metal concentrations in isolated subcellular fractions of tissue from AD and control brains (Wenstrup, 1990). Again, mercury and bromine were elevated and rubidium diminished in various fractions were found. Diminished levels of selenium and zinc were also found.

Further evidence of mercury connected to AD came from a 1998 study conducted in Switzerland (Hock, 1998). These researchers found a significant elevation of blood mercury in AD victims compared to controls. On the other hand, Fung and associates found no elevation of urine mercury in AD patients compared to controls (Fung, 1996). However, examination of this study reveals that the AD patients had a mean number of 6.2 amalgam surfaces, while the "controls" had a mean number of 3.0 amalgam surfaces. Further, the authors claim that urine mercury measurements are "excellent biological markers for assessing possible physiological effects of elemental Hg. (p. 74)" This position is contradicted by literally reams of published scientific literature.

This latter study presents pause for thought. Why the contradictory results? Research is conducted and published in many ways. For example, the Fung study was published in a dental journal, "General Dentistry." This may be of questionable "peer review", if peer reviewed at all. Why was the study not published in an appropriate medical scientific journal? Would a research study on periodontal disease be published in a neurology journal?

This is not the only example of contradictory, but questionable, studies investigating mercury and AD. Saxe and associates at the University of Kentucky have published two studies denying a connection between dental amalgams and AD. Four of the authors of the first study, including Saxe, are dentists and the study was published in the Journal of the American Dental Association (Saxe, 1995). Again, why was the study not published in an appropriate medical scientific journal? Are dental journals the appropriate forum to investigate Alzheimer's Disease?

Once again, a review of the study reveals that the subjects in this study had a mean of only 2 posterior teeth remaining (out of the original 16, not counting wisdom teeth)! Given the very long half-life of mercury in brain tissue, the validity of such a comparison is questionable.

The second study (Saxe, 1999) was also published in the Journal of the American Dental Association. Amazingly, this study concluded that mercury is NOT taken up in brain tissue, which is contradictory to every other study that has been published! One has to wonder if this study would have been published if appropriately peer reviewed by medical scientists.

So, with the exception of the studies published in the dental journals, a significant elevation of mercury in the victims of AD has been established. The next step would be to determine if mercury can cause the type of pathology found in AD. This question must be addressed in relationship to the two hallmarks of AD: 1) Neurofibrillary tangles and 2) amyloid plaques.

The published research demonstrating that inorganic mercury can cause the hallmark neurofibrillary tangles of AD is compelling. In 1991, scientists at the University of Kentucky demonstrated that mercury interfered with tubulin, which is the basis of the microtubules of nerve structure (Duhr, 1991; Duhr, 1993). This resulted in the formation of neurofibrillary tangles.

Then scientists at the University of Calgary confirmed these findings in a study that utilized both in vitro and in vivo administration of inorganic mercury (Palkiewicz, 1994). The two research teams than combined to perform an experiment where low level mercury vapor was administered to rats (Pendergrass, 1997).
specific brain lesion (i.e., neurofibrillary tangles) found in human AD victims was found in the rats. Further, the brain mercury levels in the rats were consistent with the levels previously found in human AD victims.

Meanwhile, the U.S. government (a major financier of AD research) had decided to ignore all of this validly published research and focus on the beta amyloid factor. Based on a study by the National Institute of Aging showing that beta amyloid can make cells leak choline, the National Institutes of Health (NIH) stated: “Two subplots in the Alzheimer’s Disease mystery have converged with the discovery of a possible link between beta amyloid and choline, both long-time suspects in the development of the irreversible brain disorder.”; and “The finding suggests that beta amyloid may help cause Alzheimer’s disease through its effect on choline concentrations. Dense deposits of beta amyloid in the brain are a hallmark of Alzheimer’s disease; one hypothesis holds that the plaques themselves are a direct cause of the condition.” [The Causes of Alzheimer’s Disease: Another Twist to the Tale. NIH News & Features, National Institutes of Health, May 1995.]

Well, the newly published Olivieri study shows that mercury causes the formation of beta amyloid in neurologic tissue! The study further showed that mercury caused a significant increase in tau phosphorylation, a step in the formation of neurofibrillary tangles.

With the exception of the three studies published in dental journals, the published research clearly demonstrates the influence of exposure to mercury in the development of AD. The following has been established:

1. Human victims of AD have significantly higher levels of mercury in brain tissue compared to controls, especially those areas of the brain involved in AD (amygdala, nucleus basalis of Meynert).
2. Mercury causes the neurofibrillary tangles that are one of the hallmarks of AD. This has been demonstrated in vitro and in animal experiments.
3. Mercury causes the formation of amyloid plaques, the other hallmark of AD.
4. Humans with mercury amalgam dental fillings have elevated levels of mercury in brain tissue. This has been confirmed by animal experiments and human autopsy studies (excepting the three studies published in dental journals).

AD is not a minor affliction! It presents unimaginable anguish and suffering to its victims and their families. Bio-Probe encourages all readers to present this newsletter to state and national government representatives. If the government agencies continue to refuse to act, than legislation must be passed to oblige them to act. It is time that the information contained herein is brought to light!

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NEW ARIZONA LAW ON DENTAL MATERIALS

On 29 March 2000, the Governor of the State of Arizona signed into law a new bill (SB1155) that includes a provision requiring dentists to inform patients on dental materials.

The new bill amends the Arizona Revised Statutes to include the following provision for “Unprofessional Conduct”: “FAILING TO INFORM A PATIENT OF THE TYPE OF MATERIAL THE DENTIST WILL USE IN THE PATIENT’S DENTAL FILLING AND THE REASON WHY THE DENTIST IS USING THAT PARTICULAR FILLING.”

This provision could have astounding consequences. For example, if an Arizona dentist informs the patient that a “silver” or “amalgam” filling is being placed (and the reason for doing so), the patient will not be informed of the content of mercury, or the established release of that mercury from the filling and subsequent passage into the patient’s body. Legally, the withholding of relevant information that could materially influence the consumer’s decision constitutes fraud and deception. Therefore, it is now apparent that all Arizona dentists placing amalgam fillings are, by law, required to inform there patients that 1) the filling they are placing is 43-50% mercury, 2) it is universally acknowledged (even by organized dentistry) that mercury exits the filling and transfers to body tissues, and 3) the potential health effects of this mercury exposure are still undetermined. These are uncontested facts.

Another Arizona bill that is now under consideration
would establish a joint legislative study committee to study the need for the regulation of holistic dentistry (also identified as “alternative” or “biological” dentistry) through an independent board. Hearty congratulations to Arizona Senator Grace and Representative Gerard - sponsors of the bill - as well as the legislators who supported SB 1155, the Governor of Arizona, and all who worked diligently to affect passage of the bill.

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SCIENCE

**Mercury Induces Cell Cytotoxicity and Oxidative Stress and Increases B-Amloid Secretion and Tau Phosphorylation in SHSY5Y Neuroblastoma Cells.**

Olivieri, G; Brack, Ch; Muller-Spahn, F; Stahelin, HB; Herrman, M; Renard, P; Brockhaus, M; Hock, C.


ABSTRACT: Concentrations of heavy metals, including mercury, have been shown to be altered in the brain and body fluids of Alzheimer’s disease (AD) patients. To explore potential pathophysiological mechanisms we used an in vitro model system (SHSY5Y neuroblastoma cells) and investigated the effects of inorganic mercury (HgCl2) on oxidative stress, cell cytotoxicity, B-amyloid production, and tau phosphorylation. We demonstrated that exposure of cells to 50 microgram/L (180 nM) HgCl2 for 30 min induces a 30% reduction in cellular glutathione (GSH) levels (n = 13, p < 0.001). Preincubation of cells for 30 min with 1 microM melatonin or premixing melatonin and HgCl2 appeared to protect cells from the mercury-induced GSH loss. Similarly, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyterazolium bromide (MTT) cytotoxicity assays revealed that 50 microgram/L HgCl2 for 24 h produced a 50% inhibition of MTT reduction (n = 9, p < 0.001). Again, melatonin preincubation protected cells from the deleterious effects of mercury, resulting in MTT reduction equaling control levels. The release of B-amyloid peptide (AB) 1-40 and 1-42 into cell culture supernatants after exposure to HgCl2 was shown to be different: AB 1-40 showed maximal (15.3 ng/ml) release after 4 h, whereas AB 1-42 showed maximal (9.3 ng/ml) release after 6 h of exposure to mercury compared with untreated controls (n = 9, p < 0.001). Preincubation of cells with melatonin resulted in an attenuation of AB 1-40 and AB 1-42 release.

Tau phosphorylation was significantly increased in the presence of mercury (n = 9, p < 0.001), whereas melatonin preincubation reduced the phosphorylation to control values. These results indicate that mercury may play a role in pathophysiological mechanisms of AD. 

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**Hg2+ Induces GTO-Tubulin Interactions in Rat Brain Similar to Those Observed in Alzheimer’s Disease.**

Duhr, E; Pendergrass, JC; Kasarskis, E; Slevin, J; Haley, B.


ABSTRACT: The pathogenesis of Alzheimer’s Disease (AD) is unknown. Using SDS-PAGE & autoradiography our laboratory has shown: (I) that the tubulin in AD brain is less photolabeled by [32P] 8N3GTP than is tubulin from control brain (Ann Neurol., 26:210-5, 1989) and (II) that low microM levels of preformed Hg2+EDTA specifically blocked interactions of tubulin-[32P]8N3GTP in control human brain homogenates giving a photolabeling profile identical to AD brain (FASEB J, 4:A2151, 1990).

Elevated levels of Hg2+ have been reported in AD brain by others (Neurotoxicol, 9:1-7, 1988). Earlier work using [32P]8N3GTP with Al3+ treated rat & rabbit brain showed no differences from control with regards to tubulin photolabeling (Neurotoxicol, 9:429-42, 1988).

However, our latest data show that brain samples from Hg2+ fed rats display an abolished GTP-tubulin interaction similar to AD brain samples as determined by [32P] 8N3GTP photolabeling profiles. Removal of Hg2+ from treated rats did not reverse the effect.

These results suggest that certain complexed forms of Hg2+ must be considered as a potential source for the etiology of AD.

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**HgEDTA Complex Inhibits GTP Interactions With the E-Site of Brain Beta-Tubulin.**
**ABSTRACT:** We have found that EDTA and EGTA complexes of Hg2+, which conventional wisdom has assumed are biologically inert, are potentially injurious to the neuronal cytoskeleton. Tubulin, a major protein component of the neuronal cytoskeleton, is the target of multiple toxicants, including many heavy metal ions. Among the mercurials, inorganic mercuric ion (Hg2+) is one of the most potent inhibitors of microtubule polymerization both in vivo and in vitro. In contrast to other heavy metals, the capacity of Hg2+ to inhibit microtubule polymerization or disrupt formed microtubules cannot be prevented by the addition of EDTA and EGTA, both of which bind Hg2+ with very high affinity. To the contrary, the addition of these two chelating agents potentiates Hg2+ inhibition of tubulin polymerization. Results herein show that HgEDTA and HgEGTA inhibit tubulin polymerization by disrupting the interaction of GTP with the E-site of brain beta-tubulin, an obligatory step in the polymerization of tubulin. Both HgEDTA and HgEGTA, but not free Hg2+, prevented binding of [32P] 8N3GTP, a photoaffinity nucleotide analog of GTP, to the E-site and displaced bound [32P] 8N3GTP at low micromolar concentrations. This complete inhibition of photoinsertion into the E-site occurred in a concentration- and time-dependent fashion and was specific for Hg2+ complexes of EDTA and EGTA, among the chelating agents tested. Given the ubiquity of Hg2+ in the environment and the widespread use of EDTA in foodstuffs and medicine, these mercury complexes may pose a potentially serious threat to human health and play a role in diseases of the neuronal cytoskeleton.

**Brain Trace Elements in Alzheimer’s Disease.**
Ehmann, WD; Markesbery, WR; Alauddin, M; Hossain, TIM; Brubaker, EH. Neurotoxicology, 7(1):197-206, 1986.

**ABSTRACT:** Instrumental neutron activation analysis has been used to determine the concentrations of 16 elements in selected brain regions and separated gray- and white-matter specimens from histologically verified Alzheimer’s disease (AD) and age-matched control patients. Significantly different (p<0.05) mean concentrations of Br, Cl, Cs, Hg, N, Na, P, and Rb were observed in AD bulk brain samples compared to controls, while no significant differences were observed for Ag, Co, Cr, Fe, K, Sb, Sc, and Se. The differences that are most persistent and largest in magnitude for the pooled bulk samples, males and females, left and right hemispheres, and separated gray and white matter are the elevation of Br and Hg and the depletion of Rb in AD compared to controls. Significant interelement correlations for the elements of AD and control brains are also documented. Based on these studies, the possibility of an etiological role for trace elements in AD clearly deserves further investigation.

**Mercury Determination in Nursing Home Patients With Alzheimer’s Disease.**
Fung, YK; Meade, AG; Rack, EP; Blotcky, AJ; Claassen, JP; Beatty, MW; Durham, T. General Dentistry, 74-78, Jan-Feb 1996.

**ABSTRACT:** Trace element neurotoxicity contributing to the development of Alzheimer’s disease (AD) may be an important etiologic factor for this disorder. This clinical study was conducted to determine the urine concentrations of mercury (Hg) from patients with AD disorders. Within the confines of a nursing home, all subjects were exposed to the same environment and a diet that excluded seafood. The results of this study do not indicate that subjects with AD have a greater body burden of Hg, according to urinary excretion. This can be further evidence that Hg from amalgam restorations or diet is not related to etiology and pathogenesis of AD.

**Increased Blood Mercury Levels in Patients With Alzheimer’s Disease.**
Hock, C; Drasch, G; Golombowski, S; Muller-
Spahn, F; Willerhausen-Zonchner, B; Schwarz, P; Hock, U; Growdon, JH; Nitsch, RM.

ABSTRACT: Alzheimer's disease (AD) is a common neurodegenerative disorder that leads to dementia and death. In addition to several genetic parameters, various environmental factors may influence the risk of getting AD. In order to test whether blood levels of the heavy metal mercury are increased in AD, we measured blood mercury concentrations in AD patients (n=33), and compared them to age-matched control patients with major depression (MD) (n=45), as well as to an additional control group of patients with various non-psychiatric disorders (n=65).

Blood mercury levels were more than two-fold higher in AD patients as compared to both control groups (p=0.0005, and p=0.0000, respectively). In early onset AD patients (n=13), blood mercury levels were almost three-fold higher as compared to controls (p=0.0002, and p=0.0000, respectively). These increases were unrelated to the patients' dental status.

Linear regression analysis of blood mercury concentrations and CSF levels of amyloid beta-peptide (A beta) revealed a significant correlation of these measures in AD patients (n=15, r=0.7440, p=0.0015, Pearson type of correlation). These results demonstrate elevated blood levels of mercury in AD, and they suggest that this increase of mercury levels is associated with high CSF levels of A beta, whereas tau levels were unrelated.

Possible explanations of increased blood mercury levels in AD include yet unidentified environmental sources or release from brain tissue with the advance in neuronal death.

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ADP-Ribosylation of Brain Neuronal Proteins is Altered by In Vitro Exposure to Inorganic Mercury.
Palkiewicz, P; Zwiers, H; Lorscheider, FL.

ABSTRACT: ADP-ribosylation is an essential process in the metabolism of brain neuronal proteins, including the regulation of assembly and disassembly of biological polymers. Here, we examine the effect of HgCl2 exposure on the ADP-ribosylation of tubulin and actin, both cytoskeletal proteins also found in neurons, and B-50/43-kDa growth-associated protein (B-50/GAP-43), a neuronal tissue-specific phosphoprotein. In rats, we demonstrate, with both in vitro and in vivo experiments, that HgCl2 markedly inhibits the ADP-ribosylation of tubulin and actin. This is direct quantitative evidence that HgCl2, a toxic xenobiotic, alters specific neurochemical reactions involved in maintaining brain neuron structure.

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Mercury Vapor Inhalation Inhibits Binding of GTP to Tubulin in Rat Brain: Similarity to a Molecular Lesion in Alzheimer Diseased Brain.
Pendergrass, JC; Haley, BE; Vimy, MJ; Winfield, SA; Lorscheider, FL.

ABSTRACT: Hg2+ interacts with brain tubulin and disassembles microtubules that maintain neurite structure. Since it is well known that Hg vapor (Hg0) is continuously released from “silver” amalgam tooth fillings and is absorbed into brain, rats were exposed to Hg0 4 h/day for 0, 2, 7, 14 and 28 d at 250 or 300 microgram Hg/m3 air, concentrations present in mouth air of some humans with many amalgam fillings. Average rat brain Hg concentrations increased significantly (11-47 fold) with duration of Hg0 exposure. By 14 d exposure, photoaffinity labelling of the beta-subunit of the tubulin dimer with [alpht32P]GTP in brain homogenates was decreased 41-74%, upon analysis of SDS-PAGE autoradiograms.

The identical neurochemical lesion of similar or greater magnitude is evident in Alzheimer brain homogenates from approximately 80% of patients, when compared to human age-matched neurological controls. Total tubulin protein levels remained relatively unchanged between Hg0 exposed rat brains and controls, and between Alzheimer brains and controls. Since the rate of tubulin polymerization is dependent upon binding of GTP to tubulin dimers, we conclude that chronic inhalation of low-level Hg0 can inhibit polymerization of brain
tubulin essential for formation of microtubules.

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Dental Amalgam and Cognitive Function in Older Women: Findings From the Nun Study.
Saxe, SR; Snowdon, DA; Wekstein, MW; Henry, RG; Grant, FT; Donegan, SJ; Wekstein, DR.
ABSTRACT: The authors determined the number and surface area of occlusal dental amalgams in a group or 129 Roman Catholic sisters who were 75 to 102 years of age.
Findings from this study of women with relatively homogenous adult lifestyles and environments suggest that existing amalgams are not associated with lower performance on eight different tests of cognitive function.

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Alzheimer’s Disease, Dental Amalgam and Dental Mercury.
Saxe, SR; Wekstein, MW; Kryscio, RJ; Henry, RG; Cornett, CR; Snowdon, DA; Grant, FT; Schmitt, FA; Donegan, SJ; Wekstein, DR; Ehmann, WD; Markesbery, WR.
ABSTRACT: Background: Mercury, or Hg, is a neurotoxin that has been speculated to play a role in the pathogenesis of Alzheimer’s disease, or AD. Dental amalgam releases low levels of Hg vapor and is a potential source of Hg for a large segment of the adult population.
Methods: The authors studied 68 subjects with AD and 33 control subjects without AD to determine Hg levels in multiple brain regions at autopsy and to ascertain the subjects’ dental amalgam status and history. The subjects were from central Kentucky and Elm Grove, Wis. The authors conducted dental amalgam assessments during the lives of the majority of subjects and in some subjects at the time of autopsy only. The authors also determined three dental amalgam index scores - Event (placement, repair or removal of amalgam), Location and Time in Mouth - in addition to the numbers of and surface area of occlusal amalgam restorations. The authors determined Hg levels in multiple brain regions and performed full neuropathologic evaluations to confirm the normal status of the brain or the presence of AD.
Results: The authors found no significant association of AD with the number, surface area or history of having dental amalgam restorations. They also found no statistically significant differences in brain Hg level between subjects with AD and control subjects. Clinical Implications: Dental amalgam restorations, regardless of number, occlusal surface area or time, do not relate to brain Hg levels.

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Regional Brain Trace-Element Studies in Alzheimer’s Disease.
Thompson, CM; Markesbery, WR; Ehmann, WD; Mao, Y-X; Vance, DE.
ABSTRACT: Alzheimer’s disease (AD) brain trace-element imbalances in the amygdala, hippocampus and nucleus basalis of Meynert (nbM) are found in most cases to be consistent with those previously reported in samples derived principally from AD cerebral cortex (Ehmann et al., 1986). The elevation of mercury in AD nbM, as compared to age-matched controls, is the largest trace-element imbalance observed to date in AD brain.
In addition to the general confirmation of imbalances for Cs, Hg, N, Na, P, and Rb noted previously in cerebral cortex samples, imbalances for Fe, K, Sc, and Zn were observed in two regions and one region also exhibited imbalances for both Co and Se. Persistent imbalances for the univalent cations Na, K, Rb and Cs support arguments for a membrane abnormality in AD.
The data presented here also provide the first comprehensive simultaneous multi-element determinations in both control and AD nbM.

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Trace Element Imbalances in Isolated Subcellular Fractions of Alzheimer’s Disease Brains.
Wenstrup, D; Ehmann, WD; Markesbery, WR.
ABSTRACT: Concentrations of 13 trace elements (Ag, Fr, Co, Cr, Cs, Fe, Hg, K, Na, Rb, Sc, Se, Zn) in isolated subcellular fractions (whole brain, nuclei, mitochondria, microsomes) of temporal lobe from autopsied Alzheimer’s disease (AD) patients and