Monographs on Mercury

Where Are the References?

Right Here

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Dr Huggins took three independent study courses during his Master's Program. Each course required a monograph on mercury. They are now available here for those people who want in depth information on the biological effects of mercury and the scientific litany to support the answers. All three monographs are combined in the book. They include:

1. *Sites & Mechanisms of Methylation of Mercury (pages 1-18)*

2. *Amounts of Mercury Required to Produce Toxicity in Biological Tissues (pages* 19-40)

3. *The Fate of Mercury in Biological Tissue (pages 41-81)*

Also, look for the order form at the end of the booklet.

Sites and Mechanisms of Methylation of Mercury

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It has been known for centuries that mercury is toxic, yet it is used today in many areas that expose an unsuspecting public. There are three different oxidation states for mercury: elemental mercury (Hg), mercurous ions (Hg 1+) and mercuric ions (Hg 2+). Mercury deposition in tissues is dependant upon redox potential, pH type, abundance of other anions, and the presence of other reactive groups. Anaerobic conditions usually provide sulfide ions, allowing the formation of HgS. Aerobic conditions induce the oxidation of HgS to soluble HgSO, which ionizes to produce mercuric ions for methylation. At low pH, in the presence of mercuric ions, chemical transformation of dimethyl mercury to monomethyl mercury occurs. (In this paper the term methyl mercury refers to the monomethyl form.) After complexating with SH groups methyl mercury can be decomposed by UV light to methane and HgS.

Mercury in the form of thimerosal is a preservative used in items such as contact lens solutions and injectable immunization shots. In addition, there are many people who are not aware that mercurochrome and merthiolate are mercury compounds as well.

The weathering of mercury containing rocks has released billions of tons of mercury over time. (Presently this is elemental Hg^0 mercury.) Yearly, 230 tons of mercury from weathering and 10,000 tons from humans are put into the environment (1970 figures). (1) Much of this is from the breakdown of agricultural fungicides and the burning of coal and oil. About 4,600 tons per year of mercury is from these sources alone. (2)

Another hidden exposure is the vapor that comes off of dental silver-mercury amalgam fillings that have constituted the vast majority of dental fillings for the past 160 years. This mercury exposure is bad enough in itself, but there is one substance that is far worse, methyl mercury. I intend to suggest in this paper that the mercury evolving from dental fillings can be methylated in numerous areas of the body and thereby provide an even greater, insidious hazard.

Why are methyl mercury compounds more toxic than other forms of mercury? Their lipophilic character causes an increased mobility in organisms compared to inorganic mercury. They accumulate especially in the nervous system and liver, and whereas other organic compounds are very unstable in living organisms and decompose to inorganic mercury almost immediately, methyl mercury is quite stable. It requires 70 days to metabolize half of it. The toxic character is caused in part by its interactions with SH and SS bonds of enzymes. This bonding induces a change in conformation of the tertiary structure of proteins resulting in a loss of enzymatic activity - a serious distortion of cell metabolism. (3)

First, let us list the general composition of dentistry's silver-mercury amalgam. A powder containing approximately 33-37% silver, 3-6% copper, 12-13% tin, and 1% zinc is mixed with mercury, such that the final mixture contains 48-52% mercury. Today dental alloy manufacturers are turning to what is termed "state of the art" amalgam, or high copper amalgam which contains around 30% copper. Examples of high copper amalgam are Dispersalloy® and Titan®. It is true that copper is a requirement for normal metabolism, but that copper is in an organic or biofunctional molecule. Copper in the metallic, inorganic state, as it escapes from filling is a toxic substance. The U.S. government spends over $1 billion per year to remove this type of copper from public water supplies.

The work of Svare (4, 5, 6, 7), Vimy (8, 9), and Gay (10) documents the fact that mercury escapes from silver amalgam fillings. Svare (4) reports blood levels of 18.97 ng after chewing gum for 15 seconds, as opposed to the control levels of 1.06 ng. Vimy (8, 9) calculated mercury exposure from 35 patients with amalgam (none freshly placed) and reported an average daily mercury release of 20 mcg (no range stated) due to the presence of amalgam. He reports that some of the patients received as much as 10 times the allowable daily exposure established by some countries.

Threshold Limit Values (TLV's) from the former Soviet Union are 10 mcg per day, Germany is 1.0 mcg per day, and the U.S. maintains 50 mcg per day. (11) Trakhtenberg's (12) work in Russia alone was based on the onset of neurological impairment. None of these data considered the effects of mercury in the more dangerous methyl mercury form.

The data seems to uphold the concept that substantial amounts of elemental mercury can be released from silver amalgam fillings. The body's intrinsic capacity to excrete methyl mercury is low, and methyl mercury is far more toxic than elemental mercury. The half life of methyl mercury is generally accepted to by 70 days. Because of these two facts, methyl mercury can be considered a cumulative poison. For these reasons people should be cautious in accepting unnecessary exposures to mercury.

To this data let us add the material from Brune (13) who demonstrated that mercury is released from the high copper amalgams (30% copper) 50 times faster than from conventional amalgam (3-6% copper). It is estimated by amalgam salesmen that in 1985 over three quarters of the amalgam sold was of the high copper variety. By 1996 almost all of the amalgam sold in the U.S. was "high copper".

Now let us look to the process of mercury methylation.

Methylation and demethylation are two processes that usually take place simultaneously. Methylation is the result of mercuric ion (Hg++) interference with biochemical C transfer reactions. (14) Demethylation is brought about by non-specific hydrolytic and reductive enzyme processes. (15) When mercury is present in the human body, the biochemical equilibrium is such that methylation is favored over demethylation, however, both are taking place simultaneously. In assessing the potential damage of methyl mercury (MeHg) in the human, factors favoring methylation and demethylation should be evaluated to ascertain the probable balance.

The *redox potential*, or oxidation-reduction state of the environment affects the formation of methyl mercury from inorganic mercury. Another controlling factor in methylation is the relative amount of oxygen available in the selected environment. Varying the redox potential from -220mV to +110mV; Compeau found methylation was favored at the higher negative redox potential, whereas slightly more elemental mercury was released at +110mV than at -220mV. (16)

Demonstrating its lipid solubility properties, methyl mercury is absorbed through the intestinal wall 45 more rapidly than the mercuric ion, and is retained in the body longer than the mercuric ion. Fecal suspensions were found to be able to methylate mercury as measured by thin-layer chromatography. Methyl mercury was produced when fecal matter was incubated anaerobically with $HgCl_2$. The amount of methyl mercury produced was directly proportional to the amount of mercury ions added. These studies provide evidence that the microbial flora of the intestine of humans have the potential to transform mercuric ions to highly toxic methyl mercury and could contribute significantly to the methyl mercury burden of the body and thereby add to the risk of incurring or increasing the severity of methyl mercury poisoning. (17)

Salinity also shifts the methylation process. High salinity (2.5%)

inhibited methylation, whereas low salinity (0.4%) favored methylation. (16)

Blum and Bartha (18) studied the effects of salinity on methylation by Clostridium cochlearium. Their study clearly demonstrated an inverse relationship between salinity and methylation. The most rapid methylation occurred at 0.1% salinity. Concentrations above 2% caused relatively little additional effect on methylation.

Since many factors influence the methylation process, it is advisable to review the most influential ones in depth, then list all of the influencing factors. The first factor of major significance is vitamin B-12. The rapid conversion of methyl cobalamin into B-12 at high mercury concentrations suggests that the methyl transfer could proceed via a non-enzymatic pathway.

In vitro reactions with methyl cobalamin reacted rapidly with mercuric chloride in unbuffered aqueous solutions. The reaction yielding hydroxy cobalamin and methyl mercury chloride going to 50% completion in four minutes. (19) These authors found the ease with which the methyl group was transferred from methyl cobalamin to various mercury compounds, *in an entirely non-enzymatic system,* to be rather striking.

A proposed non-enzymatic methylation of mercury was shown by a bacterium with methyl cobalamin (CH_3 - B-12) as a donor of methyl groups in the presence of ATP and a mild reductant. (20)

Imura found that methylation proceeded at a *remarkably high rate* when methyl cobalamin and inorganic mercury were mixed. Their results indicated that mercuric chloride was essential for the liberation of the methyl group from methlycobalamin in the formation of methyl mercury. "Our results show that highly toxic methyl mercury is easily generated from inorganic mercury in the presence of methyl cobalamin." (21)

In 1974 Bertilsson (22) found that mercury was methylated in a neutral water solution by a *purely abiotic* reaction. The methyl donor was methyl cobalamin; and the reaction was very fast, almost quantitative under *both* aerobic and anaerobic conditions. Microbial activity is usually a prerequisite for synthesis of methyl mercury in nature unless other methyl forms are present or tetraethyl lead or methyl tin is present.

Mercury-tolerant mutants were very effectively methylating when an excess of cysteine of homocysteine was present in the substrate. Experiments suggested that methylation might be an "incorrect" synthesis of methionine which is normally formed through methylation of homocysteine.

Cysteine and Vitamin B-12 increase the capacity for methylation in Clostridium cochlearium. Most experiments do not allow for discrimination between methylation and demethylation. This is not a true representation of the kinetics of the reactions. Experimental design should include

1) redox potential
2) inorganic mercury concentration
3) temperature
4) microbial activity
5) sulfide concentration. (22)

Methyl cobalamin has been associated with increased ability of bacteria to methylate. Neurospora has been found to induce high quantities of methyl mercury, but does not utilize the B-12 analog in metabolism. Investigations were done to identify the Neurospora mechanism of mercury methylation. High homocysteine was found to enable the most efficient synthesis of methyl mercury. L-cysteine was also found to stimulate methyl mercury production. Care should be exercised when recommending cysteine as a supplement to detoxify patients.

When homoserine was added in equal amounts to homocysteine,

the yield of methyl mercury doubled. The methyl group is transferred to the mercury atom which is complexed to homocysteine. Methylation might then be regarded as an incorrect synthesis of methionine, and since methionine is not being produced, methyl mercury production is continued. If methionine is artificially introduced into the culture medium, methylation of mercury ceases. It is quite plausible that the mutant bacteria that are resistant to the mercuric ions are constitutive mutants. The control of one of the last enzymes in methionine biosynthesis would be impaired, giving rise to a continuous methylation of mercuric ions. (24)

Kinetic studies showed:

1. Monomethyl mercury is the predominant product of methylation of mercury (near neutral pH).
2. The rate of methylation is higher in aerobic systems than in anaerobic systems for a given mercuric compound concentration and microbial growth rate.
3. Higher microbial growth produces higher methylation rates under both aerobic and anaerobic conditions.
4. Methylation rates could be hampered by the addition of sulfide to some anaerobic systems.
5. Temperature affects methylation rates in accordance with its effects on the metabolic rate of the methylating organism. (23)

The anaerobic bacterium Clostridium cochlearium has been shown to produce considerable quantities of methyl mercury from a wide variety of mercury compounds. When Vitamin B-12 is added the quantities of methyl mercury increase even more. No methyl mercury was produced from HgS. Much emphasis has been placed on anaerobic methylation, however, aerobic methylation has been observed in Enterobacter aerogenes, Pseudomonas fluorescens, Mycobacterium phlei, Escherichia coli, Aspergillus niger, Scopulariopsis brevicaulis, and Saccharomyces cerevisia. (25) Therefore much methyl mercury can be produced

in both aerobic and anaerobic environments. (26)

A number of micro-organisms, aerobic, facultative and obligate anaerobes contain an enzyme, methionine synthetase, which plays a role in synthesis of methionine from homocysteine. Cobalamin constitutes part of this enzyme, suggesting the same enzyme may catalyze the formation of methyl mercury. Tetrahydrofolic acid is an integral part of the reaction. (3)

Lexman, et al., (3) suggests that after the mercuric ion penetrates the cell, the low local redox potential would reduce in to Hg^0. If it is formed, dimethyl mercury may leave the cell now, and at low pH, and in the presence of mercuric ions be converted to monomethyl mercury.

One of the most significant discoveries about the action of methyl mercury was made by Smith in 1967. (27) He examined 55 species of bacteria that carried multiple drug-resistance factors to various antibiotics. He found that eleven of these were resistant to mercury and that resistance was due to an extrachromosomal genetic factor called a plasmid. (26)

Plasmids are small DNA molecules which are identical in chemical composition to the bacterial chromosome, but which are separate autonomously replicating structures. They often contain the genes for resistance to a variety of chemicals as well as for enzymes. Plasmids are transferred from one generation to the next during normal replication of the bacterial cell. (26)

One of the more notable plasmids is the penicillinase plasmid Staphylococcus aureus that , in addition to conferring resistance to penicillin, also provides resistance to mercury. This is the staphylococcus that notoriously gives problems in hospitals. An interesting question to pose is, does introduction to mercury stimulate antibiotic resistance? Or does introduction of antibiotics give heavy metal resistance? (26)

The physiological basis for mercury resistance in organisms containing the plasmid is attributed to the cell's ability to convert lethal mercuric compounds to methyl mercury which is more volatile. Volatile compounds can escape from the cell and transfer toxicity from the bacterium to the surrounding host. This is especially harmful in the case of methyl mercury. (26)

E. Coli possesses volatilization capacities which are dependent upon NADPH. The conversion rate of mercury to volatile forms has been calculated to be 10μM Hg2+ to Hg at 4-5 nanomol Hg2+ per minute per 10^6 cells. (28) A variety of bacteria have been found that can transfer plasmids to E. Coli, which result in increased volatilization of mercury. (29)

Twenty-three mercury resistant cultures were studied for methylation capacity. Fourteen were Escherichia and Enterobacter, three were Staphylococcus, three were Streptococcus and three were Bacillus. All Escherichia, Enterobacter and Bacillus strains were more mercury resistant than the Staphylococcus and Streptococcus. It was concluded that bacterial production of methyl mercury may be a means of resistance and detoxification against mercurials in which inorganic mercuric ions are converted to methyl mercury and excreted into the environment. (30)

There must exist in nature a high frequency for this capacity since resistant plasmids have been obtained from a wide variety of organisms such as Serratis marcescens, Ps. aeruginosa, Proteus vulgaris, Providencia, Shigella dysenteriae and Salmonella paratyphi B. Several resistant strains of E. Coli, Staphylococcus aureus and Ps. aeruginosa have been found to produce a volatile form of mercury which is soluble in organic solvents. (26, 31)

Micro-organisms seem to produce a substance that is more toxic to humans from a less toxic one. (32) Lander found that the loci determining resistance toward mercury and loci for synthesis of methionine are closely associated in Staphylococcus. This

suggests a possible relationship between methylation of mercury and methionine biosynthesis.

Yeasts have also been found to participate in detoxification of mercurial compounds. A species of Cryptococcus is a mercury resistant yeast. It apparently reduces divalent mercurial compounds to the elemental state, which accounts for its resistance. (33)

In monitoring pure cultures of bacteria, organisms in the medium containing yeast extract absorbed about twice as much mercury as organisms in medium without yeast extract. (34)

MECHANISMS OF METHYLATION

Three different pathways are available for transfer of CH_3 groups in biological systems:

1. Via S-adenosyl methionine - this is the activated form of methionine in which the CH_3 group is bound to a tertiary sulphonium ion.
2. Via N5-methyl tetrahydrofolic acid and related compounds in which the CH_3 group is bound to a quarternary ammonium ion.
3. Via methyl corrinoids in which the CH_3 group is bound to a cobalt ion coordinated by the four nitrogen atoms of a corrin ring. This is a methylated form of Vitamin B-12 (cobalamin).

CHEMICAL DEMETHYLATION

In the presence of water, methyl mercury invariably occurs as complexes either with sulfides, thiols, hydroxy or chloride. In natural conditions methyl mercury is found predominantly as

sulfur complexes. These sulfur complexes can be decomposed photochemically as follows:

$$CH_3 - Hg - S \quad \text{UV light} \quad CH_3 + HgS$$
$$CH_3 - Hg - SR \quad \text{UV light} \quad CH_3 + SR + Hg^{++}$$

Dimethyl mercury escapes to the atmosphere by:

$$(CH_3)_2 - Hg^{++} \quad \text{UV light} \quad 2\,CH_3 + Hg^0$$

Worldwide, the microbial population is becoming adapted to methyl mercury. Those species resistant to the mercuric ion survive. Demethylating organisms are resistant, and constitute and increasing and eventual dominating proportion of the microbial population after mercury pollution. (3)

Direct synthesis of methyl mercury from other organo-mercurials has never been found. (3)

MECHANISM OF REDUCING THE MERCURIC ION

This is a detoxification process

Components required:

1. NADH or NADPH.
2. A metallic mercury-releasing enzyme containing FAD as a prosthetic group.
3. Cytochrome C.
4. A sulfur compound - such as homocysteine. (3)

Pan-Hou and Imura suggest the inhibitory effects of inorganic mercury on biosynthesis of functional proteins in living bacterial cells is stronger than that of methyl mercury. Production of the volatile methyl mercury will remove the mercuric ion from the cell as a means of detoxification. (35)

Bicarbonate ion is inhibitory to methyl transfer, more so than chloride ion. Total sea salts at concentrations similar to full strength seawater (3.5% salinity), in the presence of bicarbonate, did not inhibit methylation. (36)

Cell proteins and thiols (sulfur containing compounds) inhibit methylation by binding to mercury making it unavailable for methylation. Sulfide as Na_2S when added in anaerobic conditions at 10, 50 and 100 ppm, completely prevented methylation of mercuric ion by methyl cobalamin even at the lowest sulfide concentration tested. This is consistent with the very high affinity of sulfide for mercuric ion. Lack of methylation was due to the presence of HgS. Methyl cobalamin and preexisting methyl mercury remained unaffected by sulfide. (36)

Bisogni and Lawrence (37) concluded that :

1. Microbial methylation can occur under both aerobic and anaerobic conditions.
2. Microbial methylation is also related to
   a. The presence or absence of air
   b. Growth rate or metabolic activity of methylating organisms
   c. Total concentration of mercuric ions
   d. Availability of mercuric ions
3. Predominant product of microbially mediated methylation at neutral pH in monomethyl mercury.
4. Temperature affects methylation rate only as it affects microbial activity of methylating organisms. This seems contradictory to the usual chemical concept of an increase in temperature of 10 degrees centigrade, causing a doubling of the speed of a chemical reaction.

And now to summarize this information of methylation-demethylation:

FACTORS AFFECTING METHYLATION

Factor	Stimulate	Inhibit
aerobic condition	stimulate	
anaerobic condition	stimulate	inhibit (due to S presence)
enzymatic reactions	stimulate	
non-enzymatic reactions	stimulate	
amount of mercury present	directly proportional	
pH	if neutral	if high or low
temperature	increase in temperature increases speed of methylation up to cell death	
salinity	low	high
presence of sulfide	inhibit	
methylation (Vitamin B-12)	stimulate	
cell proteins	inhibit	
thiols	inhibit	
methylating bacteria	stimulate	
bicarbonate	inhibit	
intestinal absorption	higher absorption gives more mercury for methylation	
mercury methylating yeasts	stimulate	
high redox potential	stimulate	inhibit
antibiotics	stimulate	
certain plasmids	stimulate	

From this data it becomes clear that there are *multiple opportunities* for mercury to be methylated within the human body. Considering the hazard methyl mercury offers to healthful physiology, it is suggested that mercury escaping from common dental silver-mercury amalgam fillings constitutes an unnecessary source of a toxic element, and should be eliminated from use in dentistry.

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Amounts of Mercury Required to Produce Toxicity in Biological Tissues

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Toxicity of Mercury in Biological Systems

From the early days of 1819, when mercury was first used as a tooth filling material (as silver-mercury amalgam), until 1982, mercury was assumed to stay chemically bonded within the filling. At this time the ADA was forced to publish the results of research that proved that mercury did escape from fillings. In contrast, American researchers from the 1840's to the 1890's, as well as European Dr. Alfred Stock in the 1920's, issued warnings and published scathing articles in opposition to mercury's use, but to no avail. In 1889 Northwestern Dental School's Dean, Dr. G.V. Black, the long acclaimed Father of Modern Dentistry, put the issue to rest for American dentists by issuing a proclamation that mercury did not come out of the filling. This was intended to end the amalgam controversy in America.

Research by Gay (1), Svare (2), and Vimy (3, 4) describes mercury being released from fillings and measured, as well as equated to the "body burden" of mercury. This rather destroyed the dental association's claims of non-toxicity. Apparently, silver mercury amalgam fillings have a highly dynamic surface, forming not only the corrosion products of interreactions between its metals (mercury, copper, tin, zinc and silver) but releasing mercury vapor as well. All of these metals indivudually, as well as their corrosion products are known to be cytotoxic, so there is some question as to the wisdom of placing them in a biologically and electrically active area like the mouth. This discussion will primarily address

the ultimate fate of mercury at the cellular level and its contribution to toxicity at that level.

It has been claimed that most forms of mercury, except the relatively inert mercury sulfide (HgS), are toxic. Basically, the term " toxicity" refers to the disruption of a normal critical pathway that results in *cell death*. This is different from "hypersensitivity" which leads to immune reactivity, but not necessarily to cell death. First, let us examine the various implications of the term toxicity, then look to mechanisms of toxicity, and finally to the effects of toxicity on specific tissues.

There are six basic factors that determine the toxicity of a foreign substance. They are as follows:

1. The dose and duration of exposure to the chemical.
2. The rate of exposure to the chemical (or uptake) versus the body's rate of detoxification.
3. Accessability of the foreign substance to its target tissue.
4. The biological role of the target tissue.
5. The ability of the target tissue to repair, replace or compensate for its damaged cells.
6. The nature and volume of products released from the injured cell. These products may stimulate or be cytotoxic to adjacent uninjured tissue.

Most toxic reactions result in the inactivity of some biological process. There are generally two mechanisms for inactivation. One is for the toxin to form a complex with an enzyme, binding to a cell surface receptor site, or to a cofactor. The other is to bring about a physiochemical change. This can occur in a simple fashion by altering pH, ionic concentration of the adjacent medium, solubility, oxygen saturation, or it can create more complex changes. Examples of more complex mechanisms are changes in redox potential, altering cell membrane transport (function) or actually disturbing the DNA within the cell.

Necrosis or cell death is usually the result of these biological changes, but there are even more ways by which a cell may die from exposure to mercury, should it survive the initial exposure. Alteration of cell membrane permeability leads to a *toxic hypoxic condition*. An alteration of pH will bring about the same condition. Most of the other toxic effects lead to a reduction of ATP which eventually leads to cell death via loss of energy sources, or suppression of RNA protein synthesis.

Other mechanisms of toxicity that may potentially result in cell injury or cell death are, inhibition of sulfhydryl enzymes (particularly those in the cell membrane) such as Na-K-ATPase. (5) There is also the possibility of mercury's reaction with critical cellular macromolecules that may not be reversible. (7) A highly influential aspect of mercury's cytotoxic effect is that Hg^{++} contributes to cell injury by depletion of reduced glutathione, generation of oxygen radicals (9), and lipid peroxidation (10).

The potential fate of dentally released mercury has been traced through the literature using as guidelines four of the afore mentioned six basic factors that determine toxicity.

There are many articles addressing dose-related toxic reactions, but it is difficult to correlate these articles due to different measurement systems. Some researchers report in micrograms per gram, some in micromolar amounts, and some in parts per million. In presenting the following material there is an attempt to convert all values to one of two systems, sometimes both. One is micrograms per gram, which is also called parts per million (ppm). The other is micromolar and µM.

Chinese hamster ovary cells are frequently used in tissue culture research to determine the toxic effects of exposures to heavy metals: $HgCl_2$ was extremely cytotoxic to Chinese hamster ovary (CHO) cells in culture. One hour at a 75 µM (15 ppm) concentration of this compound reduced cell plating efficiency

to 0 and cell growth was completely inhibited at 7.5 μM (1.5 ppm).

Injury to the cell membrane has been purported to be the basis of Hg^{++} cytotoxic action. (16) It is difficult to precisely extrapolate concentrations of $HgCl_2$ that produce cell membrane injury compared to the levels that cause DNA lesions. This is because of differences in culture media, lack of mercury uptake measurements in other systems, etc. The levels of Hg^{++} that produce DNA lesions are at least as low, if not lower, than those that injure the cell membrane. (17)

Heavy metals were found to slow or stop cell growth at very low concentrations (1 to 60 μM or 0.2 to 12 ppm). All interferences appeared to occur during the S-phase (synthesis phase) of mitosis. Cadmium was found to be the most toxic of the 6 metals studies, followed in descending order by mercury, cobalt, copper, nickel, and lead. The S-phase blockage produced by the metals was consistent with their genotoxic or carcinogenic activity since such activity indicates a selective interaction with DNA metabolism. (15) It is interesting to note that with the exception of lead, all of the heavy metals mentioned in this article are commonly used in dental materials.

Nerve tissue is reported to be highly sensitive to mercury, yet these references show tissues sensitive at both high and low levels. Shrivastav (83) found that 25 μM (5 ppm) methyl mercury (MeHg) decreased the conductance of axons. Concentrations as low as 0.5 μM (0.1 ppm) depolarized the nerve membrane significantly. These studies clearly indicate that the effects of mercury on the biological membranes could contribute significantly to malfunctions of the nervous system.

Several physiological responses leading to cell death were observed in Choi's work. (94) He found that astrocyte culture cells were totally destroyed when exposed to 0.01 μM (0.002

ppm or 2 ppb) methyl mercury chloride for one hour. Nakazawa (95) found complete inhibition of cell multiplication by methyl mercury chloride at 4 μM (0.8 ppm), compared to the more concentrated 25 μM (5 ppm) of mercuric chloride required. To achieve 50 percent inhibition of radioactive thymidine incorporation into cells 5 μM (1 ppm) of mercuric chloride was required, where only 1 μM (0.2 ppm) methyl mercury chloride was needed. Prasad (96) found that rat glioma cells were destroyed at 1 μM (0.2 ppm) with mercuric chloride, and 0.19 μM (0.04 ppm) for methyl mercury chloride. This takes on even more significance when we find that *most of the mercury in the human body is localized in the glial cells.* (97)

Mercury ions can penetrate the blood-brain barrier and enter the nerve cells from the blood stream. (32) Steinwell, (33) showed an impairment of the blood-brain barrier within hours of administration as either mercuric chloride or methyl mercury. Chang has shown that minute amounts (less than 1 ppm) are capable of impairing the blood-brain system leading to extravasation of normally barred plasma solutes. (34)

Later, in 1973, Chang (41) using enzyme histochemistry demonstrated a decrease in activity of succinic dehydrogenase, ATPase, and alkaline phosphatase in the rat brain following mercury intoxification (1 mg mercury/kg/or 1 ppm body weight for four weeks). There was a simultaneous increase in acid phosphatase indicating an accumulation of lysosomes in the nervous system. The number of neruosomal lysosomes may be used as a quantitative indicator for mercury toxicity within the nervous system. The decrease in these three enzymes is believed to indicate mercury caused damage to the mitochondria, cell membrane, and blood-brain barrier.

Every day we are exposed to mercury from air, food, and water. Estimates show that dental amalgams far exceed the daily exposures to all other non-occupational exposures. Mercury

appears in the urine and feces. As long as a person excretes as much as he/she absorbs, he/she should have no accumulation problem. Should excretion levels drop, then he/she may suffer from what we term "retention toxicity".

Just how much mercury can we absorb from fillings? Vimy and Lorscheider (3) calculated a daily dose of 20-29 micrograms per day from fillings they studied. Dag Brune (98) calculates around 3 micrograms of mercury per square centimeter per day as a daily dose from fillings.

How much of this exposure accumulates in body tissues? Freden (100) found as much as 380 ppm mercury accumulated in gingival tissue. Gingival tissue is gum tissue immediately adjacent to fillings and also immediately adjacent to the bone that supports the teeth. Periodontal disease is the term applied to about 85% of the population who have diseased bone and gums surrounding the teeth. Is this related to the 85% who reportedly have amalgams?

Preliminary studies performed at the University of Colorado at Colorado Springs determined that 0.4 ppm (2 μM) concentration of mercury would kill bone cells in culture. If it is possible that the 380 ppm could share 0.4 ppm with the immediately underlying bone there could emerge a whole new concept as to the etiology of the massive amount of periodontal disease experienced in this country (an American Dental Association estimate of 85% of the population). As we shall see, though, mercury is attracted to many areas of the body which result in toxic destruction.

Now that it has been shown that the presence of minute amounts of mercury can seriously damage nerve and other tissues, let us look at the relative ease or difficulty of access mercury has into our tissues. Does our body have an adequate defense mechanism against mercury intrusion to merit implanting it directly into a living body structure like a tooth? There seems to be very little to

prevent mercury from entering a cell. It appears to be one of the most readily absorbed metals. As shown by Canton and Costa (6) the uptake of $HgCl_2$ into the cells is more rapid and in greater volume than other toxic ions they studied. This includes cadmium, mercury, and copper. This accelerated uptake may be due to mercury's ability to form lipid-soluble complexes that facilitate its cellular entry. (13)

Mercury concentrations in RBC's are considered to be the most reliable index available of exposure to MeHg. (84) Since mercury vapor has already been shown in this paper to be released from the surfaces of silver mercury amalgam fillings, it would be appropriate to follow its route of entrance into the body and examine uptake potentials. Mercury can be easily transported from the mouth to the lungs via inhalation. Once in the lungs, only cell membrane permeability stands between it and direct access to the blood stream, its contents, and all that it touches.

Erythrocytes incorporate mercury vapor at high rates and the present concept is that atomic mercury becomes oxidized in the cell interior. This means that once mercury enters the cell, the oxidized form can not leave the cell through the membrane due to its electrical charge.

The following excerpts demonstrate the ease with which mercury appears to be incorporated into the red cells. In incubations with a low concentration of red blood cells, there was an increased rate of uptake of mercury into the tissues. (88) This would suggest that as the hematocrit drops, the patient might experience even less oxygen transport than the low figures would indicate.

By comparison, the rate of mercury uptake into red blood cells is considerably reduced in the presence of 2 μM ethanol. (88) Since peroxidase is required for the oxidation of both mercury vapor and ethanol, an increase in one reduces the oxidative capacity of the other. (It is curious to note that alcohol consumption is known

to be high among dentists. Could this be a unconscious way of reducing the chemical stress that mercury is putting on their systems?)

Accessability to tissues is apparently no problem for dentally introduced mercury. Mercury vapor is inhaled into the nasal passages where it has immediate access to the brain and lung tissues. From the lungs it has a strong affinity for red and white blood cells. Should mercury remain in the mouth, however, it is readily incorporated into the foods, and can run the gamut of the stomach, small and large bowels with access, via absorption, into the blood vascular system.

Perhaps of greater significance are reports of reactions in the tissues that have already been invaded by mercury. Due to its insidious nature, mercury can inflict many types of damage on a variety of tissues. Some of these are described in the following citations.

Since Hg^{++} has effects similar to those created by X-ray exposure, like being able to produce oxygen radicals in cells (9), and depleting cellular reduced glutathione levels (18), the DNA must be considered a target site of mercury's toxic action. At first glance the scenario appears opposite to what one would expect. Basically, low levels of mercury can create mutations and/or carcinogenic activity due to its ability to induce DNA lesions. At slightly higher levels, mercury kills the cells before they have time to mutate or turn cancerous. However, at levels in between, mercury may start mutations or carcinogenic activity where it would not normally start these processes. This happens because at these levels the mercury has not killed the cells, but has slowed normal DNA repair activity. Additionally, the DNA lesions induced by $HgCl_2$ may result in miscoding during DNA replication; however, $HgCl_2$ has been shown to inhibit cell growth specifically in S-phase (15), and therefore miscoding during DNA replication must occur at concentrations of $HgCl_2$ that allow this process to proceed in order

to achieve a mutagenic response in a surviving cell. These mechanistic findings may help explain the low mutagenic/carcinogenic activity displayed by $HgCl_2$ in a number of experimental systems. (19)

The DNA lesions produced by $HgCl_2$ must be considered in a different way from the DNA lesions induced by other agents. For example, the single strand breaks induced by nickel compounds and CaCrO are repaired (20, 21) while the strand breaks induced with $HgCl_2$ are not readily repaired. (6) due to the critical function of DNA in the cell, and the fact that repair enzymes are inhibited by $HgCl_2$ and can not mend the DNA lesions, cell death may result directly from mercury exposure. In fact one study shows that a 1-hour exposure to 50-75/μM $HgCl_2$ results in a high percentage of cell death and at these concentrations strand breaks and Hg binding to DNA are considerable. $HgCl_2$, however, produced DNA-DNA crosslinks which with time progressively increased in extent. These crosslinks are probably due to the ability of $HgCl_2$ to interact with the bases directly (6) while the single strand breaks may result from the production of oxygen radicals by $HgCl_2$ or by its interaction with DNA bases. (9) Such radicals have been postulated to mediate the X-ray induced DNA damage of cells.

Since it has been shown that mercury has easy access to enter tissues and even affects intracellular integrity by altering DNA structure, it is even more significant that we question the advisability of routinely implanting mercury fillings in humans. Now let us examine the intracellular aspects of mercury entry into the nervous system.

Mercury accumulation in nerves seems to concentrate in the Purkinje cells. Purkinje cells are rich in -SH groups, and their histochemical reactions are greatly reduced after mercury poisoning. This suggests that large amounts of -SH groups in the Purkinje cells may act as inert sites and offer a neutralizing effect

on mercury's action inside the cell, resulting in an apparently higher mercury tolerance. (48, 49)

Visual tissues are an extension of brain tissues, so it would seem logical to look for visual disturbances as a frequent result of exposure to mercury. In doing so, these important relationships were found:

Clinical studies of epidemic poisonings in Japan and Iraq have reported a disturbance of visual perception as one of the most consistent signs of mercury induced neurological impairment in humans. (51, 52, 53, 54) Patients typically exhibited a concentric narrowing of the visual fields and reduced visual acuity. Experimental studies of methyl mercury poisoning in neonatal and adult monkeys suggested that impaired scotopic vision, particularly a reduction in visual acuity, is the earliest sign of neurotoxicity and the most sensitive indicator of exposure. (55, 56, 57)

The neurologic impairment underlying these visual anomalies appears to be central in origin. Lesions of the primary visual cortex have been described in human autopsy material, (58, 59) whereas in most cases the retina, optic nerve, and lateral geniculate nucleus were reported to be normal. (59) These cortical lesions were characterized by diffuse neuronal degeneration and cell loss, with a proliferation of glial cells and marked astrocytosis. Similar lesions have been reported in the visual cortex of experimental animals following exposure to methyl mercury. (55, 60, 61, 62)

Shrivastav (83) found that 25μM (5 ppm) MeHg decreased the conductance of axons. Concentration as low as 0.5 μM (0.1 ppm) depolarized the nerve membrane significantly. These studies clearly indicate that the effects of mercury on the biological membranes would contribute significantly to malfunctions of the nervous system after exposure to mercury.

One of the common denominators of mercury toxicity is alteration of the cell membrane. Mechanisms for disruption of cell membrane function by mercury were examined next.

Injury to the cell membrane has been purported to be the basis of Hg^{++} cytotoxic action. (16) As found before, it is difficult to precisely extrapolate concentrations of $HgCl_2$ that produce cell membrane injury to the levels that cause DNA lesions because of differences in culture media, lack of Hg uptake measurements in other systems, etc., the levels of Hg^{++} that produce DNA lesions are at least as low as, if not lower than, those that injure the cell membrane. (17)

Since Hg^{++} has X-ray like effects in being able to produce oxygen radicals in cells (9), and deplete cellular reduced glutathione levels (18) the DNA must be considered a target site of its toxic action.

The fact that biological membranes are generally rich in sulfhydryl (-SH groups) may explain the preferential binding of mercury to the membranous structures. (48) Damage to the cell membrane by mercury is probably due to cross-linking of the protein structures within the membrane resulting in abnormal strain in the membrane structures which leads to impairment of membrane functions as well as an increase in permeability ("leaky membrane" phenomenon). (80) Mercury's damage to the blood-brain barrier is probably due to destruction of the endothelial and glial membranes. (81)

Brown and Yoshida (82) proposed that organic mercury mainly altered cell membrane structures, and appeared to interfere with protein production in nerve cells.

Erythrocytes incorporate mercury vapor at high rates and the present conception is that atomic mercury becomes oxidized in the cell interior. This prevents it from being able to permeate outwards, through the membrane due to its charge. (86)

The reason that mercury from fillings was hard to isolate as a potential cause of multiple disorders is that its action is not directed toward just one target. Many cell structures become potential targets. We have just seen evidence of nerve tissue, and cell membrane being target tissues. Others will follow. Clinical evidence suggests that there is probably a genetic predisposition for certain weaknesses. Mercury has been found to create patterns of toxicity in isolated tissues, but few, if any, researchers have been aware that a large potential source of mercury is from fillings in the mouth.

Toxins may also alter DNA, which is the genetic code for cellular duplication (called replication). One of the classical alterations that has been documented from X-ray damage is the single strand break. (6) DNA is described as a dual spiral helix, which is like two strands of thread twisted together. Exposure to X-ray can break one of these strands, thus the term *single strand break*, or SSB, has been used to describe this form to toxic reaction. X-ray induced SSB's are rapidly repaired (within one hour) under normal body conditions. (6) Repair is accomplished enzymatically, activity by a repair polymerase.

Single strand breaks are produced by $HgCl_2$. The similarities of the DNA damage induced by X-rays and $HgCl_2$ would suggest that $HgCl_2$ possesses more mutagenic and carcinogenic activity than has been reported. This assumes the existence of a good correlation between DNA damage and mutation or carcinogenesis, as has been suggested for X-rays (11) and other established carcinogenic agents. (12) An obvious difference between Hg^{++} and X-rays is that following damage with X-rays, the cell does not have to contend with the continuous presence of Hg^{++}, and is therefore able to recover more rapidly. A comparison has been made between the ability of cells to repair DNA damage induced by both of these agents and found that X-ray induced damage is rapidly repaired (within one hour), in contrast to that produced with $HgCl_2$, which actually increases in extent during a similar

one hour repair period following removal of extracellular mercury.

Since unfaithful repair of DNA has been highlighted as a critical element in the potency of X-rays in inducing mutations and transformations (13), Costa's results suggest that further work in understanding the low mutagenic potential of mercury, despite its potent DNA-damaging activity, should be directed toward a study of its effect on repair enzymes activated following DNA damage by X-rays and other chemical agents.

Less DNA damage appeared to be required to produce the same level of cell killing with $HgCl_2$ as compared with X-rays. The observations that cells are unable to repair the single strand breaks induced with $HgCl_2$ and that low concentrations of $HgCl_2$ (10 μM) (2 ppm) inhibit the rejoining of single strand breaks induced by X-rays suggest that $HgCl_2$ may act on DNA homeostasis by inhibiting DNA repair processes. Thus, in contrast to X-rays, DNA damage induced by $HgCl_2$ was not readily repaired and may represent an irreversible injury that leads to cell death. (14)

Although single strand breaks in the DNA were the primary lesion induced by $HgCl_2$, they were not the only lesion caused by this agent. DNA-DNA cross-links develop with time following exposure to $HgCl_2$ probably resulting from its ability to interact directly with the DNA bases. (14) Studies have demonstrated that $HgCl_2$, as well as a number of other toxicologically important metals, produce an S-phase specific cell cycle blockade, suggesting that mercury specifically interfaces with events involved in DNA replication. (15) The literature seems to substantiate DNA damage, but the reason we do not see more genetic defects is probably due to cell destruction rather than alteration. Attention should be directed to the fact that dental offices contain notably high levels of mercury vapor, and that X-rays are a common diagnostic procedure. Perhaps consideration should be given to this situation in light of the duplicity of exposure frequently inflicted upon dental patients.

It is hard to determine which one of the adverse effects of mercury is worse. But, we have not seen all of them yet. Since the field of immunology has gotten a great deal of attention lately, it will be considered next. The immune system is paramount to our survival because the action of its cells protect us from everything from infectious to malignant diseases. Any compromise of this system can be considered a reduction in a person's health capacity.

Developments in methodology have made it possible to determine far more discrete alterations in the immune system than ever before. The advent of *flow cytometry* has made it possible to label tissue cells with fluorescence tagged monoclonal antibodies. For purposes of explanation, let us look at a common application of flow cytometry. Lymphocytes are the primary white blood cells involved in our body's immune system. There are now identifying "markers" for several subsets of lymphocytes. Those termed B-lymphocytes produce a substance called immunoglobulins, which are molecules capable of destroying pathogenic bacteria, viruses, and foreign chemicals. Gamma globulin is a fairly well known example of an immunoglobulin. Gamma globulin injections are frequently administered to boost a persons immune capacity. It is interesting to note that mercury (in the form of thimerosal), a known immunosuppressant, is used as a preservative in injectable gamma globulin preparations.

B-lymphocytes produce immunoglobulins (also called antibodies) under the directions of the T-lymphocytes. One subset of lymphocytes called T-4 lymphocytes stimulate the B-lymphocytes to become macrophages and produce antibodies. Another subset called the T-8's operate the feedback mechanism that slows T-4 activity when an adequate supply is reached, in order to prevent antibody overproduction.

When the cells get improper messages, the immune system can be turned against its own tissues, and auto-destruction occurs. This disease state is termed auto-immune disease and includes

disorders like AIDS, arthritis, multiple sclerosis, collagen disease and systemic lupus erythematosus.

An example of the mechanism of auto-immune disease is readily duplicated with heavy metals. The primary purpose of the immune system is to detect and destroy foreign materials. In immunology vernacular, the surveillance immune system looks for "self" and "non-self" cells. One lymphocyte can look at a lymphocyte next to it, see its twin brother, so to speak, and relax knowing it has identified a "self" cell. A mercury atom, or mercurial compound can attach itself to the same lymphocyte and give it a slightly different appearance. Now the original lymphocyte looks and sees its twin as "non-self", for he himself does not have mercury in his outer coat. He now cries "enemy" and starts a process to destroy the "non-self" appearing lymphocyte wearing mercury. This destruction process is called auto-immune disease. Many heavy metals can attach to tissues in the body and elicit the same self destruction process. For this reason alone heavy metals should be avoided in the human body.

Multiple Sclerosis is auto-immune in nature, and is characterized by the presence of auto-antibodies against neural structures and peripheral lymphocytes. Reinherz and his group found a selective loss of suppressor cells (sometimes to non-detectable levels) in peripheral blood of MS patients during periods of exacerbation. Suppressor cells reappeared during periods of disease remission. "These results suggest that immunoregulatory abnormalities contribute to the pathogenesis of MS." (22)

Reinherz's results showed a definite correlation between the absence of suppressor cells (today termed T-8 cells) and disease activity. Tests performed at the University of Colorado at Colorado Springs show that toxic dental materials (nickel and mercury) both cause a drop in T-8 suppressor cells which can demonstrate recovery within a few days after toxic metal removal. T-11 cells and T-4 to T-8 ratios improve after metal removal.

These results are presented here to further emphasize Reinherz's article from the dental aspect. Their data are part of research being made ready for publication and are not intended to represent the full impact of that research.

CASE #1

	July 11 BEFORE	Dental Surgery July 12	July 17 AFTER
Immunologic Reports			
T-11	40%		78%
T-4	15%		56%
T-8	13%		26%
B-1	9%		18%
Ratio:	1.15		2.15
Complete Blood Count (CBC)			
RBC	4.82		5.21
Hgb	13.9		15.1
Hct	40.7		44.2
Plat	404,000		341,000
WBC	13,600		9,900
Mono	1 (136)		4 (396)
Lymph	42 (5712)		24 (2376)
Segs	51 (6936)		67 (6633)
Eos	5 (680)		4 (396)
Baso	1 (136)		1 (99)
Viability	81%		98%

CASE #2

	August 25 BEFORE	September 9 AFTER	January 14 FOLLOW-UP
T-11	20%	53%	90%
T-4	14%	32%	65%
T-8	7%	17%	58%
B-1	5%	16%	26%
Ratio	2:1	1.8:1	1.1:1

Mercury mediated auto-immune disease in the rat is an interesting model by which to evaluate the potential effect of a toxic agent on immunocompetent cells. Indeed mercury induces in BN rats a lymphoproliferation, the production of a variety of autoantibodies some of which are responsible for an auto-immune glomerulonephritis and a polyclonal increase in total serum IgE levels. It has been demonstrated that susceptibility is genetically controlled partly by major histocompatibility complex (MHC)-linked genes (27, 28, 29) and that $HgCl_2$ is able to induce T and/or macrophage-dependent polyclonal activation or B cells in susceptible animals. (30)

Rat recipient's T and B cells also proliferated in vivo as a response to syngeneic mononuclear SC incubated in vitro with $HgCl_2$. A 2500 times higher quantity of free $HgCl_2$ was required to induce proliferation in recipients. This latter observation suggests that a population of cells is modified by $HgCl_2$ which in turn can stimulate other cell populations in the recipient. Preliminary data also show that irradiated DC and T cells from BN rats injected with $HgCl_2$ stimulate in vitro normal BN rat mononuclear cells. None of these effects could be observed when SC from LEW rats treated with $HgCl_2$ were used, and interestingly, LEW are resistant to the induction of $HgCl_2$ induced auto-immune disease. This supports the hypothesis that modified syngeneic cells which are responsible for the proliferation of T and B cells in the DPLN

might also be responsible for the polyclonal stimulation and therefore for the auto-immune disease observed in BN rats injected with $HgCl_2$. It is of note that the auto-immune disease induced in BN rats is self-limited and the fact that SC from BN rats who had received $HgCl_2$ for 14 days had no effect, though unexplained, might be relevant. (31)

Other hypotheses can be put forward. It is possible that $HgCl_2$ modifies a non-MHC-encoded structure. These modified cells would then stimulate autologenous T cells which would trigger B cells. It has been shown in the mercury model that both MHC and non-MHC linked genes are involved in the induction of auto-immune abnormalities. One can therefore speculate that T cells are stimulated when the determinant modified at the cell surface is presented in association with the appropriate self-Ia and/or that only B cells with appropriate Ia could be triggered. Another possibility is that macrophages and T cells from BN rats release nonspecific activating factors upon $HgCl_2$ exposure. These factors could then trigger B cells recognizing autoantigens to produce autoantibodies. (31)

Whatever the fine mechanism involved, two facts suggest that the phenomenon we have reported is important in the induction of the auto-immune disease due to $HgCl_2$. First, SC from BN rats who received $HgCl_2$ were able to induce the proliferation as early as six days after the first injection of $HgCl_2$. The modification of SC is therefore the first abnormality of the immune system during the course of the disease. Second, SC from resistant LEW rats were unable to induce any proliferation. (31) From these citations it is evident that mercury launches a multiphasic attack against the immune system. With such a plethora of chemicals now invading our bodies through air pollution, unclean water, artificial substances in food, clothing and shelters, it may not be wise to add mercury - a known immune modulator - to an already over bombarded immune system.

The impairment of the blood-brain barrier, together with the possible inhibition of certain associated enzymes by mercury, is responsible for the reduction of amino acid uptake by the nervous system after mercury administration. (35)

Yoshino, in looking at the subcellular distribution of mercury in the rat nervous system, found nearly all the mercury in the protein fraction. It was concentrated mainly in the mitochondria, microsomal fractions and supernatant while the nuclear fraction contained a minimal amount. (36)

Chang has found (38) localization of mercury within the intact nervous tissues as compared to the tissue homogenates. In general increased exposure results in more mercury being found in nerve cells, glia, and nerve fibers as the intoxication process progresses. It is important to note that the route of entry did not influence the amounts or distribution of mercury in the nerve cells or fibers.

Both organic and inorganic compounds create devastation of the nervous system, but most organomercuric compounds create multifold, more serious damage. For this reason much smaller doses - less than 1 ppm - can produce measurable changes. The observation that more mercury was localized in the nervous system after methyl mercury poisoning than after mercuric bichloride poisoning also supports the general belief that organic mercury is more neurophilic and neurotoxic than inorganic mercury. (39)

Yoshino (36) observed that incorporation of amino acids into brain tissues was drastically reduced following mercury intoxication. It was postulated (33) that the reduction of amino acids into the nervous system was a consequence of the impairment of the blood-brain barrier by MeHg.

Yoshino, in another publication, (40) reported *normal* levels of several sulfhydryl enzymes (ATPase, succinic dehydrogenase,

and others) even after the onset of MeHg stimulated neurological symptoms. He postulated that the development of neurological symptoms and lesions was not a consequence of an inhibition of enzymatic functions or reduction in oxygen consumption in the nervous system, but rather the reverse could be true.

While it has been known for several decades that mercury compounds are neurotoxic, Chang (42) was the first to show a change in RNA content in neurons due to mercury compounds. Although MeHg is known to be more neurotoxic than inorganic mercury (32, 43, 44) Chang (43) found that inorganic mercury induced a more acute and severe effect on neuronal RNA than MeHg. This more rapid and drastic change in neuronal RNA content after inorganic mercury poisoning may be explained by the fact that inorganic mercury binding to nucleosides is almost ten times stronger than to MeHg. (45)

Since RNA provides the chief "machinery" for protein synthesis, it is believed that the change in protein synthesizing activity is reflected in the effect of mercury on RNA. Campagnoni and Costa have published several excellent papers on the effects of mercury and nickel. From my interpretation of the literature it appears that their reports on RNA content are a giant step forward. While many researchers stand on the knees of those published before them, these men appear to be among the real pioneers in heavy metal research.

Campagnoni (77) describes a special form of RNA in the rat nervous system. This RNA represents about 10 percent of the total RNA. It is of interest to note that this form is almost identical to RNA in spinal ganglia formed after mercury intoxication. The RNA detected after mercury intoxication may not be "abnormal" RNA, but normal RNA existing in abnormal quantities. This RNA is a minor component of neuronal RNA, but its nature and function are unknown. This special form of RNA may be responsible for the production of metallothionine which in turn provides

protection to these neurons from mercury toxicity. This may explain the manifestation of recovery and tolerance relative to intoxication. If induction of DNA lesions and active repair of these lesions are important for mutagenicity or carcinogenicity of a chemical agent, then mercury may be expected to have weak mutagenic activity at low concentrations but at higher concentrations where DNA repair activity was inhibited there should be less mutagenic activity. Additionally, the DNA lesions induced by $HgCl_2$ may result in miscoding during DNA replication; however, $HgCl_2$ has been shown to inhibit cell growth specifically in S-phase (15) and therefore miscoding during DNA replication must occur at concentrations of $HgCl_2$ that allow this process to proceed in order to achieve a mutagenic response in a surviving cell. These mechanistic findings may help explain the low mutagenic/carcinogenic activity displayed by $HgCl_2$ in a number of experimental systems. (19)

It should be noted that enhanced DNA content does not necessarily imply that methyl mercury-induced damage is completely repaired in brain regions which contain replicating cells at the time of toxic exposure. Morphological damage after developmental exposure to high levels of methyl mercury indicates intrusion of glial elements into damaged brain areas. Furthermore, low levels of methyl mercury are known to stimulate astroglial replication in tissue cultures, a factor which could contribute to a major portion of the elevation in DNA seen in cerebral cortical and cerebellar regions.

From the data presented there is apparently no safe dosage of mercury, and its effects on biological tissues range from interruption of cell membrane function to disruption or destruction of the mitotic processes. Few natural elements, even other heavy metals, rival mercury in its ability to alter normal cellular function. There is certainly enough evidence of toxicity of mercury to question the wisdom of its continued use as the major restorative material world wide for replacing decayed portions of teeth.

Submitted to the University of Colorado at Colorado Springs in partial fulfillment of requirements for a Master's degree in basic science by:

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The Fate of Mercury in Biological Tissues

Prepared by:
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Now that it is well established that mercury comes out of dental silver-mercury amalgam fillings, (1,2,3,4) it becomes important to investigate the fate of that mercury. Multiple mercuric corrosion compounds sluff off fillings; elemental mercury escapes also. This elemental mercury if swallowed can form mercuric chloride by combining with the hydrochloric acid in the stomach, which can in turn be methylated into insidious methyl mercury.

Methyl mercury and inorganic mercury can both cause tissue destruction in the body. Scientific literature is teaming with mechanisms of mercurial inactivation processes, direct effects on brain tissue, vision, heart, cell membrane dysfunction, intracellular mechanisms of toxicity, amounts necessary to produce that toxicity, yet dentistry still pours tons of mercury (literacy) into people's mouths every month. Two hundred tons per year would amount to greater than 16 tons per month implanted into Americans alone.

Nickel and other non-gold metals (also called non-precious) have now almost entirely (over 80%) replaced the market for dental crowns. Both mercury and nickel produce toxic effects at the tissue level. The following materials were selected to substantiate and demonstrate the suggestion that these heavy metals are too poisonous to be used by dentistry in the uninformed, trusting patients. They are also presented for the dentists for in their defense, dentists have been protected from this information by the appointed leaders of their trade association resulting in the practicing dentist being uninformed about toxicity in their patients.

No one is able to answer the question, "Why does dentistry continue to place mercury?" Frequently it is said that there is nothing in the literature that directly relates fillings to disease. This is no longer true, the facts are out there. As late as 1982 an official ADA response to the questions, "Does mercury escape from fillings?" was "No". We now know that that response is not true.

The primary purpose of this series of papers is to show that it is documented and easy to show that significant amounts of mercury escape. Section II showed that it is well documented that mercury has many methods of methylation into methyl mercury which is 100 fold more toxic than elemental mercury. The present section, No. III, will deal with a sample of the voluminous documentation of mercury and methyl mercury's role in producing toxic effects in various tissues. Hopefully these papers can correlate the evidence that has been present for over 2 decades in such a fashion that it becomes evident that dental mercury fillings constitute a hazard to patient's health.

First, let us describe the various implications of the term toxicity, then look to mechanisms of toxicity, and finally to the effects of toxicity on specific tissues. Toxicity has been claimed for most forms of mercury, except the relatively inert mercury sulfide (HgS). Basically toxicity is the disruption of a normal critical pathway that results in cell death. This is totally different from hypersensitivity which does not necessarily lead to cell death.

As stated in Monograph II, there are six basic factors that determine the toxicity of a foreign substance. They are as follows:

1. The dose of and duration of exposure to the chemical.
2. The rate of exposure to chemical versus the body's rate of detoxification.
3. Accessability of the foreign substance to its target tissue.
4. The biological role of the target tissue.

5. The ability of the target tissue to repair, replace or compensate for its damaged cells.
6. The nature and volume of products released from the injured cell. They may stimulate or be cytotoxic to adjacent uninjured tissue.

Most toxic reactions result in the inactivity of some biological process. There are generally two mechanisms for inactivation. One is for the toxin to form a complex with an enzyme, binding to a cell surface receptor site, or to a cofactor. The other is to bring about a physicochemical change. This can occur simply by altering pH, ionic concentration, solubility, or oxygen saturation, or it can be more complex. Examples of more complex mechanisms are changes in redox potential, altering cell membrane transport (function) or actually disturbing the DNA within the cell. Single strand breaks of DNA are an effective method and are common with primary dental materials like mercury and nickel.

Necrosis or cell death is the result of these toxic changes, but there are several ways by which a cell may die. Alteration of cell membrane permeability leads to a toxic hypoxic condition, as can an alteration of pH. Most of the other toxic effects lead to a reduction of ATP which leads to cell death via loss of energy sources, or suppression of RNA - protein synthesis.

Other mechanisms of toxicity that can potentially result in cell injury or cell death are inhibition of sulfhydryl enzymes, particularly those in the cell membrane such as NaK - ATPase (5) may be involved in cell injury. (6) There is also the possibility of mercury's reaction with critical cellular macromolecules that may not be reversible. (7) A very influential aspect of mercury's cytotoxic effect is that Hg++ contributes to cell injury by depletion of reduced glutathione, (8) generation of oxygen radicals, (9) and lipid peroxidation. (10)

Toxins may also alter DNA which is the genetic code for cellular duplication (called replication). One of the classical alterations that has been documented for X-ray is the single strand break. (6) DNA is described as a dual spiral helix, which is like two strands of thread twisted together. Exposure to X-ray can break one of these strands, thus the term, single strand break (SSB), has been used to describe this form of toxicity.

Single strand breaks are produced by $HgCl_2$. The similarities of the DNA damage induced by X-rays and $HgCl_2$ would suggest that $HgCl_2$ possesses more mutagenic and carcinogenic activity than has been reported, assuming the existence of a good correlation between DNA damage and mutation or carcinogenesis as has been suggested for X-rays (11) and other established carcinogenic agents (12). An obvious difference between Hg^{++} and X-rays is that following damage with X-rays the cell does not have to contend with the continuous presence of Hg^{++} and is therefore able to recover more rapidly. A comparison has been made between the ability of cells to repair DNA damage induced by both of these agents and found that X-ray induced damage is rapidly repaired (within one hour), in contrast to that produced with Hg which actually increases in extent during a similar one hour repair period following removal of extracellular mercury. (6) Repair is accomplished by enzymatic activity of a repair oriented reductase polymerase.

Canton and Costa report herein that a much lower level of DNA damage is required to produce a given cytotoxic response with $HgCl_2$ as compared with that produced by X-rays. They also demonstrated that the addition of $HgCl_2$ at non-cytotoxic and non-DNA-damaging concentrations was capable of inhibiting the repair of the single strand breaks caused by X-rays.

Since unfaithful repair of DNA has been highlighted as a critical element in the potency of X-rays in inducing mutations and transformations (13), Costa's results suggest that further work in

understanding the low mutagenic potential of mercury, despite its potent DNA-damaging activity, should be directed toward a study of its effect on repair enzymes activated following DNA damage by X-rays and other chemical agents.

The uptake of $HgCl_2$ into cells is more rapid and in greater volume than other toxic ions studied by Canton and Costa such as $CdCl_2$ and Cu_2So_4. This accelerated uptake may be due to mercury's ability to form lipid-soluble complexes that facilitate its cellular entry.

The induction of DNA breakage as a mechanism of cell injury by $HgCl_2$ represents a relatively new concept in understanding its potential site of action at the cellular level.

Less DNA damage appeared to be required to produce the same level of cell killing with $HgCl_2$ as compared with X-rays. The observations that cells are unable to repair the single strand breaks induced with $HgCl_2$ and that low concentrations of $HgCl_2$ 10 μM (2 ppm) inhibit the rejoining of single strand breaks induced by X-rays suggest that $HgCl_2$ may act on DNA homeostasis by inhibiting DNA repair processes. Thus, in contrast to X-rays, DNA damage induced by $HgCl_2$ was not readily repaired and may represent an irreversible injury that leads to cell death.

Although single strand breaks in the DNA were the primary lesion induced by $HgCl_2$ they were not the only lesion caused by this agent. DNA-DNA cross-links develop with time following exposure to $HgCl_2$ probably resulting from its ability to interact directly with the DNA bases (14). Recent studies have demonstrated that $HgCl_2$, as well as a number of other toxicologically important metals, produce an S-phase-specific cell cycle blockade, suggesting that mercury specifically interfaces with events involved in DNA replication (15).

Injury to the cell membrane has been purported to be the basis of

Hg^{++} cytotoxic action. (16) Although it is difficult to precisely extrapolate concentrations of $HgCl_2$ that produce cell membrane injury to the levels that cause DNA lesions because of differences in culture media, lack of Hg uptake measurements in other systems, etc., the levels of Hg^{++} that produce DNA lesions are at least as low if not lower than those that injure the cell membrane (17).

Since Hg^{++} has X-ray like effects in being able to produce oxygen radicals in cells (9), and deplete cellular reduced glutathione levels (18) DNA must be considered a target site of mercury's toxic action. If induction of DNA lesions and active repair of those lesions are important for mutagenicity or carcinogenicity of a chemical agent, then Hg may be expected to have mutagenic activity at low concentrations, but at higher concentrations where DNA repair activity was inhibited there should be less mutagenic activity. Additionally, the DNA lesions induced by $HgCl_2$ may result in miscoding during DNA replication; however, $HgCl_2$ has been shown to inhibit cell growth specifically in S-phase (15) and therefore miscoding during DNA replication must occur at concentrations of $HgCl_2$ that allow this process to proceed in order to achieve a mutagenic response in a surviving cell. These mechanistic findings may help explain the low mutagenic/ carcinogenic activity displayed by $HgCl_2$ in a number of experimental systems (19).

The DNA lesions produced by $HgCl_2$ must be considered in a different way from the DNA lesions induced by other agents. Due to the critical function of DNA in the cell, its concentration and the fact that repair enzymes are inhibited by $HgCl_2$ and cannot mend the DNA lesions, cell death may result directly from these genetic effects. In fact one study shows that a 1-h exposure to 50-75 μM $HgCl_2$ results in a high percentage of cell death and at these concentrations strand breaks and Hg binding to DNA are considerable. $HgCl_2$, however, produces DNA-DNA crosslinks which with time progressively increase in extent. These crosslinks

are probably due to the ability of $HgCl_2$ to interact with the bases directly (6) while the single strand breaks may result from the production of oxygen radicals by $HgCl_2$ and also by its interaction with DNA bases (9). Such radicals have been postulated to mediate the X-ray induced DNA damage of cells. Heavy metals were found to slow or stop cell growth at very low concentrations (1 to 60 μM or 0. 2 to 12 ppm) . All interferences appeared to occur during the S-phase (synthesis phase) of mitosis. Cadmium was found to be the most toxic followed in descending order by mercury, cobalt, copper, nickel and lead. The S-phase blockage produced by the metals was consistent with their genotoxic or carcinogenic activity since such activity indicates a selective interaction with DNA metabolism. (15)

It is hard to determine which adverse effect of mercury is the worst. Since the field of immunology has gotten so much attention lately it will be considered next. The immune system is paramount to survival because the action of its cells protect us from everything from infectious diseases to malignant diseases. Any compromise of this system should be considered a reduction of a persons health capacity.

Developments in methodology have made it possible to determine far more discrete alterations in the immune system than ever before. The advent of flow cytometry has made it possible to label tissue cells with fluorescent tagged monoclonal antibodies. For purposes of explanation, let us look at a common application of flow cytometry. Lymphocytes are the primary white blood cell involved in our body's defense system, known as the immune system. There are three basic types of lymphocytes. Those termed B-lymphocytes produce a substance called immunoglobulin, which ia a group of molecules capable of destroying pathogenic bacteria, viruses, and foreign chemicals. Gamma globulin is a fairly well known example of an immunoglobulin. Gamma globulin shots are frequently administered to boost a persons immune capacity. It is interesting to note that a known

immunosuppressant, mercury (in the form of thimerosal) is used as a preservative in all injectable gamma globulin preparations.

B-lymphocytes produce immunoglobulin (also called antibodies) under the direction of the T-lymphocytes. One subset of lymphocytes called T-4 lymphocytes stimulate the B-lymphocytes to produce antibodies. Another subset called the T-8's operate the feedback mechanism that slows antibody production.

When the body gets improper messages, its immune system can be turned against its own tissues, and auto destruction occurs. This disease state is termed auto-immune disease and includes disorders like AIDS, arthritis, multiple sclerosis, collagen disease and systemic lupus erythematosus.

An example of the mechanism of auto immune disease is easily demonstrated with heavy metals. The primary purpose of the immune system is to detect and destroy foreign materials. In the vernacular, the surveillance immune system looks for "self" and "non-self" cells. One lymphocyte can look at a lymphocyte next to it, see its twin brother, so to speak, and relax knowing it has identified a "self" cell. A mercury atom, or mercurial compound can attach itself to the same lymphocyte and give a slightly different appearance. Now the original lymphocyte looks and sees its twin as "non-self", for he himself does not have mercury in his outer coat. He now cries "enemy" and starts a process to destroy the non-self" appearing lymphocyte. This process is called autoimmune disease. Heavy metals attached to any tissue in the body can elicit the same identical self destruction process, For this reason, heavy metals should be avoided in the human body.

Multiple sclerosis is a disease that is auto-immune in nature, and is characterized by the presence of auto-antibodies against nerve tissue and peripheral lymphocytes. Reinherz and his group found a selective loss of suppressor cells (sometimes to non-detectable levels) in peripheral blood of MS patients during periods of

exacerbation. Suppressor cells reappeared during periods of disease remission. "These results suggest that *immunoregulatory* abnormalities contribute to the pathogenesis of MS." (22)

Their results showed a definite correlation between the absence of suppressor cells (termed T-8 cells) and disease activity. Tests performed at the University of Colorado at Colorado Springs show that toxic dental materials (nickel and mercury) both cause a drop in T-8 suppressor cells which can demonstrate recovery within a few days after toxic metal removal. T-11 cells and T-4 to T-8 ratios improve after metal removal. These results are presented here to further emphasize Reinherz's article from the dental aspect. These data are representative of changes seen in actual patients undergoing the toxin removal procedures.

With such similarities in immune response between onset and remission of MS compared to the presence and absence of dental materials it is not deemed wise by this author to place toxic dental materials into the person who has MS or who may have a genetic predisposition to that disease.

Lymphocytes from individuals exposed to methyl mercury have a higher incidence of structural chromosomal aberrations than controls. (23) The most common type of aberration found is described as "pulverized metaphase". These have been reported by Skerfving, et al (24) in 1974, as occurring in lymphocyte of humans exposed to methyl mercury through eating fish. These pulverized intracellular products probably represent the cytological effects of toxicity leading to cell death. In another report it was thought that methyl mercury interacted with sulfhydryl groups on tubulin and interfered with microtubule polymerization. (25) Such destructive effects of microtubules can result in C-mitosis and aneuploidy (26) which is diagnostic of active growing cancer.

Mercury auto-immune disease in the rat is an interesting model

to evaluate the potential effects of a toxic agent on immunocompetent cells. Indeed this agent induces in BN rats a lymphoproliferation, the production of a variety of autoantibodies some of which are responsible for an auto-immune glomerulonephritis and polyclonal increase in total serum IgE level. It has been demonstrated that susceptibility to auto-immune processes genetically controlled partly by major histocompatibility complex (MHC)-linked genes (27, 28, 29) and that $HgCl_2$ is able to induce a T and/or macrophage-dependent polyclonal activation of B cells in susceptible animals (30).

Recipients' T and B cells also proliferated in vivo in response to syngeneic mononuclear SC incubated in vitro with $HgCl_2$. A 2500-fold higher quantity of free $HgCl_2$; was required to induce proliferation in recipients. This latter observation suggests that a population of cells is modified by $HgCl_2$ which in turn can stimulate other cell populations in the recipient. Preliminary data also show that irradiated SC and T cells from BN rats injected with $HgCl_2$ stimulate in vitro normal BN rat mononuclear cells. None of these effects could be observed when SC from LEW rats treated with $HgCl_2$ were used; and interestingly, LEW are resistant to the induction of $HgCl_2$ - induced auto immune disease. This supports the hypothesis that modified syngeneic cells which are responsible for the proliferation of T and B cells in the DPLN might also be responsible for the polyclonal stimulation and therefore for the auto immune disease observed in BN rats injected with $HgCl_2$. It is of note that the auto immune disease induced in BN rats is self-limited and the fact that SC from BN rats who had received $HgCl_2$ for 14 days had no effect, though unexplained, might be relevant.

Other hypotheses can be put forward. It is possible that $HgCl_2$ modifies a non-MHC-encoded structure. These modified cells would then stimulate autologous T cells which would trigger B cells. It has been shown in the mercury model that both MHC and non-MHC linked genes are involved in the induction of auto

immune abnormalities. One can therefore speculate that T cells are stimulated when the determinant modified at the cell surface is presented in association with the appropriate self-Ia and/or that only B cells with the appropriate Ia could be triggered. Another possibility is that macrophages and T cells from BN rats release nonspecific activating factors upon $HgCl_2$ exposure. These factors could then trigger B cells recognizing autoantigens to produce autoantibodies. Whatever the final mechanism involved, two facts suggest that the phenomenon we have reported is important in the induction of the auto-immune disease due to $HgCl_2$. First, SC from BN rats who received $HgCl_2$ were able to induce the proliferation as early as six days after the first injection of $HgCl_2$. The modification of SC is therefore the first abnormality of the immune system during the course of the disease. Second, SC from resistant LEW rats were unable to induce any proliferation. Experiments in vivo are in progress to determine the respective roles of T cells, T cell subsets and macrophages and to elucidate the mechanism of B cell activation. (31)

Since it is such a short distance from the filling to the brain, and there is ready access from the oral cavity to the nasal cavity and close proximity to brain tissue, it would seem reasonable to find neurological problems stemming from the efflux of methyl mercury from fillings. Again the literature contains many articles of neurologic effects from methyl mercury and mercury exposure, but there is little awareness of the fact that the fillings in the mouth provide a constant source of mercury exposure.

Mercury ions penetrate the blood-brain barrier and enter the nerve cells from the blood stream. (32) Steinwell (33) showed an impairment of the blood-brain barrier within hours of administration as either mercuric chloride or methyl mercury. Chang has shown that minute amounts (less than 1 ppm) are capable of impairing the blood-brain barrier system leading to extravasation of normally barred plasma soluted. (34)

The impairment of the blood-brain barrier, together with the possible inhibition of certain associated enzymes by mercury, is responsible for the reduction of amino acids uptake by the nervous system after mercury administration. (35) Yoshino (36) demonstrated that the calcarine cortex and cerebellum showed higher mercury concentrations than any other part of the brain after MeHg administration.

Somjen (37) reports that after a chronic exposure to radio labeled MeHg the highest concentration was in the spinal dorsal root ganglia. This was followed by the subcortical part of the forebrain. Spinal cord and peripheral nerves contained significantly less Hg than the sensory ganglia. The distribution seems to correlate well with pathological findings in these areas.

Yoshino (36) in looking at the subcellular distribution of mercury in the rat nervous system found nearly all the mercury in the protein fraction. It was concentrated mainly in the mitochondria microsomal fractions and supernatant while the nuclear fraction contained a minimal amount.

Chang (38) has found localization of mercury within the intact nervous tissues as compared to the tissue homogenates. In general, increased exposure produced more mercury in nerve cells, glia, and nerve fibers as the process of intoxication progresses. It was important to note that the route of entry did not influence the amounts or distribution of mercury in the nerve cells or fibers.

The observation that more mercury was localized in the nervous system after methyl mercury poisoning than after mercuric bichloride poisoning also supports the general belief that organic mercury is more neurophilic and neurotoxic than inorganic mercury. (39)

Yoshino (36) observed that incorporation of amino acids into brain tissues was drastically reduced following mercury intoxication.

It was postulated (33) that the reduction of amino acids into the nervous system was a consequence of the impairment of the blood - brain barrier by MeHg. Yoshino (40) found normal levels of several sulfhydryl enzymes (ATPase, succinic dehydrogenase and others) even after the onset of MeHg stimulated neurological symptoms. He postulated that the development of neurological symptoms and lesions was not a consequence of an inhibition of enzymatic functions or reduction in oxygen consumption in the nervous system, but rather the reverse could be true.

Later, in 1973, Chang (41) using enzyme histochemistry demonstrated a decrease in activity of succinic dehydrogenase, ATPase, and alkaline phosphatase in the rat brain following mercury intoxification (1 mg mercury/kg or 1 ppm body weight for four weeks). There was a simultaneous increase in acid phosphatase indicating an accumulation of lysosomes in the nervous system. The number of neurosomal lysosomes may be used as a quantitative indicator for mercury toxicity within the nervous system. The decrease in these three enzymes is believed to indicate damage on the mitochondria, cell membrane, and blood - brain barrier by mercury.

MeHg produces complex changes in the metabolic responses of the brain, suggesting that the coordination of energy metabolism to functional activity has been impaired. It is interesting to note that alterations in metabolism of the brain occur at doses far below those producing overt toxicity.

While it has been known for several decades that mercury compounds are neurotoxic, Chang (42) was the first to show a change in RNA content in neurons due to mercury compounds. Although MeHg is known to be more neurotoxic than inorganic mercury (32,43,44) Chang (43) found that inorganic mercury induced a more acute and severe effect on neuronal RNA than MeHg. This more rapid and drastic change in neuronal RNA content after inorganic mercury poisoning may be explained by

the fact that inorganic mercury binding to nucleosides is almost ten times stronger than to MeHg. (45)

Somjen (46) found that mercury exposure produced a retarded conduction velocity, an elevation of extracellular threshold and a reduction in the amplitude of the compound spike. The spike potential of sensory ganglion neurons was significantly prolonged, indicating retardation in repolarization. The author suggests that nerve biopsy may be a more reliable index of MeHg poisoning than electroneurography.

Developing tissues of infants or in the fetuses are highly susceptible to mercury interference. Some of these reasons can be explained by abstracting the literature.

Damage sustained from methyl mercury exposure will depend upon the stage of development of the brain at the time of the exposure. Neuronal replication in the midbrain and brainstem is already past its peak at birth. In contrast, cerebellar cells replicate primarily postnatally. Cellular development in the cerebral cortex spans the whole prenatal period.

Differences in concentration of methyl mercury (1 mg/kg vs. 2.5 mg/kg used here) can cause differences in response. Sometimes methyl mercury will kill cells outright, thus reducing the number of cells present and other times it stimulates a compensatory increase; and increased contents can be measured. DNA synthesis in cultured human fetal astrocytes is inhibited by high concentrations of methyl mercury, but stimulated at lower concentrations.

DNA content in the midbrain and brainstem was markedly *lowered* by methyl mercury exposure as reported in Slotkin's (47) study, while RNA content tended to be *elevated.*

A different effect was found in the cerebral cortex where DNA

was markedly elevated in the high dose group and virtually unchanged at the low dose. RNA in the cerebral cortex was increased with both the low and high dose.

Results obtained in this study indicate that the effects of methyl mercury on the developing brain are regionally specific and support the hypothesis that the cellular responses to this organomercurial are dependent upon the maturational stage at which exposure occurs. In the midbrain plus brainstem, a region where major phases of the earliest cellular replication occur, methyl mercury caused a dramatic reduction in DNA: since the amount of DNA per cell is constant, this represents a corresponding loss of cells in this region. In contrast, DNA in the cerebral cortex was unaffected at the lower dose of methyl mercury and was substantially elevated at the higher dose; the stimulation was accompanied by a surge in RNA as well.

Cerebral cortical replication occurs later than in the midbrain plus brainstem and extends well into the period in which methyl mercury exposure was occurring; thus, it can be hypothesized that the stimulation of nucleic acid levels represents a compensatory reaction to the methyl mercury-induced cell loss, a factor which would not operate where replication had already ceased (as in the midbrain plus brainstem). If this is so, then an even more pronounced effect should be seen in the cerebellum, a region which undergoes replication primarily in the postnatal period. Indeed, examination of cerebellar DNA and RNA did indicate stimulation with even greater sensitivity, since in this case elevations were seen at either dose level. These characteristics are distinctly different from those seen with exposure of mature animals to methyl mercury, where effects on protein synthesis appear to be primary; developing rats displayed only small effects on protein content even when nucleic acid levels were markedly abnormal.

It should be noted that enhanced DNA content does not necessarily

imply that methyl mercury-induced damage is completely repaired in brain regions which contain replicating cells at the time of toxic exposure. Morphological damage after developmental exposure to high levels of methyl mercury indicates intrusion of glial elements into damaged brain areas. Furthermore, low levels of methyl mercury are known to stimulate astroglial replication in tissue cultures, a factor which could contribute to a major portion of the elevation in DNA seen in cerebral cortical and cerebellar regions.

In chronic cases atrophy of the brain is observed. Atrophy is most marked in the medial aspect of the occipital lobe, particularity in the calcarine regions. In most cases there is gross atrophy of the cerebellar folia and extensive thinning of the cerebellar gray matter. Pathological changes in the cerebellar cortex were found in all cases. In Minamata Bay exposures they noted that the earlier in life (especially fetal) the more wide spread the pathology, and the later the involvement, the more localized the lesions were.

Mercury accumulation in nerves seems to concentrate in the Purkinje cells. Purkinje cells are rich in -SH groups, therefore their histochemical reactions are greatly reduced after mercury poisoning. This suggests that the large amounts of -SH groups in the Purkinje cells may act as inert sites and offer a neutralizing effect on mercury's action inside the cell, resulting in an apparent higher mercury tolerance. (48,49)

Time versus deposition studies disclosed that mercury was first detected histochemically in the Schwann cell, between 12 and 24 hours after the administration of MeHg or inorganic compounds. (38) After four days mercury was found in the axoplasm. It was also evident that there was a difference in lesions produced by organic and inorganic mercury. $HgCl_2$, produced axonal degeneration, vacuolation and collage of nerve fibers. Occasional myelin destruction could be observed. After MeHg poisoning the myelin sheaths lost their lamination. Extensive axoplasmic

degeneration, axonal collapse and myelin destruction were the most prominent lesions. (50)

Visual tissues are an extension of brain tissues, so it would seem logical to look for visual disturbances as the most frequent result of exposure to mercury.

Clinical studies of epidemic poisonings in Japan and Iraq have reported a disturbance of visual perception as one of the most consistent signs of neurological impairment in humans. (51,52,53,54) Patients typically exhibited a concentric narrowing of the visual fields and reduced visual acuity. Experimental studies of methyl mercury poisoning in neonatal and adult monkeys suggested that impaired scotopic vision, particularly a reduction in visual acuity, is the earliest sign of neurotoxicity and the most sensitive indicator of exposure. (55,56,57)

The neurologic impairment underlying these visual anomalies appears to be central in origin. Lesions of the primary visual cortex have been described in human autopsy material, (58,59) whereas in most cases the retina, optic nerve, and lateral geniculate nucleus were reported to be normal. (59) These cortical lesions were characterized by diffuse neuronal degeneration and cell loss, with a proliferation of glial cells and marked astrocytosis. Similar lesions have been reported in the visual cortex of experimental animals following exposure to methyl mercury. (55,60,61,62)

Clinical signs of neurologic impairment were relatively consistent among the nine methyl mercury-treated animals. All animals exhibited some degree of visual impairment. The visual placing response was weak or absent, whereas vibrissa placing and forelimb placing responses appeared normal in most animals. Unlike control animals, they failed to avoid a visual cliff and demonstrated difficulty in negotiating obstacles when placed in a novel environment. Myoclonic jerking of the hind limbs was observed in four methyl mercury-treated animals. Episodes were

marked by brief periods of rapid involuntary contractions of the hind limb extensor muscles. In one case this localized myoclonic jerking culminated in a generalized motor seizure. Cortical pathology was noticeably different in the four convulsive animals and their results are presented separately.

Aspinous and sparsely-spinous stellate neurons have been shown to form symmetric synapses in the visual cortex of the rat. (63) These neurons contain glutamic acid decarboxylase, (64) the enzyme that synthesizes the neurotransmitter, gamma-aminobutyric acid (GABA). Physiologic and pharmacologic evidence indicates that these intracortical neurons mediate GABAergic inhibition in the neocortex. The functional implication of a relatively selective impairment of GABAergic neurons in the visual cortex that follows methyl mercury poisoning is consistent with the visual abnormalities reported in such cases. (55, 51, 52, 56, 53, 57, 54) Neurons of the visual cortex rely on GABA-mediated inhibition for many of their receptive field properties. The iontophoretic application of GABA antagonists such as bicuculline has been shown to produce a reversible inhibition of both orientation and directional specificity in cortical neurons. (65, 66) A loss of orientation specificity in neurons of the visual cortex, after an impairment of GABAergic neurons, would account for the decreased visual acuity reported in cases of methyl mercury poisoning. A loss of directional specificity may contribute to the concentric narrowing of the visual field, because visual field perimetry requires the detection of a moving stimulus.

The most conspicuous difference noted in the visual cortex of convulsive animals was the spongy appearance of that area and the presence of the perivascular cuffs of reactive astrocyte processes. Similar pathologic changes have been documented in the cerebral cortex in response to prolonged seizure activity produced by the administration of bicuculline. (67) In our study, the extensive damage observed throughout the visual cortex of

convulsive animals probably reflects a seizure-induced pathology that is secondary to methyl mercury impairment of GABAergic interneurons of the cerebral cortex.

The cellular mechanism underlying the preferential degeneration of stellate neurons and symmetric synapses in nonconvulsive methyl mercury-treated animals is not clear, although evidence suggests that an inhibition of mitochondrial respiration is involved. Altered mitochondrial respiration has been demonstrated in synaptosomes isolated from the brains of rats exhibiting clinical signs of methyl mercury poisoning. (68) A reduction in oxygen consumption has been demonstrated in brain slices prepared from methyl mercury poisoned rats (69) and in brain slices prepared from normal rats when methyl mercuric chloride is added to the incubation medium. (70) Ultrastructural changes in neuronal mitochondria, resembling the pathology that follows hypoxic injury, have been reported in rat visual cortex after neonatal administration of methyl mercury at a subclinical dose. (71) The decreased concentration of ATP that follows partial or complete phosphorylation uncoupling, would be expected to result in an inhibition of various synthetic reactions in the developing neuron. Numerous studies have shown that methyl mercury inhibits myelin formation and protein synthesis in the central nervous system, with consequent demyelination, loss of ribosomes, and eventual degeneration of neurons. (72)

The GABAergic stellate neurons of the visual cortex appear to have a higher metabolic rate than other cortical neurons. The cytoplasm of the stellate cell body has been characterized by a greater number of mitochondria and densely packed ribosomes. (64, 72) Axon terminals forming symmetric synapses contain more than twice the number of mitochondrial profiles than terminals forming asymmetric synapses. (73) These morphologic characteristics suggest that GABAergic neurons are more dependent on aerobic metabolism than other classes of cortical neuron. Thus, GABAergic stellate neurons of the cerebral cortex

could be expected to have a lower threshold to a methyl mercury-mediated inhibition of mitochondrial respiration.

The results of the following studies demonstrate a selective degeneration of aspinous or sparsely-spinous stellate neurons and their symmetric synapses in the visual cortex after neonatal administration of methyl mercury. Neuronal degeneration would account for the visual impairment observed in methyl mercury-poisoned individuals. However, reduced visual acuity and concentric narrowing of the visual field have been shown to be reversible in some cases, (52, 56) implying that neuronal degeneration is not a prerequisite. Further research is needed to document methyl mercury-induced changes in neuronal metabolism, particularly in GABAergic neurons of the cerebral cortex, which precedes neurological impairment and cortical pathology. (74)

Many researchers have overlapped their work on vision and DNA. Since DNA single strand breaks seem to be one of the primary toxic responses to heavy metals, it seems likely that other reactions could be occurring within the DNA. Upon exploration it was noted that other avenues were worthy of examination. Interesting that the paths of toxicity crisscross so often.

The result of Choi's (94) study suggests that methyl mercury chloride has more deleterious effects on DNA synthesis (as found from the thymidine incorporation test) than mercuric chloride. This shows that the relative reduction of inorganic mercury transport across the blood-brain barrier is only part of the story in mercury toxicity. Methyl mercury is clearly more capable of producing brain damage.

Protein synthesis was rapidly inhibited as found in Ally's work, (75) and in yeasts, the inhibition of protein synthesis on cytoplasmic ribosomes stops nuclear DNA synthesis. (76)

An imbalance in adenylate pools (ATP and ADP) can result in an increased frequency of mutations as DNA synthesis becomes more prone to error. (77)

Since RNA is the chief "machinery" for protein synthesis, it is believed that the change in protein synthesizing activity is reflected in the effect of mercury or RNA.

Campagnoni (77) described a special form of RNA in the rat nervous system. This RNA represents about 10 percent of the total RNA. It is of interest to note that this form is almost identical to RNA in spinal ganglia formed after mercury intoxication. This RNA detected after mercury intoxication may not be "abnormal" RNA, but a normal RNA existing in abnormal quantities. This RNA is a minor component of neuronal RNA, but its nature and function are unknown. This special form of RNA may be responsible for the production of metallothionine which in turn provides protection to these neurons from mercury toxicity. This may explain the manifestation of recovery and tolerance relative to intoxication.

DNA repair were generally rapidly rejoined (79,80) but those caused by $HgCl_2$ were not readily reversed since $HgCl_2$ has been shown to inhibit the repair of single strand breaks induced by X-rays (6). Since irreversible DNA strand breaks are produced by $HgCl_2$ at very low concentrations, that paralleled the cellular level of mercury, it is likely that cell death may result from these lesions (6).

$HgCl_2$, however, produces DNA-DNA crosslinks which with time progressively increased in extent. These crosslinks are probably due to the ability of $HgCl_2$ to interact with the bases directly (6) while the single strand breaks may result from the production of oxygen radicals by $HgCl_2$ and also by its interaction with DNA bases (9). Such radicals have been postulated to mediate the X-ray-induced DNA damage of cells. These studies illustrate basic

differences in the way metal compounds interact with DNA in intact cells. The DNA lesions produced by $HgCl_2$ must be considered in a different way from the DNA lesions induced by other agents. For example the single strand breaks induced by nickel compounds and $CaCrO_4$ are repaired (20, 21) while the strand breaks induced with $HgCl_2$ are not readily repaired (6). Hg is an extremely reactive metal that has a high affinity for sulfhydryl groups such as those present in proteins, but the nonsulfhydryl binding sites contained in DNA bases have a considerably lower affinity. However, due to the critical function of DNA in the cell, its concentration and the fact that repair enzymes are inhibited by $HgCl_2$ and cannot mend the DNA lesions, cell death may result directly from these genetic effects. Although it is difficult to precisely extrapolate concentrations of $HgCl_2$ that produce cell membrane injury to the levels that cause DNA lesions because of differences in culture media, lack of Hg uptake measurements in other systems, etc., the levels of Hg^{++} that produce DNA lesions are at least as low if not lower than those that injure the cell membrane (17). Since Hg^{++} has X-ray like effects in being able to produce oxygen radicals in cells (9), and deplete cellular reduced glutathione levels (18) the DNA must be considered a target site of its toxic action. If induction of DNA lesions and active repair of these lesions are important for mutagenicity or carcinogenicity of a chemical agent, then Hg may be expected to have weak mutagenic activity at low concentrations but at higher concentrations where DNA repair activity was inhibited there should be even less mutagenic activity. Additionally, the DNA lesions induced by $HgCl_2$ may result in miscoding during DNA replication; however $HgCl_2$, has been shown to inhibit cell growth specifically in S-phase (15) and therefore miscoding during DNA replication must occur at concentrations of $HgCl_2$ that allow this process to proceed in order to achieve a mutagenic response in a surviving cell. These mechanistic findings may help explain the low mutagenic/ carcinogenic activity displayed by $HgCl_2$ in a number of experimental systems (19).

Another biochemical injury that mercury/methyl mercury does to the body is to damage the cell membrane. It is hard to identify which mercury injury is the most damaging, because there are so many critical areas involved, but certainly cell membrane chemistry deserves a lot of attention. All essential chemical reactions take place within the confines of a cell. Most materials for those reactions (oxygen, carbohydrates, fats, vitamins, minerals, enzymes, hormones) must be transported into the cell prior to reaction time. Waste products must be removed to make room for new reactants and to prevent contamination of ongoing reactions. Who controls all this traffic? The cell membrane. Cell membranes are primarily composed of protein and fat in near equal amounts. Complex mechanisms within the cell membrane determine which nutrients come in and what products get out of the cell. Damage to the cell membrane can alter this selective process and multiple malfunctions can result.

The fact that biological membranes are generally rich in sulfhydryl (-SH) groups (48) may explain the preferential binding of mercury to the membranous structures.

Damage to the cell membrane by mercury is probably due to protein cross-linking within the membrane resulting in an abnormal strain in the membrane structures which leads impairment of membrane functions as well as an increase in permeability ("leaky membrane" phenomenon). (81) Specifically with the blood-brain barrier by mercury, the damage is probably due to alterations in the endothelial and glial membranes. (82)

Brown and Yoshida (82) proposed that organic mercury mainly altered cell membrane structures and appeared to interfere with protein production in nerve cells.

Shrivastav (83) found that 25 μM (5 ppm) MeHg decreased the conductance of axons. Concentrations as low as 0.5 μM (0.1 ppm) depolarized the nerve membrane significantly. These studies

clearly indicate that the effects of mercury on the biological membranes would contribute significantly to malfunctions of the nervous system after exposure to mercury.

The importance of the cell membrane's ability to absorb oxygen is challenged by the red cell's ability to provide that oxygen. Indeed methyl mercury can interfere with this mechanism.

Mercury concentrations in RBC are considered to be the most reliable index available of exposure to MeHg. (85)

Erythrocytes incorporate mercury vapor at high rates and the present conception is that atomic mercury becomes oxidized in the cell interior. This prevents it from being able to permeate outwards, through the membrane due to its charge. (86,87)

In incubations with a low concentration of red blood cells, there was an increased rate of uptake of mercury into the tissues. (88) This would suggest that as the hematocrit drops, the patient might experience even less oxygen transport than the low figures would indicate.

The rate of mercury uptake into red blood cells is considerably reduced in the presence of 2 μM ethanol. (89) Peroxidase is required for the oxidation of both mercury vapor and ethanol, so an increase in one shifts the oxidative capacity toward the other.

Clausing found more than 95 percent of the methyl mercury in blood bound to red blood cells. (90) This high erythrocyte concentration places considerable influence on the rate of back diffusion of methyl mercury from the red cells into the plasma. In humans the methyl mercury that diffuses back from the red blood cells to the plasma is bound to glutathione (GSH). (91)

In describing the chemobiokinetics of methyl mercury, Doi and Tagawa (92) suggest that the distribution between methyl mercury

in red blood cell may be the result of difference in affinities for hemoglobin and glutathione. They conclude that the decisive factor for this distribution is hemoglobin, with glutathione playing a secondary role.

Oxygen availability has also been linked to seizure activity. Methyl mercury disturbs this mechanism and may in part explain why many epileptic patients stop seizure activity after dental mercury filling removal.

An impairment of GABAergic interneurons in the neocortex has been implicated in the genesis of seizure activity. Inhibition of GABA synthesis or blockage of its postsynaptic action in the cerebral cortex reduce convulsive thresholds. (93) Morphological studies have demonstrated a preferential loss of GABAergic synapses in the motor cortex at sites of experimentally induced epileptic foci. (74, 94) In the developing motor cortex, a selective degeneration of axon terminals forming symmetric synapses has been reported following hypoxia. (73) In this study seizure activity exhibited by some methyl mercury-treated rats suggested that impairment of GABAergic neurons might have occurred in motor regions of the cerebral cortex.

How much is too much? There are many articles addressing dose related toxic reactions, but it is difficult to correlate these articles due to different measurement systems. Some researchers deal in micrograms per gram, some in micromolar amounts. In presenting the following material I will try to convert everything to one of two systems, sometimes both. One is micrograms per gram which is also called parts per million and abbreviated "ppm". The other is micromolar and is abbreviated μM.

Some of this material is used elsewhere, but I felt it is worth duplicating because of the significance of the implications that can be drawn from correlating amounts available and amounts required for toxicity.

$HgCl_2$ is extremely cytotoxic to Chinese hamster ovary (CHO) cells in culture since a 1-h exposure to a 75 μM (15 ppm) concentration of this compound reduced cell plating efficiency to 0 and cell growth was completely inhibited at 7.5 μM.(1.5 ppm). (16) The level of $HgCl_2$ toxicity depended upon the culture incubation medium and has previously been shown to be inversely proportional to the extracellular concentration of metal chelating amino acids such as cysteine. When cells were exposed to $HgCl_2$ there was a rapid and pronounced induction of single strand breaks in the DNA at time intervals and concentrations that paralleled the cellular toxicity. The DNA damage was shown by a variety of techniques to be true single strand breaks and not like alkaline sensitive sites or double strand breaks. Costa and Canton believe that the DNA damage caused by $HgCl_2$ leads to cell death because the DNA single strand breaks are not readily repaired. These irreversible interactions of $HgCl_2$ with DNA may be responsible for its cytotoxic action in cells.

It is interesting to note that cysteine is mentioned as a heavy metal chelator. Some people have used it for this purpose with negative clinical results. The reason for failure may be a fact that was brought out in Section II dealing with gut flora. Cysteine in the stomach and gastrointestinal tract can be converted to methyl mercury cysteine which is 100 percent reabsorbed in the caecum portion of the large bowel. It is then transported via the hepatic portal system back to the duodenum where it can traverse the gastrointestinal tract again.

Single strand breaks of DNA were discussed elsewhere on mechanisms of toxicity, and the concentrations necessary to produce single strand breaks are only reviewed here.

Less DNA damage appeared to be required to produce the same level of cell killing with $HgCl_2$ as compared with x-rays. The observations that cells are unable to repair the single strand breaks induced with $HgCl_2$, and that low concentrations of $HgCl_2$ 10 μM

(2 ppm) inhibit the rejoining of single strand breaks induced by X-rays suggest that $HgCl_2$ may act on DNA homeostasis by inhibiting DNA processes. Thus, in contrast to , DNA damage induced by $HgCl_2$ was not readily repaired and may represent an irreversible injury that leads to cell death.

The cell membrane has been mentioned as a critical structure for biochemical integrity, and mercury concentration is confronted in the following article.

Injury to the cell membrane has been purported to be the basis of Hg^{++} cytotoxic action. (16) Although it is difficult to precisely extrapolate concentrations of $HgCl_2$ that produce cell membrane injury to the levels that cause DNA lesions because of differences in culture media, lack of Hg uptake measurements in other systems, etc., the levels Hg^{++} of that produce DNA lesions are at least as low if not lower than those that injure the cell membrane. (17)

Nerve tissue is reported to be highly sensitive to mercury, yet these references show tissues sensitive at both higher and lower levels.

It would be interesting to know at what phase of cell division death occurs and also to know how other metals compare. Dental materials to be investigated include cadmium, mercury, cobalt, copper and nickel.

Heavy metals were found to slow or stop cell growth at very low concentrations (1 to 60 μM or 0.2 to 12 ppm). All interferences appeared to occur during the S-phase (synthesis phase) of mitosis. Cadmium was found to be the most toxic, followed in descending order by mercury, cobalt, copper, nickel and lead. The S-phase blockage produced by the metals was consistent with their genotoxic or carcinogenic activity since such activity indicates a selective interaction with DNA metabolism. (15) Other

researchers position brain damage at even lower concentrations.

Mercury ions penetrate the blood-brain barrier and enter the nerve cells from the blood stream. (32) Steinwell (33) showed an impairment of the blood-brain barrier within hours of administration as either mercuric chloride or methyl mercury. Chang has shown that minute amounts (less than 1 ppm) are capable of impairing the blood brain system leading to extravasation of normally barred plasma solutes. (34)

Later, in 1973, Chang (41) using enzyme histochemistry demonstrated a decrease in activity of succinic dehydrogenase, ATPase, and alkaline phosphatase in the rat brain following mercury intoxification (1 mg mercury/kg/or 1 ppm body weight for four weeks). There was a simultaneous increase in acid phosphatase indicating an accumulation of lysosomes in the nervous system. The number of neurosomal lysosomes may be used as a quantitative indicator for mercury toxicity within the nervous system. The decrease in these three enzymes is believed to indicate damage on the mitochondria, cell membrane, and blood-brain barrier by mercury.

Several physiological responses to cell death were observed in Choi's work. (95) He found that astrocyte culture cells were totally destroyed when exposed to 0.01 nM (nanomolar) (0.002 ppm or 2 ppb) methyl mercury chloride for one hour. Nakazawa (96) found complete inhibition of cell multiplication by methyl mercury chloride at 4μM (0.8 ppm), compared to the more concentrated 25μM (5 ppm) of mercuric chloride required. To achieve 50 percent inhibition of radioactive thymidine incorporation into cells 5 μM (1 ppm) of mercuric chloride was required, where only 1μM (0.2 ppm) methyl mercury chloride was needed. Prasad (97) found that rat glioma cells were destroyed at 1μM (0.2 ppm) with mercuric chloride, and 0.19μM (0.04 ppm) for methyl mercury chloride. This takes on even more significance when we find that most of the mercury in the human body is

localized in glial cells. (98)

Poisoning with methyl mercury is most closely associated with damage to the CNS. The nervous system of the fetus and neonate appear to be particularly sensitive to methyl mercury producing frank morphological damage at high dosages (2.5 ppm) and subtle biochemical and behavioral disturbances at levels which cause classical teratologic effects. A considerable body of evidence shows that methyl mercury inhibits macromolecule synthesis in mature brain tissue which contributes to alterations in neural function.

Vimy and Lorscheider (3) calculated a daily dose of 20 to 29 micrograms per day from fillings they studied. Dag Brune (99) calculates around 3 micrograms of mercury per square centimeter of filling per day as a daily dose from amalgam fillings. Hamilton (100) estimates the average human's mercury intake from food and drink to amount to around 20 micrograms daily.

How much of this exposure accumulates in body tissues? Freden (101) found as much as 380 ppm mercury in gingival tissue. Gingival tissue is gum tissue immediately adjacent to fillings and also immediately adjacent to the bone that supports the teeth. Periodontal disease is the term applied to about 85 percent of the population who have diseased bone and gums surrounding the teeth. Preliminary studies performed at the University of Colorado at Colorado Springs determined that 0.4 ppm (2μM) concentration of mercury would kill bone cells in culture. If it is possible that the 380 ppm can share 0.4 ppm with the immediately underlying bone, there could emerge a whole new concept as to the etiology of the massive amount of periodontal disease experienced in this country.

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