ALZHEIMER'S DISEASE, DENTAL AMALGAM AND MERCURY

STANLEY R. SAXE, D.M.D.; MERLE W. WEKSTEIN, M.P.A.; RICHARD J. KRYSCIO, PH.D.; ROBERT G. HENRY, D.M.D.; CHARLES R. CORNETT, PH.D.; DAVID A. SNOWDON, PH.D.; FORD T. GRANT, D.M.D.; FREDERICK A. SCHMITT, PH.D.; SARA JEAN DONEGAN, S.S.N.D., D.M.D.; DAVID R. WEKSTEIN, PH.D.; WILLIAM D. EHMANN, PH.D.; WILLIAM R. MARKESBERY, M.D.

ABSTRACT

Background. Mercury, or Hg, is a neurotoxin that has been speculated to play a role in the pathogenesis of Alzheimer's disease, or AD. Dental amalgam releases low levels of Hg vapor and is a potential source of Hg for a large segment of the adult population.

Methods. The authors studied 68 subjects with AD and 33 control subjects without AD to determine Hg levels in multiple brain regions at autopsy and to ascertain the subjects' dental amalgam status and history. The subjects were from central Kentucky and Elm Grove, Wis. The authors conducted dental amalgam assessments during the lives of the majority of subjects and in some subjects at the time of autopsy only. The authors also determined three dental amalgam index scores—Event (placement, repair or removal of amalgam), Location and Time In Mouth—in addition to the numbers of and surface area of occlusal amalgam restorations. The authors determined Hg levels in multiple brain regions and performed full neuropathologic evaluations to confirm the normal status of the brain or the presence of AD.

Results. The authors found no significant association of AD with the number, surface area or history of having dental amalgam restorations. They also found no statistically significant differences in brain Hg level between subjects with AD and control subjects.

Conclusions. Hg in dental amalgam restorations does not appear to be a neurotoxic factor in the pathogenesis of AD. The authors found that brain Hg levels are not associated with dental amalgam, either from existing amalgam restorations or according to subjects' dental amalgam restoration history.

Clinical Implications. Dental amalgam restorations, regardless of number, occlusal surface area or time, do not relate to brain Hg levels.

Alzheimer's disease, or AD, is a major health problem in late adult life and affects up to 4 million people in this country.[1] The etiology and pathogenesis of this disease are not known. Levels of numerous trace elements, including aluminum, iron, zinc and mercury, or Hg, have been reported to be imbalanced in patients with AD,[2] and it has been speculated that this imbalance may play a role in the disease. Hg levels have been reported to be elevated in some brain regions in patients with AD and in the microsomal subfraction in several studies of patients with AD and age-matched control subjects.[3-5] Hg is a neurotoxin, with metallic Hg causing erethism[6,7] and methyl mercury causing Minamata disease.[8] Toxicity can occur from inhaling Hg vapors, a potential source of which is dental amalgam.[9-13]

Dental amalgam is used extensively as a tooth restoration material; 93.8 percent of dentate adults have treated or nontreated coronal dental caries.[14] Hg constitutes approximately 50 percent of dental amalgam.[15] When abraded, as in the process of chewing, amalgam surfaces release very low levels of Hg vapor.[16-20] Public concern about dental amalgam's possible toxic effects has been the result of scientific literature reports on Hg vapor and cell function[21]; on correlations between dental amalgam and reported symptoms[22,23]; and Hg levels in blood, urine and organs, including the brain.[24-32] It also has been stimulated by media reports suggesting adverse health effects.[33]

We conducted an investigation to determine the relationship among brain Hg levels, AD and dental amalgam exposure.

CRITERIA FOR DENTAL AMALGAM INDEX SCORES.

CRITERIA		SCORE
EVENT INDEX SCORES		
Event		
Initial placement		3
Repair, replacement, crown preparation		3
LOCATION INDEX SCORES		
Tooth Surface	**Premolar**	**Molar**
Less than one-half occlusal	1	1.3
Equal to or more than one-half occlusal but not mesial or distal	2	2.5
Distal occlusal or mesial occlusal	3	3.7
Mesial occlusal distal	4	4.9
Mesial occlusal distal, buccal or lingual extension	5	5.9
Mesial occlusal distal, buccal plus lingual extension	6	6.9
Mesial or distal or bucal or lingual—a single nonocclusal surface	1	1
TIME IN MOUTH INDEX SCORES (IN YEARS)		
Occlusal Surface		
Less than five		2
Five to nine		4
Ten to 19		6
Twenty or more		8
Nonocclusal Surface		1
OPPOSING DENTITION SCORES		
Opposing dentition at time of assessment		2
Opposing dentition within past five years, but not at time of assessment		1
No opposing dentition within past five years		0

METHODS

Subjects. In this study, we compared Hg levels in the autopsied brains of 68 subjects with AD, as well as of 33 control subjects without AD whom we thoroughly studied while they were living. The subjects were from central Kentucky and Elm Grove, Wis. We also compared the subjects' dental amalgam status and histories.

AD subjects from central Kentucky were from the University of Kentucky's Alzheimer's Disease Research Center, or ADRC. All central Kentucky subjects with AD had a clinical diagnosis of probable AD using the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association Work Group criteria.[34] Control subjects from central Kentucky were participants in the ADRC research volunteer brain donation program who died and were autopsied. These volunteers were recruited from a pool of 4,500 community residents. We determined that all control subjects were cognitively and neurologically normal by performing annual cognitive testing; we also ascertained that they had no history of substance abuse or psychiatric illness.

The test and control subjects from Wisconsin were members of the School Sisters of Notre Dame, or SSND, religious congregation who were participants in the Nun Study, an epidemiologic study of aging and AD.[35] The analysis for the present study was restricted to Roman Catholic sisters who resided in a retirement and health care facility in Elm Grove, Wis.[36] Details of the SSND evaluation have been published.[37]

We included in this study only subjects who met the strict histopathologic criteria for AD[38,39] or our control criteria and who had no significant confounding disorders such as strokes, cerebral hypoxia or other disorders that could cause dementia.

Dental amalgam assess-

ments. Evaluation included determining the number and location of existing teeth plus assessing existing amalgam restorations and past amalgam experience. We determined the number and surface area of existing occlusal amalgam restorations by using an intraoral video camera (Oralcam, Oral Safe Corp.). From the recorded videotaped image's surface area, we calculated measurements in square millimeters with a Kontron Image Analyzer computing system (Kontron Electronics Inc.).

We determined the dental care history of each subject's 32 teeth, using the history obtained from the subjects and/or the written dental records and oral radiographs obtained from their dentists. We then recorded dental amalgam placement, repair or removal for each tooth, as well as the dates on which teeth were removed.

We established three dental amalgam indexes: Event, Location and Time in Mouth. We gave an Event Index score of 3 to each tooth for every placement, repair, removal or sectioning of an amalgam restoration (Box, "Criteria for Dental Amalgam Index Scores"), because these events have been recognized as realizing Hg in amounts greater than the Hg released from abrasion. We assigned Location Index scores based on the surface of each tooth containing an amalgam restoration. We weighted occlusal surfaces greater than nonocclusal surfaces, and we weighted molar occlusal dental amalgam restorations greater than those for premolars, as molars have larger surface areas. In addition, we gave an opposing dentition score if an occlusal amalgam restoration came into contact with an opposing tooth. We determined Time in Mouth Index scores by the maximum length of time any one amalgam restoration existed on a tooth surface (Box, "Criteria for Dental Amalgam Index Scores"). We added up scores for all teeth to yield the three amalgam index scores for each subject.

TABLE 1

SUBJECTS' DEMOGRAPHIC CHARACTERISTICS.

DEMOGRAPHIC CHARACTERISTICS	SUBJECTS WITH ALZHEIMER'S DISEASE (N = 68)	CONTROL SUBJECTS (N = 33)	*P*-VALUE
Number of Subjects From Central Kentucky	n = 58	n = 21	Not Applicable
Number of Subjects From Elm Grove, Wis.	n = 10	n = 12	Not Applicable
Number of Men (percentage)	n = 31 (45.6)	n = 6 (18.2)	.007
Mean Age at Death in Years (SE*)	80.9 (± 1.0)	84.6 (± 1.2)	.03
Mean Years of Education (SE)†	13.9 (± 0.5)	15.3 (± 0.5)	.10

* SE: Standard error.
† Data are unavailable for nine subjects with Alzheimer's disease.

We elicited information regarding nondental Hg exposure from the environment, diet (for example, fish) and medications in detail from subjects or from family members of subjects with AD.

Autopsy method. In subjects from central Kentucky, we removed the brain using standard procedures and then removed specimens from the left cerebral hemisphere for trace elements analysis and histopathologic studies. We took cerebral cortex specimens from the intact left cerebral hemisphere so that landmarks were present to ensure precise localization. We removed specimens from the olfactory bulb, tract and nucleus, frontal pole, middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, and hippocampus and parahippocampal gyrus. We pressed the specimens gently between hardened ashless filter paper to remove excess blood and other fluids and then placed them in virgin polyethylene bags and froze them at -70 C.

We fixed the right cerebral hemisphere, brainstem and remainder of the cerebellum in 4 percent formaldehyde for 10 days after which time we sectioned them. We evaluated coronal sections of the right cerebral hemisphere and serial, transverse sections of the brainstem and cerebellum for any macroscopic abnormalities. For diagnosis, we took sections from the hippocampus, amygdala, basal ganglia, thalamus, midbrain, pons, medulla and cerebellum of both the right and left hemispheres.

We used the same protocol to remove brain specimens for trace element analysis from the left cerebral hemispheres of the subjects from Wisconsin.

TABLE 2

SUBJECTS' DENTAL STATUS AT TIME OF BRAIN REMOVAL.

DENTAL STATUS	SUBJECTS WITH ALZHEIMER'S DISEASE (N = 68)	CONTROL SUBJECTS (N = 33)	*P*-VALUE
Number Dentate (percentage)	50 (73.0)	24 (72.7)	.43
Mean Number of Anterior Teeth (SE*)	6.7 (0.6)	7.7 (0.9)	.35
Mean Number of Premolars (SE)	3.5 (0.4)	3.9 (0.6)	.59
Mean Number of Molars (SE)	3.1 (0.4)	2.7 (0.5)	.58
Mean Number of Amalgam Restorations in Dentate Subjects (SE)	3.7 (0.5)	4.6 (0.8)	.29
Mean Surface Area of Occlusal Amalgam Restorations in Square Millimeters (SE)†	86.9 (14.3)	99.3 (22.3)	.63

* SE: Standard error.
† Data are missing for two dentate subjects with Alzheimer's disease and four dentate control subjects.

Mercury analysis. We used instrumental neutron activation analysis, or INAA, for 95 percent of the tissue analyses in this study.[40,41] The INAA detection limit in this study was 2.8 nanograms of Hg per gram of tissue, wet weight basis. This lower detection limit represents an improvement over the 6.0 ng Hg/g tissue-detection limit found in earlier studies.[3]

Approximately 5 percent of the samples yielded left-censored (below the minimum detectable amount by INAA) values for Hg. For tissue-Hg levels below 2.8 ng Hg/g, we developed a simple radiochemical neutron activation analysis, or RNAA, procedure based on the precipitation of ^{203}Hg as mercury chloride, or $^{203}Hg_2Cl_2$, for the recovery of these values. This protocol was used to lower the detection limit to 1.6 ng Hg/g. Details of the RNAA procedure were published.[42] To ensure accuracy and test the validity of the procedures, we analyzed several National Institute of Standards and Technology standards using INAA and RNAA. Our results were in agreement with recommended or certified values.[43]

Neuropathologic diagnosis. We processed all specimens in paraffin blocks in the standard manner. Sections were stained with hematoxylin and eosin, a modified Bielschowsky's method, 10 D-5 (Athena Neuroscience) and ubiquitin immunohistochemistry.

We quantified senile plaques, or SPs, and neurofibrillary tangles, or NFTs, in the middle frontal gyrus, middle temporal gyrus, inferior parietal lobule, occipital area 18, hippocampal CA1 and subiculum, and the parahippocampal gyrus. We counted SPs in the Bielschowsky's-stained sections using a ×10 microscope objective (2.35 mm^2) in the five most involved fields of each brain region. We counted NFTs using the Bielschowsky's-stained sections and a ×20 microscope objective (0.586 mm^2) in the five most involved fields of each section. In this study, we required subjects with AD to have abundant SPs in the frontal, temporal and parietal lobes (that is, a mean greater than or equal to 36 SP per 2.35 mm^2 field in any lobe[38]) and NFTs in at least one neocortical lobe and in the hippocampus, amygdala and entorhinal cortex.

When we conducted neuropathologic evaluations of control subjects, we found only age-related gross and microscopic brain alterations.

Statistical analysis. We tested for differences in demographic and dental assessments between the AD and control groups by independent Student's *t*-statistics for interval level measurements and by χ^2 tests for nominal level measurements. We recorded Hg measurements by brain region. When no tissue was available to determine Hg levels, we recorded a missing response (15.2 percent of the possible Hg level determinations). When we encountered a lower detection limit and found that it was not augmented by the RNAA procedure, we used the lower detection value in the analysis (4.6 percent of the samples with adequate tissue). Because Hg values in different brain regions were highly correlated, we created a summary measurement representing the neocortical load by averaging the Hg measurements from the frontal pole, superior and middle temporal gyri and inferior parietal lobule, provided that we had

TABLE 3

AMALGAM INDEX SCORES OF SUBJECTS' DENTAL EXPERIENCE.

DENTAL HISTORY AND AMALGAM INDEX SCORES	ALL SUBJECTS			DENTAL HISTORY MORE THAN 20 YEARS		
	Subjects With Alzheimer's Disease (n = 68)	Control Subjects (n = 33)	*P*-Value	Subjects With Alzheimer's Disease (n = 35)	Control Subjects (n = 23)	*P*-Value
Mean Dental History in Years (SE*)	22.5 (1.7)	25.8 (3.0)	.07	33.4 (1.9)	35.3 (3.4)	.60
Event Index Score (SE)	34.8 (4.9)	47.5 (8.5)	.17	44.0 (8.6)	42.3 (10.8)	.90
Location Index Score (SE)	23.1 (4.1)	19.4 (2.3)	.40	21.3 (3.9)	19.6 (5.0)	.79
Time in Mouth Index Score (SE)	25.8 (3.5)	33.2 (6.3)	.27	31.4 (6.0)	29.7 (8.1)	.86
Opposing Dentition Score (SE)	3.8 (0.7)	4.5 (1.2)	.57	3.8 (1.1)	3.1 (1.3)	.69

* SE: Standard error.

Hg values for at least two of these brain regions.

Within each brain region and neocortical load, we tested for an AD vs. control comparison of mean Hg values by independent Student's *t*-statistics. We determined statistical significance for these comparisons at the $P < .05$ level after adjusting the *P*-values for unequal group variances using Satterthwhaite's method when the F ratio for comparing group variances was significant at the $P < .01$ level. We used an analysis of covariance to compare mean Hg values after adjusting the group means for covariates consisting of the demographic and dental measurements. Because there was considerable skewness in the Hg measurements, we log-transformed these measurements before we conducted correlation analyses. We determined the association between the logarithm of Hg values and those of dental assessments by Pearson product moment correlation for each brain region of interest. To guard against the type 1 error (or to avoid rejecting the null hypothesis of zero correlation when the fact in the hypothesis is true) when examining many correlation coefficients, we determined statistical significance was at the $P = .01$ level.

Within each brain region and neocortical load, we compared mean mercury values of control subjects and subjects with Alzheimer's disease.

We based multivariate analyses on a series of stepwise regression models with separate regression models for each brain region and neocortical load. In each regression model, the list of potential predictor variables were the demographic and dental assessments, including group status (control vs. AD), and the interactions between each demographic measurement and dental assessment. We fit two regression sets. One was for dentate subjects only. The other was for all subjects, both dentate and edentulous, but without surface area and number of amalgam restorations as possible predictors. We present results for the latter set because the results are similar in both sets. We set statistical significance to enter the regression model at $P < .15$, while we set statistical significance to remain in the regression model at $P < .05$.

RESULTS

Demographic characteristics of subjects are provided in Table 1. The assessment and brain Hg analysis between the subjects in central Kentucky and the subjects in Wisconsin showed no difference, allowing us to combine subjects into single AD and control groups. There were significantly more men in the AD

TABLE 4

MEAN LEVELS OF MERCURY IN NANOGRAMS PER GRAM, WET WEIGHT, FOR SUBJECTS BY BRAIN REGION.

BRAIN REGION	SUBJECTS WITH ALZHEIMER'S DISEASE, NANOGRAM PER GRAM (SE,* SAMPLE SIZE)	CONTROL SUBJECTS, NANOGRAM PER GRAM (SE, SAMPLE SIZE)	*P*- VALUE
Frontal pole†	61.1 (28.9, 68)	51.4 (17.0, 33)	.77
Inferior parietal lobule	39.2 (14.0, 64)	34.9 (11.0, 30)	.81
Superior and middle temporal gyrus	40.1 (12.9, 67)	39.4 (12.2, 33)	.97
Neocortical load	47.2 (14.7, 68)	44.3 (13.3, 33)	.89
Hippocampus and parahippocampal gyrus	20.3 (5.2, 66)	30.1 (14.7, 31)	.54
Olfactory‡	41.7 (11.5, 57)	88.9 (31.1, 24)	.16

* SE: Standard error.
† Includes data for middle frontal gyrus (area 9).
‡ Includes olfactory bulb, tract and nucleus.

group (45.6 percent) than in the control group (18.2 percent) ($P = .007$). Subjects with AD died at an earlier mean age than did control subjects (80.9 years ± 1.0 standard error, or SE, vs. 84.6 years ± 1.2 SE, $P = .03$). We found no significant difference in dental status at time of brain removal between subjects in the AD group and subjects in the control group (Table 2). We also found that no significant difference in dental amalgam index scores existed between subjects in the AD and control groups, based either on comparison of all subjects or on subjects having dental histories dating back more than 20 years (Table 3).

We found no significant difference in mean Hg values between the two subject groups in any brain region (Table 4). We conducted an analysis of covariance (covariates being all demographic and dental measurements and scores) for each brain region and found no significance. This indicates that group differences are not the reason for the lack of significance. One explanation for lack of significance could be the high degree of skewness associated with the Hg levels. This is illustrated in the histograms of neocortical load Hg values (Figure). Our analysis of nondental exposure to Hg revealed no unusual exposure in any subject, thus eliminating these nondental factors as possible explanation for skewness. A reanalysis of the data using the logarithm of the Hg readings as the dependent variable produced a significant difference in the olfactory region in which the geometric mean Hg level was significantly higher in control subjects than it was in subjects with AD (30.9 vs. 14.0 ng/g tissue wet weight, respectively, $P = .03$).

Using Pearson product moment correlation, we showed that two dental assessments significantly correlated with the logarithm of the Hg values. In the control group, we found a significant correlation between the Event Index score and the logarithm of the Hg values in the frontal pole ($r = .46$, $P < .006$, n = 33) and the olfactory region ($r = .52$, $P < .009$, n = 24), and between Time in Mouth Index score and the logarithm of the Hg values in the olfactory region ($r = .52$, $P = .009$, n = 24). Among AD subjects, years of education was correlated with the logarithm of Hg values in the temporal region ($r = .34$, $P < .009$, n = 58) and in the olfactory region ($r = .37$, $P < .009$, n = 49).

Stepwise regressions showed that years of education was the only significant predictor of the logarithm of Hg in neocortical load ($P = .008$) and three regions of the brain: the frontal pole ($P = .01$), inferior parietal lobule ($P = .01$) and hippocampus ($P = .03$). Years of education explained, at most, 7.5 percent of the total variation in the logarithm of Hg values in each of these brain areas. In the temporal lobe, years of education

(P = .007) and, in the AD group, being a woman (P = .04) explained 14 percent of the total variation in the logarithm of Hg. Years of education (P = .01), Time in Mouth score (P = .01) and the number of premolars in the AD group (P = .02) explained 20 percent of the total variation in the logarithm of Hg values in the olfactory region.

We computed the residuals, which are the differences between the observed and predicted values of the logarithm of Hg, for each of the subset models. We then plotted these residuals against each of the independent variables. These plots showed that the best subset models were adequate fits to the data. Except in the olfactory region, none of the dental measurements explained a significant proportion of the total variation in the logarithm of Hg values in any brain region.

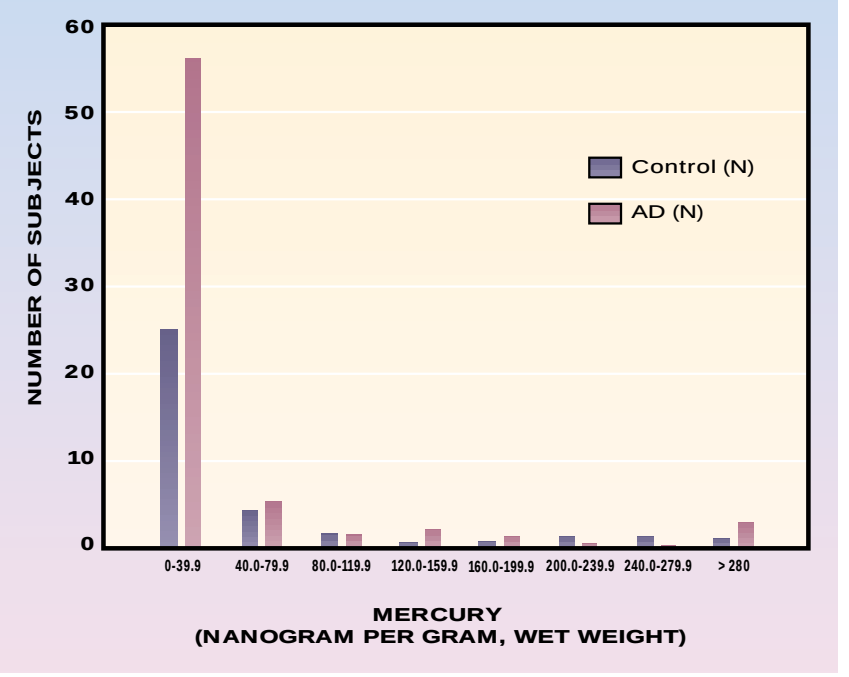

Figure. Histogram of mercury levels in neocortical load for the control and Alzheimer's disease, or AD, groups. Measurements in nanograms per gram, wet weight, greater than 200 are 236, 248 and 319 in the control group and 384, 622 and 698 in the AD group.

DISCUSSION

We found no significant association of AD with the number, surface area or duration of dental amalgam restorations. We did not observe any significant difference in brain Hg levels between subjects with AD and control subjects. Thus, this is the first thorough clinical pathological correlative study in humans to show that Hg in dental amalgam restorations does not appear to be a neurotoxic factor in the pathogenesis of AD.

We designed this study to address the limitations of earlier studies, particularly the lack of information about past dental amalgam restoration experience, documented cognitive and neuropathologic status of controls, and environmental, dietary and occupational exposure to Hg.

> We found no significant association of Alzheimer's disease with the number, surface area or duration of dental amalgam restorations.

Some of the strengths of our study include the large number of subjects; the lower levels of Hg detection[3]; the use of the RNAA procedure that yielded detection levels to 1.6 ng Hg/g[43]; the recruitment of volunteer living subjects who had access to their health and dental records and underwent yearly cognitive testing; the fact that subjects were from two groups of volunteers from two geographical regions; the fact that we could gather information from living subjects by directly questioning control subjects and family members of subjects with AD; and that the existence of and access to dental records provided complete dental histories for subjects.

The fact that Hg vapor is released from dental amalgam restoration surfaces is no longer questioned.[18,44-48] However, how to calculate a daily dose of Hg released from amalgam restorations by use of intraoral Hg vapor measurement and then estimating body burden of Hg—especially brain burden—is an unresolved debate.[44,49-51] Surrogate measures of body burden of Hg, including blood and urine Hg levels, have been used by others to hypothesize an increase in brain burden of Hg resulting from amalgam restorations. Blood measures have been equivocal.[47,52,53] Although urine levels have been shown to relate to the number of existing amalgam restorations[15,54,55] and are indicative of exposure, there is no evidence in the cited studies that

these levels are predictive of brain Hg levels. Therefore, our finding of no association of brain Hg levels with dental amalgam restorations implies that surrogate measures—blood and urine—of brain Hg level are not valid measures of the relationship between amalgam restorations and brain Hg levels. Our study measured brain Hg levels and existing amalgam restorations for each subject and demonstrated no significant association in subjects with or without AD.

A previous INAA study of bulk brain specimens revealed a significant elevation of Hg in subjects with AD in combined data from 12 brain regions compared with that of control subjects.[3] Examination of the individual brain regions, however, revealed that only the levels in the cerebellum were significantly elevated in subjects with AD. Thompson and colleagues[4] found an elevation of Hg in the amygdala, hippocampus and nucleus basalis of Meynert, although only the latter reached significance. In a subcellular fractionation study, Wenstrup and colleagues[5] reported a significant elevation of Hg in the microsomal fraction of multiple neocortical areas of subjects with AD compared with levels in that area in control subjects, as well as a nonsignificant elevation in combined bulk neocortical regions in subjects with AD. In another INAA study of 29 subjects with AD and 21 control subjects, no significant differences were found in Hg in the amygdala, pyriform cortex or olfactory pathway.[56] Large subject-to-subject variations in Hg levels were found in that study and in a study of non-neural tissue from healthy control subjects.[57] In our study, numerically high Hg values were found in the frontal pole, inferior parietal lobule, temporal lobe and the total neocortical load in AD. Despite our having a large subject population, none of these elevations in subjects with AD met a $P < .05$ significance level. Another study found no difference in Hg levels in seven brain regions of subjects with AD and control subjects.[58]

Two autopsy studies relating Hg levels to the number of tooth surfaces containing dental amalgam have been reported.[30,31] Brain Hg levels significantly increased with increasing amalgam load. Information about dental amalgam restoration history, duration of occlusal amalgam restorations, other Hg exposure or cognitive status was not reported in these studies, as the subjects were sudden-death victims in a coroner's autopsy series.

CONCLUSIONS

In summary, we found no association between brain Hg levels and dental amalgam and no differences in dental amalgam experience between subjects with AD and control subjects. We found no significant difference in brain Hg levels between subjects with AD and control subjects. Our results do not support the hypothesis that dental amalgam is a major contributor to brain Hg levels. They also do not support the hypothesis that Hg is a pathogenetic factor in AD. This study demonstrates that dental amalgam is not a major public health risk factor for AD. ■

Dr. Saxe was a professor of periodontics and geriatric dentistry and director, Geriatric Oral Health Program, College of Dentistry, University of Kentucky, Lexington, when this article was written. He now is retired. Address reprint requests to Dr. Saxe at 2001 Lakeside Drive, Lexington, Ky. 40502-3016.

Ms. Wekstein was the project director and the geriatric oral health coordinator, College of Dentistry, University of Kentucky, Lexington, when this article was written. She now is retired.

Dr. Kryscio is a professor, Department of Statistics, University of Kentucky, Lexington.

Dr. Henry is director of geriatric dental services, Department of Veterans Affairs Medical Center, Lexington, Ky., and an associate clinical professor, College of Dentistry, University of Kentucky, Lexington.

Dr. Cornett was a research fellow, Department of Chemistry, University of Kentucky, Lexington, when this article was written. He now is an assistant professor, Department of Chemistry and Physical Sciences, College of Mount St. Joseph, Cincinnati.

Dr. Snowdon is an associate professor, Department of Preventive Medicine, University of Kentucky, Lexington.

Dr. Grant was a postdoctoral fellow in geriatric dentistry, College of Dentistry, University of Kentucky, Lexington, when this article was written. He now is director of geriatric dentistry, department of dentistry, Carolinas Medical Center, Charlotte, N.C., and director, Special Care Dental Center, Huntersville-Oaks Nursing Home, Huntersville, N.C.

Dr. Schmitt is an associate professor, Department of Neurology, College of Medicine, Sanders-Brown Center on Aging, University of Kentucky, Lexington.

Dr. Donegan is director of dentistry, Notre Dame of Elm Grove, Elm Grove, Wis., and an associate professor of restorative sciences, Marquette University School of Dentistry, Milwaukee.

Dr. Wekstein is a professor of physiology and biophysics; assistant director, Alzheimer's Disease Research Center; and an associate director, Sanders-Brown Center on Aging, University of Kentucky, Lexington.

Dr. Ehmann is professor emeritus and former chairperson, Department of Chemistry, University of Kentucky, Lexington.

Dr. Markesbery is a professor of pathology and neurology; director, Alzheimer's Disease Research Center; and director, Sanders-Brown Center on Aging, University of Kentucky, Lexington.

This work was supported by National Institute of Health grants RO1 AG 10664, 1-P01-AG 05119, 5-P50-AG 05144 and RO1 AG 09862.

The authors acknowledge the central Kentucky dental practitioners and the members and staff of the School Sisters of Notre Dame religious congregation in Elm Grove, Wis., for their support and cooperation. The authors thank Paula Thomason for her editorial assistance, Brian Hallahan for his technical assistance and Jenny Howell, D.M.D., for her clinical assistance.

1. Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a com-

munity population of older persons. JAMA 1989;262:2551-6.
2. Markesbery WR, Ehmann WD. Brain trace elements in Alzheimer's disease. In: Terry RD, Katzman R, Bick KL, eds. Alzheimer's disease. New York: Raven Press; 1993:353-67.
3. Ehmann WD, Markesbery WR, Alauddin M, Hossain TI M, Brubaker EH. Brain trace elements in Alzheimer's disease. Neurotoxicology. 1986;5:197-206.
4. Thompson CM, Markesbery WR, Ehmann WD, Mao YX, Vance DE. Regional brain trace-element studies in Alzheimer's disease. Neurotoxicology 1988;9:1-7.
5. Wenstrup D, Ehmann WD, Markesbery WR. Trace element imbalances in isolated subcellular fractions of Alzheimer's disease brains. Brain Res 1990;533:125-31.
6. Clarkson TW. The toxicology of mercury. Crit Rev Clin Lab Sci 1997;34:369-403.
7. Goldblatt D. Pollutants and industrial hazards. In: Rowland LP, ed. Merritt's textbook of neurology. 8th ed. Philadelphia: Lea & Febiger; 1989:924-5.
8. Kurland LT, Faro SN, Siedler H. Minamata disease. World Neurol 1960;1:370-90.
9. Clarkson TW, Hursh JB, Sager PR, Syversen TLM. Mercury. In: Clarkson TW, Friberg L, Nordberg GF, Sager PR, eds. Biological monitoring of toxic metals. New York: Plenum Press; 1988:199-246.
10. World Health Organization. Environmental health criteria 118: Inorganic mercury. Sect. 5, Environmental levels and human exposure. Geneva: World Health Organization; 1991:35-46.
11. Goering PL, Galloway WD, Clarkson TW, Lorscheider FL, Berlin M, Rowland S. Toxicity assessment of mercury vapor from dental amalgams. Fundam Appl Toxicol 1992;19:319-29.
12. Richardson GM. Assessment of mercury exposure and risks from dental amalgam: Final report. Ottawa: Medical Devices Bureau, Environmental Health Directorate, Health Canada. August 18, 1995.
13. Choi BC. Risk assessment in dentistry: health risks of dental amalgam revisited. J Can Dent Assoc 1996;62:328-32.
14. Winn DM, Brunelle JA, Selwitz RH, et al. Coronal and root caries in the dentition of adults in the United States, 1988-91. J Dent Res (special issue) 1996;75:642-51.
15. Department of Health and Human Services' Committee to Coordinate Environmental Health and Related Management. Dental amalgam: A scientific review and recommended public health service strategy for research, education and regulation—Final report of the Subcommittee on Risk Management of the Committee to Coordinate Environmental Health and Related Programs, Public Health Service. Washington, D.C.: Department of Health and Human Services; 1993.
16. Gay DD, Cox RD, Reinhardt JW. Chewing releases mercury from fillings (letter). Lancet 1979;1:985-6.
17. Svare CW, Peterson LC, Reinhardt JW, et al. The effect of dental amalgams on mercury levels in expired air. J Dent Res 1981;60:1668-71.
18. Vimy MJ, Lorscheider FL. Intraoral air mercury released from dental amalgam. J Dent Res 1985;64:1069-71.
19. Vimy MJ, Lorscheider FL. Serial measurements of intraoral air mercury: estimation of daily dose from dental amalgam. J Dent Res 1985;64:1072-5.
20. Mackert JR Jr. Factors affecting estimation of dental amalgam mercury exposure from measurements of mercury vapor levels in intra-oral and expired air. J Dent Res 1987;66:1775-80.
21. Lorscheider FL, Vimy MJ, Summers AO. Mercury exposure from "silver" tooth fillings: emerging evidence questions a traditional dental paradigm. FASEB J 1995;9:504-8.
22. Siblerud RL, Motl J, Kienholz E. Psychometric evidence that mercury from silver dental fillings may be an etiological factor in depression, excessive anger, and anxiety. Psychol Rep 1994;74:67-80.
23. Berglund A, Molin M. Mercury vapor release from dental amalgam in patients with symptoms allegedly caused by amalgam fillings. Eur J Oral Sci 1996;104:56-63.
24. Vroom FQ, Greer M. Mercury vapour intoxication. Brain 1972;95:305-18.
25. Magos L. Mercury and mercurials. Br Med Bull 1975;31:241-5.
26. Gerstner HB, Huff JE. Clinical toxicology of mercury. J Toxicol Environ Health 1977;2:491-526.
27. Clarkson TW. Mercury: an element of mystery. N Engl J Med 1990;323:1137-9.
28. Eley BM, Cox SW. The release, absorption and possible health effects of mercury from dental amalgam: a review of recent findings. Br Dent J 1993;175:355-62.
29. Pendergrass JC, Haley BE. Inhibition of brain tubulin-guanosine 5'-triphosphate interactions by mercury: similarity to observations in Alzheimer's diseased brain. Met Ions Biol Syst 1997;34:461-78.
30. Nylander M, Friberg L, Lind B. Mercury concentrations in the human brain and kidneys in relation to exposure from dental amalgam fillings. Swed Dent J 1987;11:179-87.
31. Eggleston DW, Nylander M. Correlation of dental amalgam with mercury in brain tissue. J Prosthet Dent 1987;58:704-7.
32. Weiner JA, Nylander M. The relationship between mercury concentration in human organs and different predictor variables. Sci Total Environ 1993;138:101-15.
33. Poison in the Mouth (transcript). "60 Minutes," CBS television, December 16, 1990; "Panorama," BBC television, July 11, 1994.
34. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984;34:939-44.
35. Snowdon DA, Kemper SJ, Mortimer JA, Greiner LH, Wekstein DR, Markesbery WR. Linguistic ability in early life and cognitive function and Alzheimer's disease in late life: findings from the Nun Study. JAMA 1996;275:528-32.
36. Saxe SR, Snowdon DA, Wekstein MW, Riley KP, Greiner PA, Markesbery WR. Dental amalgam and cognitive function in older women: findings from the Nun Study. JADA 1995;126:1495-501.
37. Snowdon DA, Greiner LH, Mortimer JA, et al. Brain infarction and the clinical expression of Alzheimer's disease. JAMA 1997;277:813-7.
38. Khachaturian ZS. Diagnosis of Alzheimer's disease. Arch Neurol 1985;42:1097-105.
39. Mirra SS, Heyman A, McKeel D, et al. The consortium to establish a registry for Alzheimer's disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991;41:479-86.
40. Ehmann WD, Ding XX, Khare SS, et al. Activation analysis in a multitechnique study of trace element imbalances in age-related neurological diseases. J Radioanal Nucl Chem 1993;168:223-31.
41. Ehmann WD, Vance DE. Studies of trace element involvement in human disease by in vitro activation analysis. J Radioanal Nucl Chem 1996;203:429-45.
42. Cornett CR, Samudralwar DL, Ehmann WD, Markesbery WR. Sensitivity improvements in the determination of mercury in biological tissues by neutron activation analysis. J Radioanal Nucl Chem 1995;195:117-21.
43. Cornett CR. Trace elements in Alzheimer's disease tissues and environmental sources of mercury (dissertation). Lexington, Ky.: University of Kentucky; 1995.
44. Vimy MJ, Lorscheider FL. Dental amalgam mercury daily dose estimated from intraoral vapor measurements: a predictor of mercury accumulation in human tissues. J Trace Elem Exp Med 1990;3:111-23.
45. Aronsson AM, Lind B, Nylander M, Nordberg M. Dental amalgam and mercury. Biol Met 1989;2:25-30.
46. Berglund A, Pohl L, Olsson S, Bergman M. Determination of the rate of release of intra-oral mercury vapor from amalgam. J Dent Res 1988;67:1235-42.
47. Snapp KR, Boyer DB, Peterson LC, Svare CW. The contribution of dental amalgam to mercury in blood. J Dent Res 1989;68(5):780-5.
48. Mackert JR Jr. Dental amalgam and mercury. JADA 1991;122(9):54-61.
49. Berglund A. Estimation by a 24-hour study of the daily dose of intra-oral mercury vapor inhaled after release from dental amalgam. J Dent Res 1990;69(10):1646-51.
50. Marek M. Interactions between dental amalgams and the oral environment. Adv Dent Res 1992;6:100-9.
51. Mackert JR Jr., Berglund A. Mercury exposure from dental amalgam fillings: absorbed dose and the potential for adverse health effects. Crit Rev Oral Biol Med 1997;8(4):410-36.
52. Abraham JE, Svare CW, Frank CW. The effect of dental amalgam restorations on blood mercury levels. J Dent Res 1984;63(1):71-3.
53. Barregård L, Sällsten G, Järvholm B. People with high mercury uptake from their own dental amalgam fillings. Occup Environ Med 1995;52(2):124-8.
54. Kingman A, Brown LJ, Albertini T. Correlations between urinary mercury concentrations and amalgam exposure among NIDR amalgam study participants (abstract). J Dent Res 1994;73:1644.
55. Weiner JA, Nylander M. An estimation of the uptake of mercury from amalgam fillings based on urinary excretion of mercury in Swedish subjects. Sci Total Environ 1995;168(3):255-65.
56. Samuldralwar DL, Diprete CC, Ni BF, Ehmann WD, Markesbery WR. Elemental imbalances in the olfactory pathway in Alzheimer's disease. J Neurol Sci 1995;130(2):139-45.
57. Aalbers TG, Houtman JP, Makkink B. Trace-element concentrations in human autopsy tissue. Clin Chem 1987;33(33):2057-64.
58. Fung YK, Meade AG, Rack EP, Blotcky AJ. Brain mercury in neurodegenerative disorders. J Toxicol Clin Toxicol 1997;35(1):49-54.