Reduced Levels of Mercury in First Baby Haircuts of Autistic Children

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Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

Keywords Amalgam, Autism, Hair, Mercury, Thimerosal

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Autism has been defined by symptoms rather than causes since it was first characterized by Kanner in the 1940s (Eisenberg and Kanner 1956). Since Rutter's (Rutter 1978) further elaboration of diagnostic standards in 1976, the prevailing standards for diagnosis (*Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition [DSM III] 1980; 3rd edition—revised [DSM-III-R] 1987; 4th edition [DSM IV] 1994) have included impairment in three domains: social relatedness, communication, and behavior. In a small number of cases, either genetic (Wahlstrom et al. 1986; Bolton et al. 2002; Steffenburg et al. 1996) or environmental (Stromland et al. 1994; Williams and Hersh 1997; Aronson, Hagberg, and Gillberg 1997) causes have been established, but the vast majority of cases remain idiopathic.

The need to account for the relative contribution of genetic and environmental causes has taken on increased importance in light of possible sharp increases in the incidence of autism. Early prevalence studies in the United States (Burd, Fisher, and Kerbeshian 1987; Treffert 1970; Ritvo et al. 1989) and the United Kingdom (Lotter 1966; Wing and Gould 1979; Deb and Prasad 1994) reported low rates of autism—generally less than 5 per 10,000—among children born before 1990. Studies of populations born in the 1990s, however, show far higher (Bertrand et al. 2001; Baird et al. 2000) and increasing (Department of Developmental Services 1999; Kaye, del Melero-Montes, and Jick 2001; Taylor et al. 1999) rates of autism and autism spectrum disorders (ASDs), in some cohorts as high as 55 per 10,000 for autism and 80 per 10,000 for ASDs.

These increases clearly point to the rising importance of environmental factors and raise the possibility of an etiological role for toxic exposures: either prenatal, postnatal, or in some cumulative pattern that combines the effect of maternal, gestational, and infant exposures. One group (Bernard et al. 2001) has hypothesized a causal connection between mercury exposure and the symptoms of autism.

Until recently, thimerosal, a preservative containing 49.6% ethyl mercury, was used in three childhood vaccines: hepatitis B, *Haemophilus influenzae B* (Hib), and diphtheria-pertussis-tetanus (DPT). Hib and hepatitis B were introduced to the U.S.

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infant vaccination schedule in October 1990 and November 1991, respectively. In addition, most varieties of Rho D immunoglobulin injections, administered to Rh-negative mothers during pregnancy, contained thimerosal until late in the 1990s. The Institute of Medicine has investigated the connection (Stratton, Gable, and McCormick 2001) between mercury exposure from thimerosal-containing vaccines and neurodevelopmental disorders, including autism, and found insufficient evidence to accept or reject a causal connection, but concluded that such a connection was biologically plausible and recommended a comprehensive research program.

In addition to ethyl mercury, other possible sources of early mercury exposure include fetal exposures to inorganic mercury inhaled by the mother from dental amalgam fillings (Drasch et al. 1994; Vimy et al. 1997) and to methyl mercury intestinally absorbed as a consequence of maternal fish consumption.

Little is known about the specific patterns of mercury absorption, distribution, metabolism, or excretion in human infants. The large majority of infants immunized with the full complement of thimerosal-containing vaccines have not been diagnosed with an adverse effect, such as neurodevelopmental delay. Nevertheless, ecological analysis of the timing of the increases in autism incidence and the increased exposure to mercury in thimerosal-containing vaccines fails to exclude a causal relationship between the two trends of rising autism incidence and rising mercury exposure (Blaxill 2001).

Fully prospective studies of the role of mercury exposure in autism have not yet been designed and even retrospective studies are highly constrained by availability of relevant biological samples. Many families do, however, retain locks of infant hair, especially the first baby haircut. These samples provide an opportunity for analysis when other opportunities have passed. Although hair mercury levels provide only a partial insight into the excretion patterns of autistic infants, they offer substantial availability advantages and can provide a useful test of the plausibility of the autism-mercury hypothesis.

In a clinical practice, one of the study authors (ASH) submitted hair samples from autistic patients for commercial laboratory testing for toxic metal exposure. Most of these mercury hair levels were found to be low, contrary to a first-order hypothesis of heavy metal toxicity in autism. She then asked patients to submit first baby haircut samples for analysis, thereby testing a sample that would more accurately reflect early exposures. With two exceptions (these coming from a different commercial laboratory than her preferred source and the source used in the current study), these samples yielded hair mercury levels that were consistently close to zero.

Based on this observation, and on the possibility that impaired mercury excretion might be an important susceptibility factor underlying recent increases in autism, she expanded her investigation. She increased the sample of autistic first baby haircut samples and collected a set of age- and gender-matched control baby haircut samples. Notably, the control samples were collected under the condition that the child received all their childhood vaccinations on schedule, in order that they would show comparable postnatal exposure levels. Consequently, this study does not attempt to examine the role of childhood vaccine exposures in autism. Although there are limits to the design, we believe that our study effectively examines the null hypothesis of no differential excretion rates in the hair of infants subsequently diagnosed with autism.

MATERIALS AND METHODS

Patient Recruitment and Profile (Table 1)

All autistic patients were referred to the clinical practice of ASH with a confirmed diagnosis of DSM IV autism by either a pediatric neurologist or developmental pediatrician. The mother of each autistic child was interviewed for exposure information using a structured survey questionnaire. The autistic children were between the ages of 2 and 15 at the time of interview, with a median age of 7. Although the location of primary residence was slightly skewed to the Midwest and Southeast, in part due to proximity to the clinical practice, the autistic patients provided a good cross-section of the different regions of the United States, with an additional 6% coming from England, Canada, and Mexico. Boys outnumbered girls, with a male:female ratio of 3.5:1, consistent with the typical population prevalence in autism studies (Fombonne 1999).

Conditions of Autistic Baby Hair Collection

Autistic patients sent their baby hair samples directly to Doctor's Data Inc. (DDI, West Chicago, IL) following DDI's instructions for the hair minerals test. First baby haircut samples had

TABLE 1
Study group profiles

	Autistic group	Control group
Number of males/ females (ratio)	73/21 (3.5:1)	34/11 (3.1:1)
Median year of birth (range)	1994 (1985–1999)	1994 (1990–1999)
Median months at baby haircut timing (range)	17.7 (11–24)	17.8 (12–24)
Residence[a]		
Northeast	15%	22%
Midwest	28%	22%
Southeast	25%	22%
Mountain/Plains/ South Central	14%	20%
West	12 %	13%
International	6%	0%

[a]Northeast: CT, MA, VT, NY, NJ, PA. Midwest: IL, OH, MI, WI, MN, MO. Southeast: FL, GA, NC, SC, VA, LA, MS, AL, AR, KY, TN. Mountain/Plains/South Central: CO, KS, ND, SD, NE, UT, ID, TX, OK, AZ. West: CA, WA, NV. International: Canada, Mexico, England.

been collected by the parents between 11 and 24 months of age, with a mean age at haircut of 17.7 months. The minimum sample amount was 0.25 g. Before deciding to standardize on a single testing source, a minority of hair samples (20 in all) were collected in the course of clinical treatment of additional autistic patients and sent to a separate commercial laboratory—not DDI—that performs similar testing. These results were reviewed by the clinic but excluded from this study. Results from this other laboratory were similar to those from DDI, showing low hair mercury levels in autistic patients. Two of the 20 excluded test results included mercury levels that were higher than the reported levels for any of the autistic subjects in the present study.

Controls Recruitment and Baby Hair Collection

Normal controls were recruited through an appeal to autism parent groups and through autism newsletters. None of these control children or parents were interviewed in person, but each of the mothers was interviewed over the telephone. Hair collection procedures were the same as for the autistic patients. The inclusion criteria for controls included the following: no developmental disabilities or chronic illness of any kind, no siblings on the autistic spectrum, and completion of the recommended childhood vaccinations on schedule. These controls were recruited with the objective of matching the autistic patients in terms of gender and age profile (see Table 1). Although not a condition of recruitment, the state of residence was quite similar to the autistic sample, minimizing the possibility of regional exposure bias.

Hair Analysis Methods

Laboratory testing was conducted using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) in blinded fashion to the clinical status of the hair provider. Hair specimens were collected based on retained samples of a minimum required amount of 0.25 g of hair from the child's first haircut. In the laboratory, the hair specimens were further cut and washed using a modified method developed by the International Atomic Energy Agency (IAEA) (Ryabukin 1998). Aliquots (about 0.2 g) of the washed hair samples were digested with nitric acid in a CEM (CEM Corporation, Matthews, NC, USA) microwave oven with temperature feedback control. All element determinations were made on an ICP-MS (Elan 5000; Perkin-Elmer, Norwalk, CT, USA) using a flow injection sample uptake system (FIAS 400; Perkin-Elmer). Accuracy was assessed and verified using a hair standard reference material (SRM) from China (GBW 09101, 30 elements). Puchyr et al. (1998) have described this method and analytical performance in detail.

Clinical Observation of Subjects Based on Clinic Visits

All previously diagnosed autistic patients were also observed by ASH in a clinical setting. A repetitive diagnostic interview was not conducted, but an overall assessment of each child was performed and each was assigned an autism severity level: severe, moderate, or mild. The definitions for these categories were as follows: (a) severe—no expressive language at all, very little evidence of receptive language, constant divergent gaze in presence of clinician or parent, no toy play, "people treated as objects"; (b) mild—some expressive language, including short phrase speech and ability to communicate wants and needs, responsive to commands indicating functional receptive language, some eye contact, some appropriate toy play, obvious connection with parents and/or other family members; and (c) moderate—subjects not meeting criteria for either the severe or mild groups.

Data Collection for Other Maternal Exposures

ASH interviewed the mothers of autistic and normal children to obtain information on mercury exposure during and after gestation. Exposure measures were developed from survey questions in four categories: (a) maternal amalgams during pregnancy were estimated by direct observation by the mother (either using a mirror, or counted by her husband) of amalgam surfaces at time of interview less new fillings since the gestation period; (b) exposures through Rho D immunoglobulin injections during pregnancy were self-reported by the mother; (c) childhood vaccinations, including the timing of exposures to hepatitis B, DPT, and Hib vaccines, were obtained based on a joint review of the child's pediatrician's records; and (d) fish consumption during pregnancy in four categories was estimated using a four-level scale. The four levels estimated were based on the relative frequency of meals in which fish was consumed: "heavy" was once a week or more, "moderate" was less than weekly and more than monthly, "little" was less than once a month, with the final category being "none."

Statistical Analysis

Data were analyzed using Microsoft Excel version 9.0.3821 SR-1. Comparison of distributions of autistics and controls was made using the two-tailed test. Multiple-regression analysis on the normal hair sample was performed with the hair mercury level as the dependent variable and three independent variables. For amalgam fillings, we used the square of the number of fillings. We chose an exponential curve for several reasons: mothers with more fillings would be likely to have larger fillings and larger exposed surface areas per filling; multiple amalgams would be more likely to react with each other and release higher levels of mercury due to galvanism or abrasion; higher levels of amalgam exposure could lead to greater retention in maternal tissue; and mothers with large numbers of fillings would be more likely to have had recent dental work done. In addition, the exponential relationship produced consistently superior statistical correlations. For fish diets, we assigned a monthly number of fish meals for each response level: 5 per month for "heavy" consumption, 2.5 per month for "moderate" consumption, 0.5 for "little" consumption, and 0 for no consumption. For vaccine exposure,

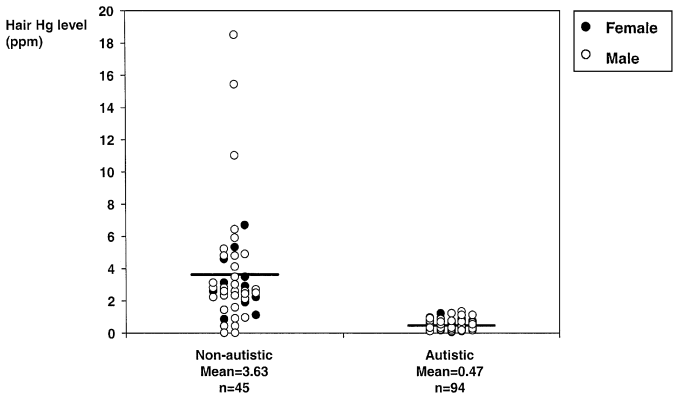

FIGURE 1

A plot of the birth hair mercury levels of nonautistic versus autistic children. Solid circles represent individual female subjects and open circles represent individual male subjects.

we used the total number of micrograms received through all thimerosal-containing vaccines.

ANALYSIS OF DATA AND RESULTS

Hair Mercury: Autistics Versus Controls

Figure 1 shows the results of the analysis comparing the excretion of mercury in baby hair of autistic children and normal controls. The hair levels of mercury in autistic children were significantly lower than in controls ($p < .0000004$), with a mean of 0.47 ppm compared to a mean of 3.63 ppm in the control group.

Rho D Immunoglobulin and Amalgam Exposure: Autistics Versus Controls

Despite the lower levels of mercury excretion in baby hair, the mothers of autistic children had higher exposures to mercury when pregnant with their autistic child than did normal controls. Table 2 shows that the number of Rho D immunoglobulin injections received by mothers in the autistic group was significantly higher than the mothers of controls (0.52 versus 0.09; $p < .0000004$). Forty-six percent of the autistic mother received Rho D immunoglobulin injections as compared to 9% of the control mothers. In addition, the number of amalgam fillings present in the mouths of mothers of autistic children exceeded the number of fillings for mothers of controls (8.35 versus 6.60; $p < .01$). Thirty-four percent of the mothers in the autistic sample had 10 or more amalgam fillings as compared to 18% of the controls.

Hair Mercury Levels Within Autistic Population: Mild Versus Moderate Versus Severe

Within the autistic sample, the level of mercury in hair was inversely correlated with the symptom severity level. Figure 2 displays the distribution of hair levels across the three subgroups of autistic children. The mean hair mercury content was 0.21 ppm in the severe group, 0.46 ppm in the moderate group (severe versus moderate: $p < .000002$), and 0.71 ppm in the mild group (moderate versus mild: $p < .0004$; severe versus mild: $p < .00000003$). Even these stark differences were somewhat moderated by a clear trend toward reduced mercury levels in female hair within the mild group.

TABLE 2

Exposure differences in autistic group as compared to controls

	Autistic group ($N = 94$)	Control group ($N = 45$)
Mercury levels in first baby haircut (ppm, mean ± *SD*)	0.47 (±0.28)[a]	3.63 (±3.56)
Rho D immunoglobulin shots during pregnancy (number per mother, mean ± *SD*)	0.53 (±0.67)[b]	0.09 (±0.29)
Amalgam fillings during pregnancy (number per mother, mean ± *SD*)	8.35 (±3.43)[c]	6.60 (±3.55)

[a] Statistically different from control group ($p < .0000004$).
[b] Statistically different from control group ($p < .0000004$).
[c] Statistically different from control group ($p < .01$).

Differences Within Autistic Population: Gender and Developmental Patterns

Table 3 provides information on other differences between the three subgroups within the autistic sample. In addition to the differences in hair mercury levels, the gender distribution varied substantially across the three subgroups. The mild group had the highest percentage of females, at 56%, whereas the moderate and severe groups had 14% and 4% females, respectively. In Figure 2, it is also apparent that the female children in the mild

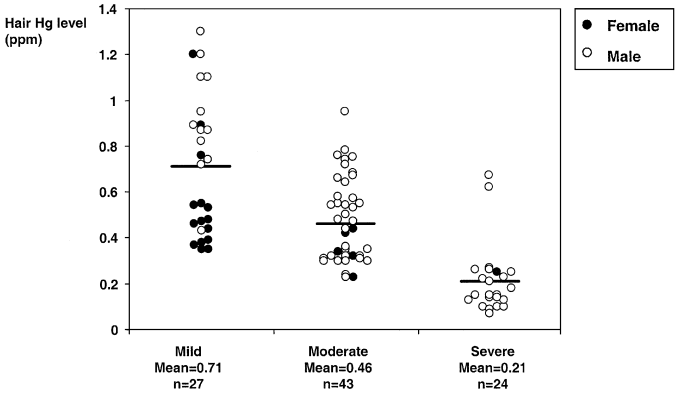

FIGURE 2

A plot of the birth hair mercury levels in autistic children based on the clinical severity of the disease. Solid circles represent individual female subjects and open circles represent individual male subjects.

TABLE 3
Differences within autistic population

	Mild group ($N = 27$)	Moderate group ($N = 43$)	Severe group ($N = 24$)
Mercury levels in first baby haircut (ppm, mean ± *SD*)	0.71 ppm (±0.3)	0.46 ppm (±0.19)[a]	0.21 ppm (±0.18)[a,b]
Males: females	12:15	37:6	23:1
Percent regressive	100	93	21

[a] Statistically different from mildly autistic group ($p < .0004$).
[b] Statistically different from mildly autistic group ($p < .000000003$).
[c] Statistically different from moderately autistic group ($p < .0000002$).

group made up over 90% of the children below the mean and only 21% of the children above the mean. In addition, the developmental patterns varied strongly across the three subgroups. The severe group was the most likely to have demonstrated consistency in symptoms from birth, only 21% displayed any pattern of developmental regression. By contrast, the vast majority of the mild and moderate groups reported some kind of developmental regression.

Correlation Between Exposure and Hair Levels

In the control sample, the levels of mercury in baby hair were significantly explained by gestational mercury exposures. Figure 3 demonstrates that a single exposure variable, maternal amalgam fillings, was strongly correlated with mercury hair levels in control children, but not in autistic children. Several different regression models were applied—including one, two, and three independent variable regressions—and the three-variable equation shown in Figure 4 provided the best statistical fit. Maternal amalgam fillings were significantly correlated with mercury levels in all regressions and on their own explain over 60% of the difference in normal hair levels. Reported maternal fish consumption during pregnancy is an additional and significant contributor to hair mercury levels. Vaccine exposure from all childhood immunizations also reached significance at the 95% confidence level. By contrast, similar regressions for autistic mercury hair levels (not shown) fail to reach significance for any exposure variable. Moreover, applying the exposure coefficients from the control group to the autistic group yields a sharply higher rate of predicted excretion levels than the actual results, whereas the predicted results based on known exposures in the control group are remarkably close to the actual results (see Figure 4). This reflects the high explanatory power ($R^2 = .79$) of the multiple regression model in the control sample.

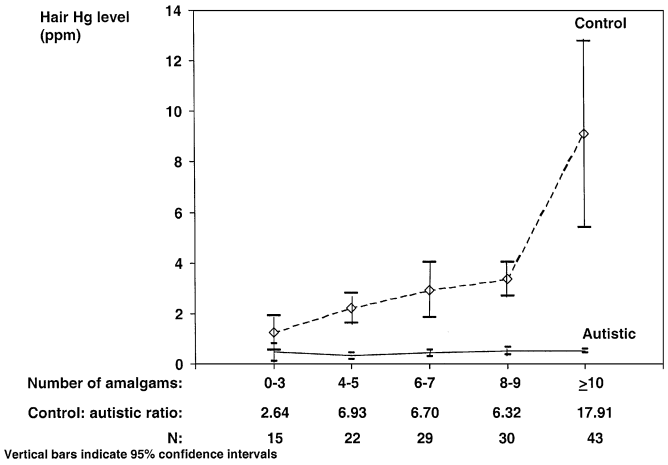

FIGURE 3
A plot of the birth hair mercury levels of nonautistic (control) children versus autistics compared to the grouped numbers of dental amalgams of the birth mothers. *N* equals the number of subjects and the control-to-autistic ratio for each subset is presented.

DISCUSSION

Past Studies Using Hair as a Marker in Autism

Although this is the first analysis of the first baby haircut of autistic infants, a number of previous studies have measured the hair contents of autistic subjects. The earliest studies (Wecker et al. 1985; Shearer et al. 1982; Gentile et al. 1983) analyzed hair from subjects born before 1981 and only one of these (Wecker et al. 1985) measured mercury. All of these studies found some significant differences between autistic and control groups. A group of autistic subjects averaging 5.7 years of age (Wecker et al. 1985) showed low hair levels of calcium, magnesium, copper, manganese, chromium, and lithium, but similar levels of mercury compared to controls. A group averaging 8 years of age (Shearer et al. 1982) showed low levels of cadmium excretion. A third group of unspecified age (Gentile et al. 1983) showed elevated levels of magnesium and potassium.

A more recent study (Holloway et al. 2001) of 50 autistic families (no age was specified) investigated both heavy metal exposures and hair levels for the purpose of testing the hypothesis of different metabolism and/or exposure levels between autistic

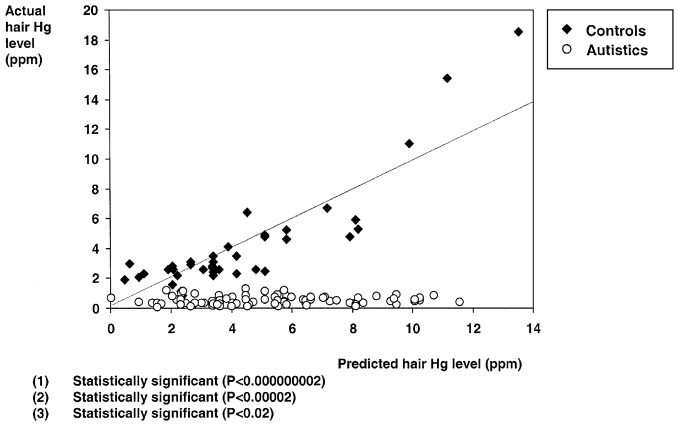

FIGURE 4

Actual hair levels in autistics and controls are compared to a predicted value. The predicted value is obtained using the regression equation for controls: Birth hair mercury level = (5.60) + 0.04 (amalgam volume$^{(1)}$) + 1.15 (fish consumption$^{(2)}$) + 0.03 (vaccine$^{(3)}$) $R^2 = .79$. Perfect prediction of actual hair levels by the regression model is represented by the dashed line. Filled diamonds represent individual nonautistic subjects and open circles represent autistics.

subjects and controls. This study used current, not baby hair, samples and found slightly reduced levels of mercury and lead in autistic hair relative to controls and significant differences in exposure, including increased maternal intake of fish and an increased rate of ear infection in infancy and associated exposure to antibiotic treatments.

Hair analysis has frequently been used as a measure of mercury exposure. In particular, it has been common practice (Grandjean et al. 1997, 1998) to measure maternal hair levels as a marker for mother-to-fetus exposures that could affect subsequent brain development. Hair mercury analysis has also been criticized as a diagnostic tool for treatment (Kales and Goldman 2002), and hair minerals test results from commercial laboratories have been criticized as inconsistent and unreliable. A recent critical review (Seidel et al. 2001) included the testing source for this study.

In our view, this recent review offered criticisms that were applied in an undifferentiated fashion to a group of laboratories and made no attempt to distinguish between proper and improper practices within the group. Much of the criticism was justified in the case of other facilities, because many of the laboratories examined made use of outdated technologies and exploited their testing results for commercial purposes, including promotion of nutritional supplements. Close reading of the analysis shows that the laboratory used in our analysis used none of the questionable practices deployed by most other laboratories and was one of only two laboratories that employ the most advanced (ICP-MS) testing equipment. We believe that the exemplary practices of DDI, the advantages of ICP-MS, the specificity of the timing of our sample collection, the fact that the laboratory was blind to the clinical status of the samples, and our use of a single, consistently calibrated laboratory protocol all combine to mitigate any concerns over the reliability of our results.

Infant Mercury Exposure and Autism

The mercury exposure levels in infants from the recommended U.S. childhood immunization schedule exceeded the threshold set by the U.S. Environmental Protection Agency (EPA) for most of the 1990s. This increased level of exposure came about as a consequence of the addition of two new thimerosal-containing vaccines, hepatitis B in November 1991 and Hib in October 1990, to the U.S. infant vaccination schedule. For example, a typical 2-month-old infant would have received an average of 0.25 μg/kg/day if immunized on schedule, as compared with the EPA threshold safety level of 0.1 μg/kg/day. This average exposure may well understate the severity of these exposures. On the days of vaccination, the bolus dose of mercury was many times the threshold. This excess exposure went undetected until the summer of 1999 when an Food and Drug Administration (FDA) review identified the problem. Subsequent to this discovery, the American Academy of Pediatrics (AAP) and the Centers for Disease Control and Prevention (CDC) (1999) issued a joint statement suspending the birth dose of hepatitis B vaccine and recommending the phasing out of thimerosal from all infant vaccines.

The special case of direct human infant exposure to thimerosal has never been studied. Yet the hypothesized (Bernard et al. 2001) relationship between mercury exposure and autism is supported by a number of ecological connections: increases in autism rates in the United States have accompanied increasing exposure to thimerosal-containing vaccines (Blaxill 2001); increases in autism rates in England (Lotter 1966, Wing and Gould 1979; Deb and Prasad 1994; Baird et al. 2000; Kaye et al. 2001; Taylor et al. 1999) followed closely on the heels of a change in the recommended immunization schedule and physician incentive structure (Salisbury and Dittman 1999) that increased the level of thimerosal exposure in the first four months of life; and rates of autism rose in Japan's Fukushima prefecture (Hoshino et al. 1982) immediately after the 1965 incident in neighboring Niigata prefecture when an chemical factory released large amounts of mercury into a local river.

Iatrogenic exposure to mercury has been shown to cause childhood disease. Mercury used in teething powder preparations in the first half of the 20th century was identified as the cause of acrodynia, a serious disease of young children that puzzled medical professionals for decades. Resistance to the evidence of mercury poisoning delayed full acceptance of the evidence for many years (Dally 1997). There are numerous parallels between the symptoms of acrodynia and autism, including loss of sociability and communication skills (Bernard et al. 2001).

Animal Models of Infant Mercury Excretion

Despite the absence of direct observation of mercury toxicokinetics in human infants, there are a number of animal models

that provide insight into the differences in the kinetics of mercury between infants and adults. Independent of the effects on the brain, these models demonstrate that several factors unique to infants can contribute to reduced excretion capacity.

Mercury is excreted mostly through the feces and is heavily dependent on the biliary secretion of inorganic mercury transported into bile by glutathione. Studies in neonatal rats (Ballatori and Clarkson 1984) have demonstrated reduced bile flow and glutathione excretion that together contribute to a reduced ability in infants to excrete methyl mercury. In a range of studies, research has demonstrated that both milk diets and antibiotic administration reduce the excretion of mercury (Kostial et al. 1979, 1981; Rowland, Robinson, and Doherty 1984), effects that are both common and amplified (Kostial et al. 1978) in suckling animals. The presence of healthy gut flora is critical (Rowland, Davis, and Grass 1978; Rowland, Robinson, and Doherty 1984) to the excretion of mercury and early exposure to mercury has even been shown to alter the composition of gut flora and promote both antibiotic-resistant and mercury-resistant strains (Summers et al. 1993). All of these mechanisms suggest that the infant period is one of both high potential variation in mercury excretion capacity and increased risk of unintended consequences arising from mercury exposure.

Mercury Excretion in Human Infants Who Became Autistic

Our findings are consistent with the hypothesis connecting mercury exposure and autism. Autistic infants released dramatically lower levels of mercury into hair than control infants. In our autistic group, this reduced level was not associated with lower levels of overall exposure, quite the contrary. In many, though not all, exposure categories, autistic infants experienced higher levels of mercury exposure. As a matter of design, we did not attempt to assess the impact of differences in vaccine exposures, because we only included controls who received a full exposure to mercury through the thimerosal in vaccines.

Autistic infants in our sample experienced increased exposure levels through maternal Rho D immunoglobulin injections (the large majority of licensed preparations sold during the study period used thimerosal as a preservative). Forty-three out of 94, or 46%, of the children in our sample were exposed to mercury through these injections, as compared to 4 out of 45, or 9%, of controls. Several of the autistic mothers received multiple injections, which resulted in a mean number of Rho D immunoglobulin injections in the autistic group of 0.52 injections per child, as compared to 0.09 among the controls. This observation is supported by a similar finding of elevated Rh incompatibility in mothers of autistic children in a previous study (Juul-Dam, Townsend, and Courchesne 2001). The level of Rh incompatibility we observed in our sample, however, is significantly greater than the rate observed in mothers in the previous study, 46% versus 12%. (The prevalence of Rh incompatibility in our controls was also higher, with a rate of 9% as compared to 3% in the previous study.) The rate of antenatal prophylaxis in our sample seems high and may be a result of increased treatment rates of Rh incompatibility as well as increasing frequency of the practice of antenatal prophylaxis, a relatively recent development. Because our study is the second report of elevated rates of autism in children born to Rh-negative mothers, this is a finding that deserves further investigation.

Increased numbers of amalgam fillings in the mother has been associated with increased fetal mercury exposure in several studies (Drasch et al. 1994; Vimy et al. 1997). Our results suggest that autistic children received increased exposure through outgassing of amalgam fillings than controls. The average level of amalgam fillings among mothers of autistic children was significantly greater than controls, with 8.35 fillings per mother in the autistic group ands 6.6 fillings among control mothers. Mothers of autistic children were far more likely to have received extensive dental work, with 35 of 94 mothers, or 37%, having 10 or more amalgam fillings as compared to 8 of 45, 18%, of controls.

Maternal dietary consumption of fish was not significantly associated with autism (data not shown).

Within the autistic group there were also strong differences in hair mercury levels. Lower hair mercury levels were significantly associated with the severity of the autistic behavior observed in the clinic. Adjusting for gender differences, these results were even stronger, because the "mildly autistic" group was disproportionately female. Within the mildly autistic group, female hair levels were almost uniformly lower than the male levels. This suggests that factors related to gender might offer a level of protection to female infants who might otherwise demonstrate more severe symptoms. By contrast, boys who displayed symptoms of similar severity nevertheless successfully released larger amounts of mercury, suggesting that boys might require high levels of mercury elimination to develop at similar rates. The increased male risk of autism has been extensively documented (Fombonne 1999; Gillberg and Wing 1999).

The control group showed a very strong correlation between measurable mercury exposure and the amount released into hair. This suggests that normal children have an ability to defend themselves against potentially toxic exposures and may demonstrate little negative effect despite exposures that were relatively large. By contrast, autistic infants who experienced comparable exposure to mercury were completely incapable of excreting mercury through hair at the levels that might have been predicted based on the excretion patterns of the control infants.

Possible Consequences of Low Hair Mercury Levels

In past reviews of potential risk from mercury in vaccines (Stratton, Gable, and McCormick 2001), the possibility of neurological damage due to exposure to thimerosal has been minimized as a "theoretical, but unproven" risk. A core concern among reviewers has been the absence of evidence that "low-dose" exposures such as those administered through vaccines have the ability to cause any detectable harm.

Our study suggests two reasons why "low dose" (where "low" is relative to demonstrably harmful or even fatal doses and not

the modeled EPA standard) exposures might raise the risk of developmental damage. First, vaccine exposures do not occur in isolation, but rather represent one among several pathways of exposure through which the fetal and infant brain might accumulate toxic levels of mercury. These pathways must therefore be evaluated in the context of cumulative exposures, any one of which might be harmless on its own but when combined with other sources might contribute to harmful overall levels. Both the autistic and the control children in our study showed increased mercury risk based on multiple sources of exposure: in the autistic group, both Rho D immunoglobulin and amalgam fillings in the mother were elevated relative to controls; in the control group, hair mercury levels were significantly correlated with maternal amalgam fillings and fish consumption as well as vaccine thimerosal exposure.

Second, the risk of any exposure will be greater if a larger fraction of the toxin is retained in tissue and not excreted quickly. Although hair is a minor pathway for mercury excretion and is far less important than feces and urine, the low levels of mercury in the hair of autistic infants support a hypothesis that these infants were retaining mercury in tissue at a higher rate than control infants. The lack of mercury in the hair of autistics may be due to a decrease in blood mercury levels feeding the hair follicles. This decrease is likely caused by the retention of the mercury inside the cells where it most likely causes its major biological damage.

When mercury is not available to the hair follicle, it is less likely to be available to the primary detoxification and excretory pathways and retained in tissue. If we presume that a portion of the tissue mercury retention is sequestered in the central nervous system and is available to cause neurological damage at sensitive points in brain development, then it is plausible that mercury-associated damage might be a meaningful element in the pathological process that leads to an outcome of autism.

Limitations of the Current Study

We recognize that there are limitations to the current study. The study was not the result of a fully prospective design, recruitment of autistic study subjects was influenced by medical care-seeking behavior, the testing facilities were not under the direct control of the investigators, and the resultant population distributions may not be representative of the autism population as a whole. Additional research is necessary both to replicate these findings in autism and to elaborate on the impact of all the major risk factors associated with toxic exposures to mercury.

CONCLUSIONS

The reduced levels of mercury in the first baby haircut of autistic infants raise clear questions about the detoxification capacity of a subset of infants. Despite hair levels suggesting low exposure, these infants had measured exposures at least equal to a control population, suggesting that control infants were able to eliminate mercury more effectively. In the case of autistic infants, those in our sample were exposed to higher levels of mercury during gestation, through dental amalgams or Rho D immunoglobulin injections in the mother. The addition of multiple postnatal exposures to mercury in childhood vaccines would have more severe consequences in infants whose detoxification capacity is reduced or who may be closer to a dangerous threshold exposure. In the case of control infants, mercury hair levels were strongly affected by exposure levels, suggesting that detoxification and excretion played an important role in ensuring normal development in children with elevate toxic exposure relative to peers. If reduced overall mercury elimination is related to hair elimination, then autistic infants will retain significantly higher levels of mercury in tissue, including the brain, than normal infants. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, our study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

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