Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study

Michael E Pichichero, Elsa Cernichiari, Joseph Lopreiato, John Treanor

Summary

Background Thiomersal is a preservative containing small amounts of ethylmercury that is used in routine vaccines for infants and children. The effect of vaccines containing thiomersal on concentrations of mercury in infants' blood has not been extensively assessed, and the metabolism of ethylmercury in infants is unknown. We aimed to measure concentrations of mercury in blood, urine, and stools of infants who received such vaccines.

Methods 40 full-term infants aged 6 months and younger were given vaccines that contained thiomersal (diptheria-tetanus-acellular pertussis vaccine, hepatitis B vaccine, and in some children *Haemophilus influenzae* type b vaccine). 21 control infants received thiomersal-free vaccines. We obtained samples of blood, urine, and stools 3–28 days after vaccination. Total mercury (organic and inorganic) in the samples was measured by cold vapour atomic absorption.

Findings Mean mercury doses in infants exposed to thiomersal were 45·6 μg (range 37·5–62·5) for 2-month-olds and 111·3 μg (range 87·5–175·0) for 6-month-olds. Blood mercury in thiomersal-exposed 2-month-olds ranged from less than 3·75 to 20·55 nmol/L (parts per billion); in 6-month-olds all values were lower than 7·50 nmol/L. Only one of 15 blood samples from controls contained quantifiable mercury. Concentrations of mercury were low in urine after vaccination but were high in stools of thiomersal-exposed 2-month-olds (mean 82 ng/g dry weight) and in 6-month-olds (mean 58 ng/g dry weight). Estimated blood half-life of ethylmercury was 7 days (95% CI 4–10 days).

Interpretation Administration of vaccines containing thiomersal does not seem to raise blood concentrations of mercury above safe values in infants. Ethylmercury seems to be eliminated from blood rapidly via the stools after parenteral administration of thiomersal in vaccines.

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Introduction

Thiomersal is a preservative used in vaccines routinely administered to infants and children. Its antimicrobial activity is due to small amounts of ethylmercury; the usual dose of paediatric vaccine contains 12·5–25 μg of mercury.[1-3] When vaccines containing thiomersal are administered in the recommended doses, allergic reactions have been rarely noted, but no other harmful effects have been reported.[4] Massive overdoses from inappropriate use of products containing thiomersal have resulted in toxic effects.[5–9]

Mercury occurs in three forms: the metallic element, inorganic salts, and organic compounds (eg, methylmercury, ethylmercury, and phenylmercury). The toxicity of mercury is complex and dependent on the form of mercury, route of entry, dosage, and age at exposure. Mercury is present in the environment in inorganic and organic forms, and everyone is exposed to small amounts.[10,11] The main route of environmental exposure to organic mercury is consumption of predatory fish, especially shark and swordfish. A 6-ounce can of tuna contains 2–127 μg (average 17 μg) of mercury.[12] Freshwater fish (eg, walleye, pike, muskie, and bass) can also contain high concentrations of mercury.

Most of the toxic effects of organic mercury compounds take place in the central nervous system, although the kidneys and immune system can also be affected.[10,11,13] Organic mercury readily crosses the blood-brain barrier, and fetuses are more sensitive to mercury exposure than are children or adults. Data about potential differences in toxicity between ethylmercury and methylmercury are few. Both are associated with neurotoxicity in high doses; in-utero poisoning with methylmercury causes problems that are similar to cerebral palsy. Findings about the effect of low-dose methylmercury exposure on neurodevelopment in infants are contradictory.[14,15] In-utero exposure could be related to subtle neurodevelopmental effects (eg, on attention, language, and memory) that can be detected by sophisticated neuropsychometric tests— although the conclusion is confounded by concomitant ingestion of polychlorinated biphenyls in the patients investigated.[14,15]

No toxic effects of low-dose exposure to thiomersal in children have been reported.[3] The effect of the small amounts of mercury contained in vaccines on concentrations of mercury in infants' blood has not been extensively assessed, and the metabolism of ethylmercury in infants is unknown. We aimed to assess concentrations of mercury in full-term infants after administration of routine vaccinations according to the schedule used in the USA, and to obtain additional information about the presence of mercury at other body sites including urine and stool. Samples of hair and breast milk were also obtained from some mothers of infants participating in the study.

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Methods

Study populations

We studied two groups of full-term infants who differed in their history of exposure to vaccines containing thiomersal. Infants in the exposure group were recruited at the Elmwood Pediatric Group, a large paediatric practice in Rochester, NY, USA, where vaccinations with thiomersal preservative were routinely given. 20 infants aged 2 months and 20 aged 6 months were studied at this practice to obtain information about the range of total thiomersal exposures likely to take place during infancy. The control group consisted of 21 infants who did not receive vaccines containing thiomersal and were recruited from the National Naval Medical Center, Bethesda, MD. All the infants were recruited during routine well-child examination and vaccination visits by the investigators (between November, 1999 and October, 2000). Written informed consent was obtained from parents for all procedures.

Vaccines

Vaccines containing thiomersal that were given to infants in the exposure group included Tripedia (diphtheria-tetanus-acellular pertussis vaccine; Aventis Pasteur, Swiftwater, PA; 0·01% thiomersal, 25 μg mercury per dose) Engerix (hepatitis B vaccine; GlaxoSmithKline, Rixensart, Belgium; 0·005% thiomersal, 12·5 μg mercury per dose), and in some children HibTITER (*Haemophilus influenzae* type b conjugate vaccine, Wyeth-Lederle, Pearl River, NY, USA; 0·01% thiomersal, 25 μg mercury per dose). Vaccines administered to the control group included Infanix (diptheria-tetanus-acellular pertussis vaccine; GlaxoSmithKline, Rixensart, Belgium), Recombivax HB (hepatitis B vaccine; Merck, West Point, PA, USA), and ActHIB (*Haemophilus influenzae* b conjugate vaccine, Aventis Pasteur, Swiftwater, PA, USA).

Procedures

We obtained vaccination histories—including type of vaccine, manufacturer, lot number, and dates of administration—from the medical records. In the exposure group, we obtained samples of heparinised whole blood, stool, and urine, during a visit 3–28 days after vaccination. Blood and urine were kept at 4°C, and stools were frozen until assessment. Urine was sampled by use of a urine bag at the clinic, and stool was taken from a diaper (nappy) provided by the parent. Whole blood and urine were obtained from the control children. At both sites, we obtained at least 50 hairs from the mother by cutting at the base near the scalp in the occipital area, to assess potential transplacental exposure of infants to mercury. Additionally, several samples of breastmilk or formula were obtained from mothers of infants at Elmwood Pediatric Group, as well as stool samples from a few infants who were not exposed to thiomersal.

We measured total mercury in all samples (and inorganic mercury in stool samples) by cold vapour atomic absorption as previously described.[16,17] The limit of reliable quantitation in this assay ranged between 7·50 nmol/L and 2·50 nmol/L, dependant on sample volume.

Population pharmacokinetic calculations

To estimate the half-life of thiomersal mercury in the blood, we developed a prediction model for the expected concentrations of mercury in blood for half-lives of mercury ranging from 1 day to 45 days, on the basis of bodyweight of the infant, the doses of thiomersal administered, and the times between the individual doses of thiomersal and when the blood was obtained. To do these calculations, we assumed that 5% of the mercury dose was distributed to blood,[7] that blood volume represented about 8% of the infant's bodyweight, and that elimination of mercury from blood followed a single-compartment model with first-order kinetics. For each possible half-life between 1 and 45 days, we then calculated the difference between the predicted and actual recorded concentrations in blood for each infant. Only measurements within the range of reliable quantitation were used in these calculations.

The best estimate of the blood half-life of mercury was judged to be the hypothetical half-life, which resulted in the smallest difference between predicted and observed values. We constructed a 95% CI based on a likelihood ratio for this estimate with the assumption that errors from the decay model were independent, additive, and normally distributed. The 95% confidence limits were the

	Infants aged 2 months		Infants aged 6 months	
	Thiomersal-exposed (n=20)	Controls (n=11)	Thiomersal-exposed (n=20)	Controls (n=10)
Bodyweight (kg)				
Mean (range)	5·3 (4·0–6·4)	NR	8·1 (6·7–10·6)	NR
Total mercury exposure (μg)*				
Mean (range)	45·6 (37·5–62·5)	0	111·3 (87·5–175·0)	0
Blood mercury (nmol/L)				
Number of samples tested	17	8	16	7
Number with mercury in range	12	1	9	0
Mean (SD)†	8·20 (4·85)	4·90	5·15 (1·20)	..
Median (IQR)†	6·15 (4·60–10·85)	4·90	5·30 (4·55–6·10)	..
Range†	4·50–20·55	..	2·85–6·90	..
Urinary mercury (nmol/L)				
Number of samples tested	12	6	15	8
Number with mercury in range	1	0	3	0
Mean (SD)†	3·8‡	..	5·75 (1·05)	..
Median (range)†	3·8‡	..	6·2 (4·55–6·45)	..
Stool mercury (ng/g dry weight)				
Number of samples tested	12	NT	10	NT
Number with mercury in range	12	..	10	..
Mean (SD)†	81·8 (40·3)	..	58·3 (21·2)	..
Median (IQR)†	83·5 (47·0–121·3)	..	58·0 (42·0–68·5)	..
Range†	23·0–141·0	..	29·0–102·0	..

NR=Not recorded. NT=not tested. *Via vaccination. †All calculations done only with samples within range of accurate quantitation. ‡Only one value so SD and range are not applicable.

Concentrations of mercury in blood, urine, and stool of infants who received vaccines containing thiomersal and those who did not

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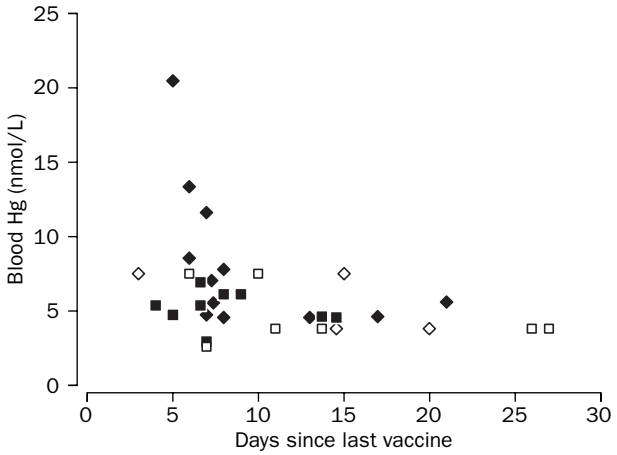

Figure 1: **Blood mercury concentrations in infants aged 2 months (diamonds) and 6 months (squares) by time of sampling**

Filled symbols represent measured values and open symbols represent samples at the limit of quantitation, either 7·50 nmol/L, 3·75 nmol/L, or 2·5 nmol/L, dependent on sample volume.

points where the curve crossed the minimum sum of squares multiplied by $1+\chi^2(1)/(n-1)$ where n is the number of data points and $\chi^2(1)$ is the upper 5% point of the χ^2 distribution on one degree of freedom.

Statistical analysis

Because this was a descriptive study we did no formal calculations for sample size. Student's *t* test and Fisher's exact test were used to compare results for the exposure and control group, with $p \leqslant 0{\cdot}05$ judged to be significant.

Role of the funding source

The sponsors of the study approved the study design but had no other involvement in the in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

61 infants were enrolled in this study (table). Among infants aged 2 months in the exposure group, samples were taken from eight within 7 days of vaccination, from five between 8 and 14 days after vaccination, and from seven between 15 and 21 days after vaccination. Among 6-month-old infants in the exposure group, samples were taken from seven between 4 and 7 days after vaccination, from eight between 8 and 14 days after vaccination, and from five between 15 and 27 days after vaccination. Samples were obtained from infants in the control group at regularly scheduled visits at 2 or 6 months of age. All children remained healthy throughout the study and during 24–36 months of follow-up.

Sufficient volumes of blood ($\geqslant$1 mL) for the measurement of mercury by the atomic absorption technique were obtained from 17 infants aged 2 months and 16 aged 6 months in the exposure group. Mercury concentrations were below the range of reliable quantitation in five of 17 blood samples from 2-month-olds, and seven of 16 blood samples from 6-month olds (p=0·48). The mean concentration of blood mercury in samples with quantifiable mercury was higher in 2-month-olds than in 6-month olds (difference 3·05 nmol/L, 95% CI 0·03–1·24, p=0·06), but was low in both these groups (table). Sufficient blood volumes for measurement of mercury were obtained from 15 infants in the control group, including eight

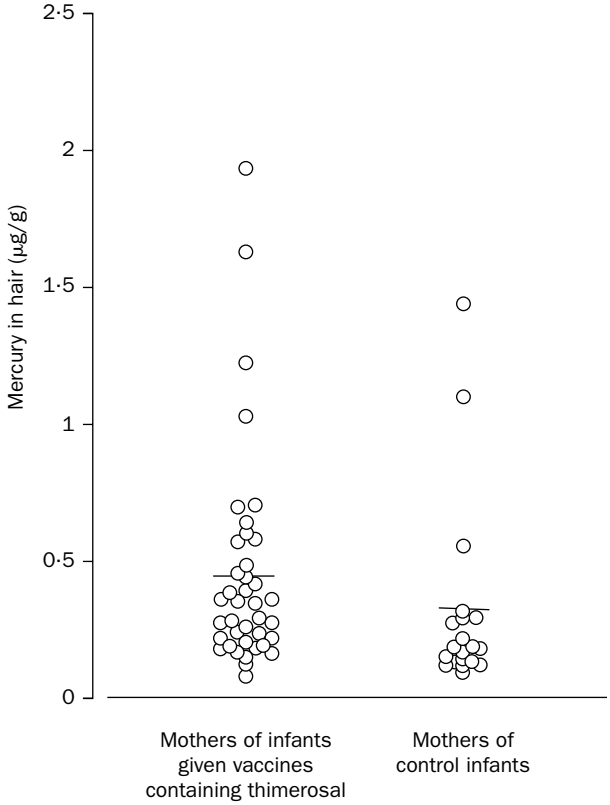

Figure 2: **Mercury concentrations in hair from mothers of infants**

Bar represents mean concentration of mercury in maternal hair.

aged 2 months and seven aged 6 months. Blood mercury was below the level of reliable quantitation in seven of the eight samples from the 2-month-olds and in all seven samples from 6-month-olds. The only detectable value from the control group was 4·65 nmol/L.

Overall, mercury concentrations were below the range of quantitation in 12 of 33 samples from thiomersal-exposed infants and in 14 of 15 unexposed infants (p=0·04). The highest level of blood mercury detected in any infant in this study was 20·55 nmol/L, which was measured 5 days after vaccination in a 2-month-old infant weighing 5·3 kg, who had received vaccines (Tripedia and Engerix B) containing a total dose of 37·5 μg mercury. The relation between time between vaccination and sampling and the concentration of mercury in the blood in the exposed group is shown in figure 1. Although mercury concentrations were uniformly low, the highest levels were recorded soon after vaccination.

Mercury was undetectable in most of the urine samples from the infants in this study. Only one of 12 urine samples from 2-month-olds, and three of 15 from 6-month-olds in the exposure group, and none of the 14 samples from the controls, contained detectable mercury. The highest concentration of urinary mercury detected was 6·45 nmol/L, in a 6-month old infant in the exposure group (table).

Stool samples were collected from infants in the exposure group. All of the stool samples from infants who received thiomersal-containing vaccines had detectable mercury, with concentrations in stools from 2-month-old infants slightly higher than those in 6-month-olds (p=0·098, table). As expected, most of the mercury in stools was inorganic. Stool samples were not obtained from control infants; therefore, to determine

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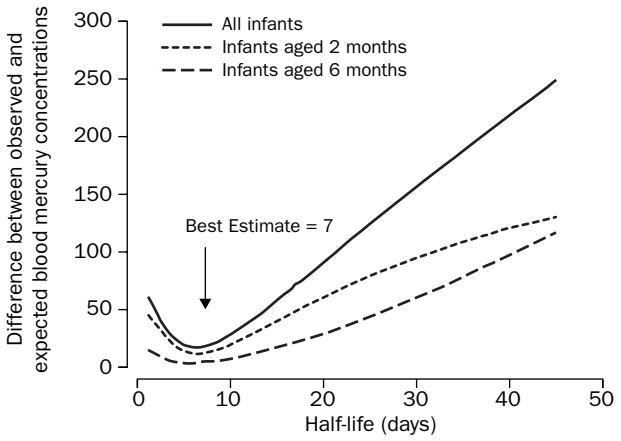

Figure 3: **Estimated blood half-life of mercury in infants who were exposed to thiomersal**

Lines represent sum of square of differences between observed concentrations of blood mercury (nmol/L) and those predicted for every individual infant on the basis of bodyweight and time of sampling, with a series of hypothetical half-lives shown on x axis. Arrow shows point with lowest value for squared difference, indicating best estimate for serum half-life.

whether dietary intake could contribute to the mercury content of stools, we also obtained samples from nine infants at Elmwood Pediatric Group who were age-matched with the infants in the exposure group and were not exposed to vaccines containing thiomersal. The mean mercury concentration in the stools of these infants was 22 ng/g dry weight (SD 16), which was significantly lower (p=0·002) than the mean of the samples collected from thiomersal-exposed infants.

Amounts of mercury measured in maternal hair are shown in figure 2. The mean concentration of hair mercury in mothers of the exposure group was 0·45 μg/g hair, whereas the mean amount in mothers of the control infants was 0·32 μg/g (p=0·22). Eight mothers of infants in the 6-month-old cohort provided breast milk samples. Concentrations of mercury in these samples were low (mean=0·30 μg/g, range 0·24–0·42 μg/g).

We estimated the half-life of mercury in blood after vaccination to be 7 days, since this result gave the smallest difference between the expected and recorded (measured) concentration (figure 3). The 95% CI around this estimate was 4–10 days. The half-life estimate was very similar when only measurements in 2-month-olds (7 days, 95% CI 4–11) or 6-month-olds (5 days, 3–9) were included, suggesting that the rate of elimination of thiomersal mercury from blood was similar in both age-groups.

Discussion

We have shown that very low concentrations of blood mercury can be detected in infants aged 2–6 months who have been given vaccines containing thiomersal. However, no children had a concentration of blood mercury exceeding 29 nmol/L (parts per billion), which is the concentration thought to be safe in cord blood;[18] this value was set at ten times below the lower 95% CI limit of the minimal cord blood concentration associated with an increase in the prevalence of abnormal scores on cognitive function tests in children. Blood mercury concentrations indicate concentrations in organs well.[18]

Although our study was not designed as a formal assessment of the pharmacokinetics of mercury, we did obtain samples of blood at various time points after exposure. Assessment of these samples suggested that the blood half-life of ethylmercury in infants might differ from the 40–50 day half-life of methylmercury (range 20–70 days) in adults and breastfeeding infants.[10,19] The concentrations of blood mercury 2–3 weeks after vaccination noted in our study were not consistent with such a long half-life, but suggested a half-life of less than 10 days. However, this conclusion is based on several assumptions and a very simple model, and does not take into account the fact that at least some of the mercury detected in the blood of the infants in this study is likely to have been derived from exposures other than vaccination. Because of the short period between vaccination and sampling, the findings of Strajich and colleagues[20] could be consistent with either a 6-day or 40-day half-life, but are otherwise consistent with the assumptions made in our model. Because we expected a 45-day half-life on the basis of methylmercury pharmacokinetics, the first blood samples were obtained 3 days after vaccination. Blood samples taken in the first 72 hours after vaccination, stool samples obtained every 24 h, and samples from premature newborn babies (weighing ⩾2000 g) given a birth dose of hepatitis B vaccine would have helped us to reach stronger conclusions. Thus, additional studies of the pharmacology of thiomersal in infants are underway.

At the times tested after vaccination, mercury excretion in urine in our study population was low. By contrast, concentrations of mercury in stool were high, and combined with the finding that stool mercury concentrations in infants who were not exposed to thiomersal were significantly lower is consistent with the hypothesis that the gastrointestinal tract represents a possible mode of elimination of thiomersal mercury in infants.

Overall, the results of this study show that amounts of mercury in the blood of infants receiving vaccines formulated with thiomersal are well below concentrations potentially associated with toxic effects. Coupled with 60 years of experience with administration of thiomersal-containing vaccines, we conclude that the thiomersal in routine vaccines poses very little risk to full-term infants, but that thiomersal-containing vaccines should not be administered at birth to very low birthweight premature infants. Decisions about the elimination of thiomersal from these vaccines must balance the potential benefit of reduced exposure to mercury against the risks of decreased vaccine coverage because of higher costs, the risk of sepsis in recipients because of bacterial contamination of preservative-free formulations, and the risks of exposure to alternative preservatives that might replace thiomersal.

Conflict of interest statement

None declared.

Contributors

M Pichichero and J Treanor contributed to the study conception and design; obtained, assessed, and interpreted data; drafted and revised the manuscript; and provided statistical expertise and supervision. E Cernichiari contributed to analysis and interpretation of data, revision of the manuscript, and technical support. J Lopreiato contributed to revision of the manuscript, and obtained data.

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References

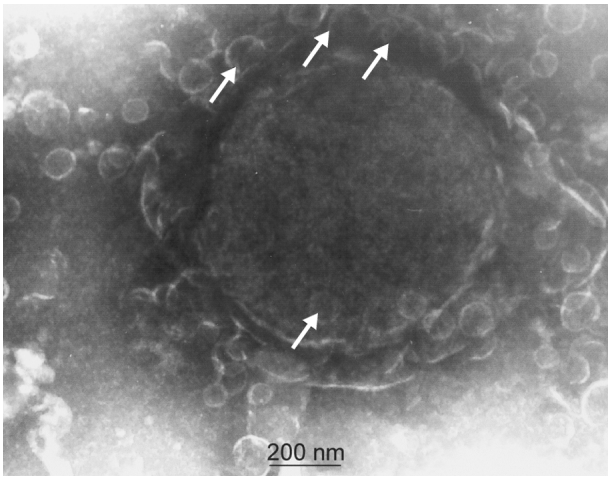
1 Clements CJ, Ball LK, Ball R, Pratt D. Thiomersal in vaccines. *Lancet* 2000; **355:** 1279–80.
2 American Academy of Pediatrics, Committee on Infectious Diseases, and Committee on Environmental Health. Thimerosal in vaccines—An interim report to clinicians. *Pediatrics* 1999; **104:** 570–74.
3 Ball LK, Ball R, Pratt, RD. An assessment of thimerosal use in childhood vaccines. *Pediatrics* 2001; **107:** 1147–54.
4 Cox NH, Forsyth A. Thimerosal allergy and vaccination reactions. *Contact Dermatitis* 1988; **18:** 229–33.
5 Axton JH. Six cases of poisoning after a parenteral organic mercurial compound (merthiolate). *Postgrad Med J* 1972; **561:** 417–21.
6 Fagan DG, Pritchard JS, Clarkson TW, Greenwood MR. Organ mercury levels in infants with omphaloceles treated with organic mercurial antiseptic. *Arch Dis Child* 1977; **52:** 962–64.
7 Matheson DS, Clarkson TW, Gelfand EW. Mercury toxicity (acrodynia) induced by long-term injection of gammaglobulin. *J Pediatr* 1980; **97:** 153–55.
8 Lowell JA, Burgess S, Shenoy S, Curci JA, Peters M, Howard TK. Mercury poisoning associated with high-dose hepatitis-B immune globulin administration after liver transplantation for chronic hepatitis B. *Liver Transpl Surg* 1996; **2:** 475–78.
9 Pfab R, Muckter H, Roider G, Zilker T. Clinical course of severe poisoning with thimerosal. *J Toxicol Clin Toxicol* 1996; 34**:** 453–60.
10 Clarkson TW. Mercury: major issues in environmental health. *Environ Health Perspect* 1992; **100:** 31–38.
11 Clarkson TW. The toxicology of mercury. *Crit Rev Clin Lab Sci* 1977; **34:** 369–403.
12 Yess NJ. US food and Drug Administration survey of methylmercury in canned tuna. *J AOAC Int* 1993; **76:** 36–38.
13 Shenker BJ, Guo TL, Shapiro IM. Low-level methylmercury exposure causes human T-cells to undergo apoptosis: evidence of mitochondrial dysfunction. *Environ Res* 1998; **77:** 149–59.
14 Grandjean P, Weihe P, White RF, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; **6:** 417–28.
15 Davidson PW, Myers GJ, Cox C, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles child development study. *JAMA* 1998; **280:** 701–07.
16 Cernichiari E, Toribara TY, Liang L, et al. The biological monitoring of mercury in the Seychelles study. *Neurotoxicology* 1995; **16:** 613–28.
17 Cernichiari E, Brewer R, Myers GJ, et al. Monitoring methylmercury during pregnancy: maternal hair predicts fetal brain exposure. *Neurotoxicology* 1995; **16:** 705–10.
18 Nielsen JB, Andersen O, Grandjean P. Evaluation of mercury in hair, blood and muscle as biomarkers for methylmercury exposure in male and female mice. *Arch Toxicol* 1994; **68:** 317–21.
19 National Academy of Sciences. Toxicologic effects of methylmercury. Washington DC: National Research Council, 2000.
20 Stajich GV, Lopez GP, Harry SW, Sexson WR. Iatrogenic exposure to mercury after hepatitis B vaccination in preterm infants. *J Pediatr* 2000; **136:** 679–81.

Clinical picture

Fatal meningococcal septicaemia with "blebbing" meningococcus

Ellen Namork, Petter Brandtzaeg

A 20-year-old previously healthy man was found unconscious in bed early in the morning. He was febrile and had an extensive haemorrhagic and cyanotic rash. He developed severe septic shock, multiple organ failure, and died 6 h later. *Neisseria meningitidis* serogroup B grew in one blood culture. Electron-microscopy of his plasma showed meningococci releasing many outer membrane vesicles ("blebs") known to harbour endotoxin (lipopolysaccharide). The endotoxin level in his plasma was 1700 endotoxin units/mL, which is equal to the activity of 170 ng/mL of purified lipopolysaccharide from *Escherichia coli*. Release of "blebs" (figure; magnified ×65 000, arrows) from rapidly growing meningococci is thought to contribute to the very high levels of endotoxin in plasma which characterise fatal meningococcal septicaemia.



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