

Respondent's Exhibit GG

18th February 2008

REPORT

THIMEROSAL VACCINE LITIGATION

INTRODUCTION

I qualified in medicine at the University of Birmingham in 1955; after holding training posts in neurology, pediatrics and general medicine, obtained membership of the Royal College of Physicians in 1958; (the equivalent of Boards in Internal Medicine) became qualified in general psychiatry (academic Diploma in Psychological Medicine, University of London, awarded with distinction) in 1961 (the equivalent of Boards in Psychiatry). I then trained in child development and specialized in child psychiatry, becoming Consultant in Child and Adolescent Psychiatry at the joint Bethlem Royal and Maudsley Hospitals in 1966, an appointment that I have held ever since. Since the early 1960s I have had extensive clinical experience in the diagnosis and treatment of autism and other autism spectrum disorders and have undertaken extensive research into these conditions¹. Together with colleagues, I am responsible for the development of what have become the accepted standardized methods for assessing children with possible autism (the Autism Diagnostic Interview Revised and the Autism Diagnostic Observation Schedule, and other related instruments). I undertook the first systematic psychiatric epidemiological study in childhood² and have extensive other experience in epidemiological studies. I have particular expertise in using epidemiological evidence to tackle the testing of hypotheses about environmentally mediated causation³. I undertook the first systematic epidemiological study examining the interconnections between neurological disorders and psychopathology⁴ and this topic has remained a focus for my clinical and research interests. With respect to the possibility that toxins may contribute to the causation of mental disorders, I have reviewed the evidence on the effects of environmental lead^{5,6}, and have done the same for the effects of the measles-mumps-rubella vaccine⁷. I have published extensively on

genetics of mental disorders (Rutter, 2006⁸) and especially on the interplay between genetic and environmental factors (Rutter, 2007⁹; Rutter, in press¹⁰).

I have won several international prizes for my work in the fields of autism, epidemiology and the study of normal and abnormal development and my standing as a research scientist is shown by my election as a Fellow of the Royal Society in 1987, as Founding Fellow of both the UK Academy of Medical Sciences (of which I am currently clinical vice president) in 1998 and Academia Europaea in 1988, and as an honorary Fellow of the British Academy in 2002, as well as election as a Foreign Associate Member of the US National Academy of Science Institute of Medicine in 1988, as a Foreign Honorary Member of the American Academy of Arts and Sciences in 1989, and as a Foreign Associate Member of the US National Academy of Education in 1990.

My full Curriculum Vitae and list of publications are attached as a separate document.

INSTRUCTIONS

With respect to the issues being considered by the Vaccine Court, I wish to make explicit that some four years ago I agreed to serve as an expert witness with respect to Thimerosal vaccine litigation. In that connection, I partially drafted a report that, in the event, was never submitted because the litigation was put on hold. Similarly, about a year before that, the same situation arose with respect to litigation over MMR. Once more, the draft report was never finalized and was never submitted because the litigation was dropped. Finally, last year I served as an expert in relation to the British General Medical Council's case against 3 pediatricians involved in Andrew Wakefield's research into autism and MMR. The issues involved there did not concern the scientific case at all but, rather, were involved strictly with the ethical conduct of the research undertaken. With respect to the Vaccine Court hearings, I have particular expertise in the steps needed to identify environmental causes of disease (Academy of Medical Sciences¹¹, Rutter, 2007¹²).

I have been asked by the Secretary of Health and Human Services to provide an expert opinion on (1) what is known about the nature of autism spectrum disorders (ASD); (2) the possible causes of ASD; (3) genetic influences on the liability to autism and their implications with respect to possible Thimerosal effects; (4) the methods for testing causal hypotheses; (5) whether or not there has been a true rise over time in the rate of ASD; (6) the epidemiological evidence on the alleged links between Thimerosal and ASD; (7) the phenomenon of developmental regression in ASD and the question of whether there might be an unusual or idiosyncratic response to Thimerosal, together with the issue of the type of evidence needed to test this hypothesis and on whether such evidence is available. I base my opinions on the available scientific evidence and, in the body of the report, I note the scientific papers that are relevant in relation to individual points. In addition, I also make use of my extensive clinical experience over the last four decades in diagnosing autism spectrum disorders and treating children and adults with these disorders.

DECLARATION

I, Michael Rutter, declare that in the preparation of my report:

In accordance with the ethical principles that I have always followed in serving as an expert witness:

- (i) I understand that my duty is to the Court and I have complied with and will continue to comply with that duty to the best of my ability.
- (ii) I have endeavored to be succinct and accurate and have covered all relevant issues around the matters that I have been asked to address.
- (iii) I have endeavored to include in my report those factors that might adversely affect the validity of my opinion.
- (iv) I have not included anything in this report that has been suggested to me by anyone (including the lawyers instructing me) without forming my own independent view of the matter.
- (v) Where there is a range of reasonable opinion, I have indicated the extent of that range in the report.
- (vi) I will notify those instructing me immediately, and confirm in writing, if for any reason my report requires any correction or qualification.

- (vii) I understand that this report will be the evidence that I shall affirm in court, subject to any correction or qualification I may make before affirming its veracity.
- (viii) Regarding potential conflicts of interest, I declare that throughout the whole of my career I have never received any funding for my research from pharmaceutical companies or any other commercial organization. I agreed to serve as an expert witness in litigation in the UK regarding the mumps - measles - rubella vaccine and received standard fees for the time spent in preparing for this role, but the litigation never resulted in a court hearing. I am, or have been, a trustee of several charities originally established by founders associated with commerce (the Wellcome Trust, Novartis Foundation, Nuffield Foundation, and Jacobs Foundation) but in all instances, the charities are completely independent in their functioning, and my main role has been in the distribution of grants, rather than in their receipt.

I believe that the facts I have stated in this report are true and that the opinions that I have expressed are correct.

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Executive Summary

- I. Autism spectrum disorders (ASD) are characterized by a combination of qualitative impairments in reciprocal social interaction, qualitative impairments in communication, and restricted repetitive and stereotyped patterns of behavior, interests and activities. These features typically first become manifest early in the second year of life. When ASD were first described, it tended to be assumed that there was always gross handicap, but it is now known that the syndrome may be present in individuals with a normal level of general intelligence.
- II. The validity of the concept of ASD as a meaningfully distinctive disorder (or group of disorders) has been shown by a range of studies indicating crucial differences from other psychiatric and developmental disorders.
- III. The scientific means of testing the validity of postulated new syndromes are well known, and well established. They have successfully shown the validity of Rett syndrome, as well as ASD, but they have not so far been applied to the hypothesized syndrome of ASD alleged to be caused by Thimerosal.
- IV. Some (probably 10 to 15%) of cases of ASD are known to be associated with specific medical conditions that are likely to be involved in the causal process. However, in the majority of instances the cause is unknown. There is good evidence that ASD are based on some kind of organic brain pathology and that this is subject to strong genetic influences. Non-genetic factors are also likely to be implicated but it is not known what these might be, other than it is probable that they operate in the prenatal rather than postnatal period.
- V. It has long been known that developmental regression occurs in about a third of cases of autism and there is no evidence that this proportion has increased over time. The degree and type of regression shows great variability and its meaning remains ill-understood. However, there is no reason to suppose that it is due to some environmental event or hazard.
- VI. The claims that Thimerosal may cause ASD are largely based on the evidence that mercury is known to be a neurotoxin that, in high dosages, damages the brain. Parallels between autism and mercury poisoning have been drawn but they are far-fetched and

unconvincing. It is pertinent that, in the clinical accounts of the effects of mercury poisoning, the occurrence of autism has not been noted. The claim that Thimerosal is responsible for the rise over time in the rate of diagnosed autism is contradicted by the evidence that the rise has continued after the withdrawal of Thimerosal in vaccines used in Scandinavia, Canada, and California.

- VII.** Within the UK and the USA, the epidemiological evidence shows no association between the dose of Thimerosal received by children and the risk of ASD. This finding is not in keeping with the claim that Thimerosal increases the risk of autism.
- VIII.** It has also been argued that, even if Thimerosal does not commonly cause ASD, it does so in a small number of unusually susceptible children. No such hypothesized susceptibility has been demonstrated. Accordingly, the causal hypothesis remains highly speculative and without research support.
- IX.** It is concluded that there is no convincing evidence that ASD can be caused by Thimerosal.

Nature of Autistic Spectrum Disorders (ASD)

Characteristics of ASD

1. As reflected in the diagnostic criteria specified in the two main internationally used systems of classification - those developed by the World Health Organization¹³, and by the American Psychiatric Association¹⁴ - autism spectrum disorders (ASD) are characterized by the combination of three main areas of symptomatology that become manifest during the first three years of life. The three domains of symptomatology are qualitative impairments in reciprocal social interaction; qualitative impairments in communication; and restricted repetitive and stereotyped patterns of behavior, interests and activities. The descriptor 'qualitative' means that the functioning in these areas is not just delayed in relation to what is expected in normal development but is abnormal, or deviant, in quality. Because of the high level of agreement across the world with respect to these diagnostic criteria, it has proved possible to develop systematic standardized methods of diagnosis based on both parental report^{15,16} and observation^{17,18}.
2. The qualitative abnormalities in reciprocal social interaction may show themselves in a variety of ways^{19,20,21}. Normal toddlers, and even older babies, are intensely social as shown by their interest in other people, the manifest pleasure that they show in social interactions, their obvious enjoyment in interactive social games, their wish to join in social activities and their desire to bring other people into their games. Similarly, very young children naturally integrate eye-to-eye gaze, facial expression, body posture and gesture when they either initiate social approaches or respond to other people's social overtures. They are aware of other people's feelings and they readily offer comfort when it is evident that someone to whom they are close is distressed. When older, they go on to form selective friendships that involve a sharing of both emotions and activities. All of these features tend to be seriously impaired in autism spectrum disorders. It is not that children with ASD are simply delayed in the acquisition of these social features; rather, their social interactions continue to lack these qualities as they grow older.
3. The qualitative impairments in communication follow a broadly similar pattern. Normal babies and toddlers make extensive social use of a range of sounds, developing into

babble, well before the time they acquire the use of spoken words. Language-related skills in thinking patterns are shown in the development of make-believe and social imitative play. Even with quite young children, after they have gained spoken language, conversations have a definite to and fro quality in which the child responds to what the other person says, as well as using words to convey their own ideas or wants. By contrast, children with ASD are impaired in the social use of language, in creativity and imagination, and in the use of reciprocity in conversational interchange. In the same way that children with an ASD are impaired in their emotional responses to other people's verbal and nonverbal overtures to them, they are impaired in their social usage of gesture and they tend to have rather stereotyped patterns of language usage.

4. The restricted, repetitive and stereotyped patterns of behavior, as evident in ASD, may show themselves in: an encompassing preoccupation with particular unusual objects; intense circumscribed patterns of interest; preoccupations with part objects or non-functional elements of play material; an apparently compulsive adherence to specific non-functional routines or rituals; specific attachments to unusual objects; and particular kinds of motor mannerisms.
5. The concept of an ASD is of a condition that involves brain abnormalities that arise early in life and which interfere with normal development in a serious way that impairs functioning across all situations and which persists over time. Numerous studies have shown that that is the case. Thus, for example, long-term longitudinal studies extending into adult life have shown the high degree of persistence of the basic problems^{22,23,24}. Individuals with an ASD may vary in their behavior according to the specifics of different situations but the autistic features will be evident to an important extent in all situations. Similarly, they may improve markedly in their functioning, although most remain substantially handicapped, but fully normal functioning in adult life is rare.

Broadening of the diagnostic concept of ASD

6. Although it was evident from the very first reports of autism onwards that autism varied greatly in the extent with which it was associated with intellectual disability or severe

social impairment, the initial view was that autism was a relatively rare condition that was qualitatively quite distinct from normality and which was usually accompanied by serious social impairment. The clinical reports and systematic studies during the 1940s to mid-1970s all reflected this concept. However, the first epidemiological study by Lotter²⁵ showed the frequency with which somewhat atypical autistic-like disorders were found. The first twin study showed that the genetic liability to autism extended beyond the boundaries of the traditional diagnosis^{26,27}. Subsequent genetic studies confirmed that this was so^{28,29,30}. The general population study undertaken by Wing and Gould³¹ also showed that atypical autistic-like disorders were even more common than autism itself. Subsequent research has amply confirmed the validity of this observation. Accordingly, the concept broadened to include a range of ASD that showed the same qualitative abnormalities but which varied somewhat in both the pattern and severity of the core diagnostic features⁷. Nevertheless, because there is no independent laboratory test for autism, or for ASD, the precise boundaries of the diagnostic concept remain somewhat uncertain³². The practical implications of these boundary uncertainties is well shown by Lord *et al*'s³³ prospective follow-up study from age 2 to age 9 of the 84 two year olds with a best estimate diagnosis of autism, 71 received the same diagnosis at 9 years, 12 shifted to a less definite autism spectrum disorder and only 1 received a diagnosis outside the autism spectrum. This represents a very high degree of diagnostic stability. By contrast, of the 46 with a less definite spectrum diagnosis, only 27 were diagnosed as having autism at 9 years and 5 received a diagnosis outside the spectrum. In other words, although most of the broader spectrum diagnoses at 2 years were thought to be autism at 9 years, a third still remained on the periphery of the spectrum and some ceased to show autism at all.

Validation of ASD syndromes

7. There is a well accepted approach to the validation of any new medical syndrome, including psychiatric syndromes^{34,35,36}. The history in relation to autism illustrates this process¹. Initially, Kanner³⁷ provided a detailed clinical description of 11 children whose pattern of behavior showed several specified common features and which seemed distinctively different from that shown by children with other developmental and

behavior disorders. His description was quite explicit in terms of what he viewed as the differentiating characteristic features. Because the syndrome had not previously been recognized at all, the next step was to find out whether other clinicians could confirm that this apparently distinctive pattern of behavior occurred in the patients that they saw. After a few years, a series of confirmatory reports appeared in the literature. The next step was to find out whether this syndrome, meaning this collection of co-occurring features, was indeed truly distinctive from other forms of mental disorder. One of the first systematic comparative studies was that undertaken by my colleagues and myself³⁸ and which did show important differences. Over the years, a range of studies from all over the world provided further validating evidence - indicating the distinctiveness of autism in relation to the particular pattern of cognitive deficits, biological features such as increased head size, neuropathological findings, an unusual age for the first development of epileptic seizures, long term course, and the rate of mild autistic-like features in other family members^{20,39,40}.

Rett syndrome

8. The history of Rett syndrome went through a broadly comparable process. That is, it began in 1966 with the identification of a set of apparently characteristic features⁴¹, better publicized in 1983 by a key report by Hagberg *et al*⁴²; this was followed by confirmatory reports by many other clinicians and researchers; and this, in turn, was followed by evidence that the course over time was distinctively different; that there was a characteristic impairment of head growth in infancy and early childhood (following a normal head circumference at birth) and the development of particular neurological features⁴³. The further validation of the syndrome was provided by the identification of a particular gene that caused the disorder⁴⁴, this being then further confirmed by animal models showing that when the gene mutation was induced in animals it produced a syndrome that very closely paralleled that found in humans^{45,46}. It is noteworthy that, prior to the general recognition of Rett syndrome, the majority of cases were given the diagnosis of some form of autism spectrum disorder⁴⁷ - despite the clinical features being rather different^{48,49}. As is usually the case, once the key characteristics of a syndrome have been identified, further work shows that atypical manifestations occur and that

these have similar correlates and consequences to the original definition of the syndrome. This happened with both autism and Rett syndrome.

The postulated syndrome of regressive autism

9. It is necessary to consider whether the same validation applies to the postulated syndrome of regressive autism that, according to some claims, is supposed to be due to Thimerosal. It is clear that there is no evidence of any comparable systematic, step-by-step, validation. Thus, up to the present time, there has been no systematic description of the defining features of this hypothesized syndrome. Reference to regression does not help very much because regression is known to occur in about one fifth to two-fifths of cases of autism as reported in all eras.

It has been argued that the regressive autism supposed to be due to Thimerosal involves symptoms associated with mercury poisoning⁵⁰ but documentation of this claim is lacking. The postulated parallels between autism and mercury poisoning are considered in more detail in paragraphs 62 to 65.

The Possible Causes of ASD

Specific medical causes of ASD

10. It is well-established that some cases of autism are associated with a specific medical condition that is likely to have played a part in causation^{51,52}. The best available evidence suggests that approximately 10-15% of cases of autism are associated with identifiable medical disorders, although uncertainty remains on precisely how they are involved in the causal processes. Thus, even with medical conditions, such as tuberous sclerosis, that have the strongest association with autism, autism develops in only some individuals. There is some indication that this may be a function of the particular part of the brain that is most affected⁵³. The most common medical conditions associated with ASD involve chromosomal abnormalities of one kind or another. With the probable exception of a particular anomaly involving chromosome 15^{30,54}, the associations with autism are weak, inconsistent, and almost certainly indirect⁵⁵. What is particularly striking, however, about the evidence is that very few of the associated medical

conditions involve some form of postnatal disease or disorder. For example, it might be supposed that encephalitis in the early years of life could involve a substantial risk for autism but that seems not to be the case⁵⁶, although there are occasional case reports of individual cases of herpes encephalitis leading to features said to resemble autism^{57,58}. Similarly, autism has not been a feature in the sequelae of severe head injuries in early childhood⁵⁹. It may be concluded that the great majority of medical conditions known to be associated with ASD involve prenatal pathology. Although, rarely, brain abnormalities acquired postnatally (such as herpes encephalitis) can give rise to ASD-like features, these are decidedly unusual.

Neural basis of ASD

11. It is now generally accepted that ASD are neurodevelopmental conditions that involve some kind of organic brain pathology⁶⁰. That this was likely was first shown by the finding from follow-up studies that about a quarter of individuals with autism who had not shown previous clinical evidence of a neurological abnormality developed epileptic seizures – most characteristically in the age period of late adolescence or early adult life^{61,62}. It is also apparent from clinical measurements of head size^{63,64,65,66}, brain scanning studies^{67,68,69,70} and post mortem brain studies^{71,72,73,74,75,76,77} that, compared with the general population, an increased proportion of individuals with an ASD have an unusually large brain. The postmortem brain studies all point to abnormalities that are likely to be prenatal in origin and what is distinctive about the brain size findings is that the increase occurs in the early postnatal period. It should be noted however, that because the studies are necessarily based on individuals who died early in life, the samples are skewed towards individuals with an ASD who were also severely retarded and suffered from epileptic seizures. The many brain imaging studies have all noted brain abnormalities in some cases of ASD^{20,39,78,79,80} and are consistent in showing a systems abnormality rather than a localized brain area abnormality^{81,82}. Neurochemical investigations, although numerous, have been singularly inconclusive in their findings apart from the well-replicated observations of raised blood serotonin levels in about a quarter to half of individuals with an ASD⁸³. Raised serotonin levels, however, are also found in other neurodevelopmental disorders. Neurophysiological studies have also

given rise to rather contradictory findings, although patterns have become clearer with more recent research⁸⁴. Like the brain imaging studies, the findings point to pervasive problems in the integration of higher brain functions rather than some specific isolated abnormality. Multiple, minor congenital physical anomalies are found in many children with an ASD, as they are, too, in other neurodevelopmental disorders⁸⁴. Like the other neural findings, their presence points to a prenatal origin.

Cognitive deficits in ASD

12. In the original descriptions of autism onwards, it has been apparent that many individuals with ASD show mental functioning that is substantially impaired. For some time, it was thought that impaired cognitive functioning might have a motivational basis but findings from a range of studies using both naturalistic and experimental strategies were consistent in indicating that cognitive deficits were intrinsic and not due to any failure in motivation⁸⁵. Numerous well-conducted studies have clearly shown that ASD are associated with relatively specific deficits in functions involved in social cognition - meaning the mentalizing processes involved in socio-emotional reciprocity and relationships^{39, 86,87,88,89,90,91}. In particular, ASD are associated with what has come to be termed a deficit in 'theory of mind'. Basically this refers to the mentalizing ability that human beings have to judge what another person is likely to be thinking on the basis of the social context or the other person's particular beliefs or experiences. Most, but not all, individuals with an ASD show such deficits and they are much less common in other conditions, although they are evident in some individuals with severe disorders of receptive language⁹². Although 'theory of mind' deficits are those that are most strongly and specifically associated with ASD, they are not the only ones that occur. It would be highly desirable to be able to diagnose ASD on the basis of these specific cognitive deficits but the exceptions are too many for this to be possible at an individual level. Although there is no serious doubt that these deficits in social cognition are fundamental to autism, their current measurement is not such that they can, as yet, be used to confirm or rule out the diagnosis of an ASD.

Genetic findings

13. The first systematic twin study of autism, undertaken by Susan Folstein and Michael Rutter^{26,27}, showed that there was evidence of a strong genetic influence. Subsequent research has amply confirmed the very strong effect of genetic influences^{28,29,30,93,94,95}.

14. Clinicians' acceptance of the likelihood of strong genetic influences on the liability to autism has been based on the consistent evidence from twin and family studies^{29,30}. In brief, the twin studies have shown a concordance rate (i.e. the proportion of pairs in which both twins show the feature) of 60-90% in monozygotic pairs as compared with 0-10% in dizygotic pairs^{96,97}. They have also shown that the concordance is not accounted for by shared prenatal or perinatal difficulties. The implication is that the large difference in concordance between identical (MZ) and non-identical (DZ) twins points to high heritability. Quantitative estimates put this at more than 90%. The same findings, however, also show that the genetic liability extends to a phenotype that is qualitatively similar to autism as usually diagnosed, but much broader and much milder^{98,99}. The family studies give rise to precisely the same conclusions¹⁰⁰. Thus, depending on what assumptions are made about the population base rate of autism in the general population, the rate of autism in siblings is some 20-100 times increased. As with the twin studies, the family data also indicate that the liability extends to a broad range of social and communicative problems and a range of circumscribed interests and repetitive behaviors. The marked fall-off in rate between monozygotic co-twins and dizygotic co-twins, plus the fall-off between first degree and second degree relatives, strongly suggests that autism is due to a relatively small number (say 2-10) of interacting genes⁹⁸. The findings are incompatible with the notion that most cases of autism are due to just one major gene (although that cannot be ruled out as an occasional occurrence) and, equally, they make it unlikely that autism is a result of a very large number of genes acting independently. The fact that the concordance rate is less than unity in monozygotic pairs, together with the other evidence from twin and family studies, also indicates the likelihood that some form of non-genetic factor is likely to play a contributory role in some instances. The fact that the concordance rate in monozygotic pairs is as high as it is, however, makes it rather unlikely that autism will be solely due to an environmental risk factor, other than rarely.

15. At present, there is no good evidence on what such non-genetic risk factors might be. It is important to note, however, that they need not concern specific environmental influences. Thus, for example, they could include the effects of developmental perturbations. Biology operates in a probabilistic fashion, setting a general pattern for development, and leaving the way open for modification of the details of what happens, as well as the operation of chance factors. Thus, for example, minor congenital anomalies are extremely common in normal children, but are rather more common in those with developmental disabilities. It is possible that the non-genetic influences on autism concern such developmental perturbations rather than a defined specific environmental risk factor. For example, Reichenberg *et al*¹⁰¹ using a large Israeli cohort, showed that a high paternal age was associated with a substantial increase in the risk for autism. Similar results have been found in two other studies^{102,103}. Possibly similarly, recent large scale collaborative research¹⁰⁴ found that copy number variations (CNV) were associated with autism in a substantial minority of individuals. CNV refer to submicroscopic deletions or duplications in individual chromosomes. They will bring about their effects through genetic mechanisms but, although their origin is not adequately understood, it may be that they are best conceptualized as developmental perturbations. The same applies to the increased rate of chromosome anomalies associated with autism. In other words, the non-genetic factors may involve random variations brought about by features that increase the risk of developmental perturbations, rather than by some specific environmental insult. It is noteworthy that these all concern prenatal effects, and not ones that took place after birth.
16. Three major issues arise out of the genetic findings. First, there is a tendency to assume that if the heritability of a liability to autism is as high as 90%, this leaves little room for any major environmental influence. It is crucial to appreciate that that is a wrong assumption. Heritability statistics are known to be both time and population-specific⁸. If environmental circumstances change in a major way, heritability figures from any time prior to that change will prove misleading. Human height provides the most obvious relevant example. It, too, has a very high heritability but, despite that being so, during

the last century there was a huge increase in the average height of people living in industrialized nations - almost certainly mainly due to greatly improved nutrition and possibly also to massive reduction in infectious disease. Similar possibilities could apply in the case of ASD. As discussed in paragraphs 29 to 40 below, unlike the situation with height, there are queries as to whether the rise in diagnosed cases of autism is mainly due to a broadening of the diagnostic concept and better ascertainment, rather than to a true rise in the incidence of the same disorder. In addition, it is necessary to ask whether there is good evidence that any postulated environmental influence actually had an impact on the incidence of autism. It is not enough to argue that such an effect could happen; rather it is essential to concentrate on the evidence as to whether it did actually happen.

17. The second issue is whether there are any genetic influences that operate in a deterministic way that is independent of environmental variations. As already noted, these play a tiny role in the etiology of ordinary varieties of autism. On the other hand, it is well known that a few cases of autism are due to tuberous sclerosis (a wholly genetic disorder)¹⁰⁵; a few to the Fragile X anomaly¹⁰⁶; a few are associated with (and possibly due to) Down syndrome^{107,108}; and, in the last few years, molecular genetic studies have identified rare cases of autism associated with several specific determinative genes^{109,110}. The findings are too recent for any definitive conclusions on their role in the causation of autism but two aspects stand out. First, these genes account for a very tiny minority of cases of ASD, and, second, their effects seem to extend beyond ASD to intellectual disability and other neurodevelopmental disorders (raising major queries on the extrapolation to ASD as such). It seems reasonable to maintain the conclusion that the general run of ASD cases that are not associated with a specific genetically caused medical condition, follow a multifactorial pattern. Where, in any individual case, there is a well established genetic cause that operates independently of environmental variations (as in the case of Rett syndrome or tuberose sclerosis), it would seem implausible that some environmental factor adds to the risk for autism. Because such instances are rare, that situation will not be considered further here.

18. The third issue is whether there is any known environmental influence that has been shown to cause ASD. The obvious example is profound institutional deprivation^{111,112}. There are strong reasons for concluding that the association is real and that it is causal. On the other hand, such deprivation has very rarely been associated with ASD in ordinary circumstances (*ie*, outside the profound deprivation that occurred in Romanian institutions) and, moreover, both the details of the manifestations and the developmental course over time are different from those usually associated with ASD. The term 'quasi-autism' rather than ASD seems appropriate and it is doubtful whether the findings have much relevance to the general run of ASD cases.

Testing Causal Hypotheses

19. Causal hypotheses almost always begin with an observation that some feature or some experience that might constitute a risk factor for some specified disease or disorder outcome is more common in individuals with that disease or disorder than in other groups with which it may be compared. There are well-established criteria that may be used to assess the likelihood that the hypothesized causal effect truly represents a causal influence^{11,12,113,114,115}. However, before considering these criteria, it is important to note the various alternatives that might account for the reported association. First, particularly when the hypothesized risk factor has to be assessed through measures that are at least partly subjective, the association may represent a biased judgment. It is well established that all of us tend to perceive what we expect to find. Accordingly, the key protection against this biasing tendency is for measurement of risk factors to be undertaken 'blind' to the group from which the sample derives - that is to say, without knowing whether the relevant sample comes from an individual with the disease or disorder or from some comparison group. Thus, for example, it was found that early claims about the association of particular urinary peptide patterns with autism¹¹⁶ could not be replicated when the analyses were undertaken without knowing from which group the samples came¹¹⁷. The same applied to the claims that abnormal serotonin reflux (*i.e.* passage of serotonin across the cell membrane) was a feature of autism¹¹⁸. Similarly, weak measurement may also give rise to misleading findings. For example, the early Scandinavian reports on the association between the fragile X anomaly (a particular form

of chromosome abnormality) and autism claimed that this was found in a quarter of cases of autism^{119,120}. Further research showed that, in fact, it probably was found in well below 5% of cases of autism¹⁰⁶. The misleading early findings date from before the time when the Fragile X anomaly could be identified by a specific DNA test. Measurement problems also probably account for the fact that neurobiological findings in autism have proved to be rather inconsistent³⁹.

20. A further possibility is that the supposed risk feature is due to some cause other than the disease or disorder being studied. For example, during the 1960s there was excitement over claims that schizophrenia was associated with particular biochemical characteristics referred to as 'the pink spot'¹²¹. In the event, further research showed that it derived from differences in drug treatment, diet and other circumstances and that there was no specific association with schizophrenia¹²². The research literature is replete with claims about hypothesized causation that proved to be fallacious once adequate methodological rigor was introduced together with 'blind' assessments. The golden rule in science is that no association, however statistically significant, should be assumed to be valid until the association has been confirmed by different studies, undertaken by different investigators, using different methodologies and samples. This is usually summarized in the requirement that the association should be a *consistent* one.
21. A further methodological consideration concerns the possible bias introduced by the study of atypical samples of one kind or another. For example, the early reports by Kanner noted the high social class of the parents of individuals with autism. Subsequent systematic research has failed to confirm this association and it seems likely that it was a consequence of the types of professional families who were aware of Kanner's standing as the most distinguished child psychiatrist of his day and who had special knowledge about autism¹²³, and therefore they sought referral to him.
22. Traditionally, it has been expected that a causal influence is more likely to be valid if the association between the risk feature or experience and the disease/disorder outcome is *strong* and if there is a *dose-response gradient* (meaning that the likelihood of the

disease/disorder developing is greater according to the severity or duration of exposure to the risk feature or experience). Both criteria provide a useful guide, but it is necessary to appreciate the constraints that operate in their application to any particular causal hypothesis. With respect to the strength 'criterion', this must be assessed in an appropriate fashion. Thus, with respect to almost all risk features, far more people escape the risk than succumb to it. Thus, only a minority of people exposed to infectious agents (viruses and bacilli) develops an infectious disease. On the other hand, the association is strong in the sense that the disease does not develop in the absence of exposure to the infectious agent. It is also important to appreciate that some risk factors only operate on particularly vulnerable or susceptible individuals. Thus, only people with an allergy to nuts develop a life threatening shock reaction to the ingestion of nuts. However, it is never acceptable simply to infer a particular vulnerability because the person develops the disease or disorder. It is necessary to have an independent means of measuring it. Thus, to follow through on the nut analogy, special skin sensitivity tests can identify such vulnerability. The *dose-response gradient* criterion might be thought not to apply to risks of an either/or categorical variety, such as whether or not someone has had vaccination involving Thimerosal. That might apply if the response to mercury was a result of an unusual idiosyncratic hypersensitivity to mercury. Nevertheless, it is still relevant in the important sense that there must be one or more mediating mechanisms and it may be expected that these should show a biological gradient. Moreover, if the postulate is a risk effect due to the direct neurotoxic effects of mercury, a dose-response gradient would be expected. This is to say, if the causal effect is real (i.e. is not just a coincidence or the effect of some other risk factor) the clear expectation is that the degree of risk should be systematically associated with either the total dose if mercury has accumulated over time, or the level of dose in any one vaccination, or the number of vaccinations involving mercury that were received.

23. *Specificity* is a further useful criterion. Other things being equal, associations that apply to a diverse range of diseases and disorders that are known to have a different underlying physiology or pathology are less likely to represent a truly causal effect. For example, Nelson and her colleagues undertook an interesting study, using blood taken during the

newborn period, to assess neuropeptides and neurotrophins in children with autism and children with non-autistic varieties of intellectual disability^{124,125}. Both groups were compared with control children who showed neither set of problems. The findings were informative in that, despite the major differences between autism and intellectual disability as investigated in other ways, the neuropeptides and neurotrophins did not differentiate the groups with and without autism, although both were strikingly different from normal. Although the lack of association with a specific diagnosis does not rule out a causal effect, it does suggest that the risks, in so far as they are real, probably are contributory in an indirect fashion (or are a consequence of, instead of a cause of, brain abnormalities) rather than features that are directly involved in the causal pathway to autism. The caveat, however, is that some risk factors do have a causal influence on a diverse range of disorders because the risk factor involves several different, but associated, risks. The risks associated with smoking cigarettes constitute an obvious example (the increased risks include lung cancer, coronary artery disease and osteoporosis - to mention just a few of the risks). In this case, however, there is other evidence that the causal effect is valid. A further caveat is that some risk factors, both genetic and environmental, have effects that span across a range of supposedly different disorders. The apparent diversity in these instances may be the result of the boundaries between diagnostic groups being defined in ways that are scientifically inappropriate. In other words, if risk effects are non-specific the onus is on the person putting forward the hypothesis to indicate the common unifying factor and to undertake the relevant research to test whether or not that is in fact operating.

24. A *timing* link constitutes one more criterion. Ordinarily, this is understood to involve two rather different concepts. First, most obviously, the exposure to the risk factor must precede the onset of the disease or disorder (risk exposure can scarcely cause something that has already happened!). The second is the expectation of a decline in likelihood of effect with increasing time after exposure. Although that does apply to some sorts of risk mechanisms, it does not apply to others. On the other hand, if there are postulated delayed effects, the onus is on the person putting forward the hypothesis to specify the conditions under which this occurs and to test for the hypothesized mediating

mechanisms. It is not acceptable just to say that the effects may be immediate, or delayed, without specifying why it is the former in one case and the latter in another. Without such specification, the hypothesis is rather meaningless and impossible to test.

25. *Biological plausibility* and *biological coherence* are also of some relevance but they really operate effectively in only one direction. That is to say, almost anything is biologically plausible. It is only when it is impossible to conceptualize any kind of biological mechanism that a causal hypothesis can be downgraded as rather unlikely to be true. Coherence means that there is a meaningful causal pathway, which may involve several different steps, but with each step open to testing (and therefore refutation) and with the pathway fitting in with what is known about the underlying pathophysiology of the condition.

26. Experimental and quasi-experimental testing is usually required in order to assess causal hypotheses in rigorous fashion. The crucial element is the development of firm predictions on circumstances that would either support or refute the causal hypothesis. For example, raised serotonin levels in the blood have been found in up to half of individuals with autism. It was possible that these raised levels could play a part in the causation of autism although the complete lack of specificity (high levels are found in a wide range of neurological and neuropsychiatric disorders) raised doubts. However, if the high serotonin levels were involved in causation, it should follow that a reduction in serotonin levels ought to have benefits for key autistic symptoms (unless the high levels led to irreversible damage). Fenfluramine is a drug that has a powerful effect in reducing serotonin levels but a series of studies showed that it had negligible effects on the main autistic phenomena¹²⁶. Similar considerations apply to the hypothesis that excess opioids carry a risk for autism¹²⁷. The supposed evidence in support mainly derives from chromatographic studies that do not allow accurate identification of the compounds in the urine. A systematic, but small scale, study by Hunter *et al*¹²⁸ failed to find any excess opioid peptides in the urine of children with autism, thereby casting doubt on the hypothesis. However, if the hypothesis were correct there is also the implication that a drug that countered opioids, such as Naltrexone, should improve autistic manifestations

but it seems that it does not, although it may have a slight beneficial effect on hyperactivity⁸³. Similar issues arose with respect to the supposed therapeutic benefits of secretin in the treatment of autism. Numerous studies showed that the claims were incorrect¹²⁹. Quasi-experimental testing is made possible by considering other circumstances that should give rise to the postulated risk effect. For example, in relation to the hypothesis that MMR (the measles-mumps-rubella vaccine) predisposes to ASD because an intestinal lesion leads to increased intestinal permeability indicates the value of focusing on other intestinal disorders that are likely to result in increased permeability. With respect to the relevant age group, celiac disease constitutes the most obvious example of that kind. So far as I am aware, there are no systematic epidemiological studies looking at the association between celiac disease and autism spectrum disorders but it is noteworthy that the most exhaustive review of biological factors in autism does not document such an association¹³⁰ and specific studies have failed to find a link between autism and celiac disease^{131,132}.

27. In some circumstances, challenge tests may provide a useful experimental approach. For example, some years ago it was argued that food additives created a risk for hyperactivity disorders. The epidemiological evidence was largely negative, but somewhat inconclusive¹³³. The possibility remained that there might be a small subgroup of children who had an unusual sensitivity to either food additives or other food products. Systematic experimental studies of children whose behavior suggested that this might be the case were undertaken by reducing children's diet to a very basic level and then gradually introducing foodstuffs one by one. The findings showed that, a few children had consistent adverse responses to certain specific food substances¹³⁴. Although this probably accounted for quite a small number of children with hyperkinetic disorders, it did seem to be a truly causal effect in this identifiable small number with an unusual idiosyncratic response, and a more recent study¹³⁵ showed the same with respect to particular food additives.
28. With non-experimental studies, one of the most serious methodological problems is dealing with possible confounding variables. Confounding variables are ones that are

associated with both the hypothesized environmental cause and the disease outcome being studied. Because of this double association, the true causal effect may be actually due to the confounder and not to the hypothesized environmental causal factor at all. Experienced epidemiologists are well accustomed to undertaking a variety of statistical tests to rule out the possible biases created by confounders that they both know about and can measure accurately. The greatest problem is provided by the possibility that there may still be bias stemming from unknown, unmeasured confounders¹¹. There is no single universally effective answer to the problem when dealing with non-experimental studies. Nevertheless, the key lies in thinking carefully about alternatives to the claimed environmental effect and devising strategies to test whether they might be operative. In considering claims regarding Thimerosal and autism, that is the approach that should be used.

Has There Been a True Rise Over Time in the Incidence of ASD?

29. As several authoritative, well conducted, reviews of the research evidence have shown^{19,136,137,138}, there is no doubt that autism is being diagnosed now much more frequently than it was at the time of the first epidemiological study nearly 40 years ago. Several questions need to be asked in relation to this evidence. First, there is the question of the quality of the more recent epidemiological studies. There can be no doubt that the quality of the larger recent studies is far superior to the earlier studies. That is because they have had a much better coverage of the general population, because they have not been so reliant on children being referred to specialized clinics and because the diagnoses have been made using well standardized and well tested diagnostic instruments. Thus, these qualities are evident in the systematic study undertaken by Chakrabarti and Fombonne^{139,140} and the follow up study of early developmental screening undertaken by Baird *et al*^{141,142}. The research designs used by these two studies are rather different but they have helpfully complementary advantages. The findings of both studies show a much higher rate of ASD than was accepted on the basis of the early studies. Because of the continuing uncertainties over the boundaries of the syndrome of autism, it is not yet possible to arrive at a firm conclusion on the true rate of ASD but probably it may be at least six per thousand for spectrum disorders as a whole,

with about a third of these accounted for by autism 'proper'^{19,138}. It may be concluded, therefore, that there can be reasonable confidence that the present estimates of a rate of autism that is much higher than was accepted in the past are likely to be valid.

30. The next question is whether the same confidence can be expressed with respect to the much lower rates found in the earlier studies. It is clear that it cannot. To begin with, most of the earlier studies rely to a considerable extent on some form of screening based on the children attending some clinical facility or some form of special school or residential unit. There was much less satisfactory coverage of children attending ordinary schools and both hospital and educational services for children with autism spectrum disorders were very much less well developed and much less freely available than is the case today. During the 1960s and 1970s few child psychiatrists and few pediatricians, and even fewer general practitioners, were experienced in the recognition and diagnosis of autism spectrum disorders. Thus, this is reflected in the finding in the Howlin and Moore¹⁴³ study that children now are receiving diagnoses of an autism spectrum disorder at a substantially earlier age than was the case some decades ago. The much less satisfactory ascertainment of cases in the 1960s and 1970s will certainly have led to cases of ASD being missed in the studies undertaken at that time.

31. Barbaresi *et al*¹⁴⁴ examined the incidence of autism in Olmsted County, Minnesota, between 1976 and 1997. The database was provided by the Rochester register, with systematic diagnostic concepts being applied to the records data. The findings showed nearly a twofold rise over this time period for developmental and neuropsychiatric disorders other than autism. There was a comparable rise for autism up to about 1988 but thereafter there was a much greater increase over the next decade. It was concluded that the rise was likely to be due to improved surveillance and a broadening of the diagnostic concept. That possibility could not be tested in a systematic fashion. It was noteworthy that the rise took place nearly 30 years after the widespread use of the MMR vaccine and at a time when there had not been any change in vaccination practice (because Minnesota required completion of vaccination before school entry).

Nevertheless, vaccination status was not determined and therefore causal links could not be tested.

32. A further question is whether concepts of ASD have changed over time and, in particular, whether they have broadened in a manner that would lead to the diagnosis of ASD now that would not have been made a generation ago. It is clearly apparent that there has indeed been a broadening of the diagnostic concept. In part, this has come about because the twin and family studies have shown that the genetic liability to autism extends well beyond the traditional concept of autism as a severely handicapping disorder usually associated with substantial intellectual disability²⁸. The most obvious way in which this is reflected in the modern epidemiological studies using systematic standardized methods is the major increase in the proportion of children with ASD who have an IQ in the normal range^{139,141,145}. One preliminary study that sought to consider whether the application of modern concepts to old data would increase the rates of ASD in early studies found that it did¹⁴⁶ and another report by a different research group¹⁴⁷ argued that the rise over time could be accounted for by methodological considerations. There is no doubt that they play a major role but it is much more uncertain whether they fully account for the rise.
33. Three studies of autism in California have attempted to determine whether the rise in rate reflects a true increase over time in incidence, but the findings are not entirely consistent. The Department of Developmental Services¹⁴⁸ used standard records data from 21 regional centers over the time period from January 1987 through December 1998. They found that the percentage increase in the diagnostic populations was substantially greater for autism (210%) than for intellectual disability (49%) and other neurodevelopmental disorders. The increase in autism as such was, however, less than that for other pervasive developmental disorders (equivalent to autism spectrum disorders other than autism). It was noteworthy that there was a significant increase over time in the proportion of individuals with autism whose intellectual functioning was in the normal range. An update using data covering 1999 through 2002¹⁴⁹ showed that earlier trends were generally continuing, with the rate of ASD still rising, and the proportion of cases

of ASD without intellectual disability also increasing. The findings are limited by the fact that they reflect administrative prevalence rather than true incidence.

34. In the United States a multi-site surveillance network¹⁵⁰ was established to assess the prevalence of ASDs among children aged 8 years in 14 areas. The findings are compatible with other surveys in showing a much higher rate of autism than was the case a generation ago. However, what is most striking about the report is the huge variation in ASD prevalence across the different States, plus variation across sites as well with respect to ethnic variation and sex differences. In my opinion, such variations provide a warning that administrative prevalence rates are likely to be open to important artifactual variations as a result of variations in service provision.

35. Croen *et al*¹⁵¹ conducted a population-based study of birth cohorts across the time period 1987 to 1994, using the same data records as the 1999 study outlined above. The study had the advantage of linkage with birth certificates and, hence, the ability to generate incidence figures, albeit based on records diagnoses. The findings showed an increase in the rate of autism from 5.8 per 10,000 for children born in 1987 to 14.9 per 10,000 for those born in 1994 (despite the fact that the latter group had a shorter period of time during which there was the opportunity of making a diagnosis). The increase in the rate of autism was somewhat greater for those without intellectual disability. Over the same time period, the rate of diagnosed intellectual disability fell from 28.8 per 10,000 to 19.5 per 10,000. The authors reasonably concluded that the findings suggested that at least part of the rise in rate was likely to be due to changes in diagnostic practice and better ascertainment.

36. Blaxill *et al*¹⁵² have pointed out that ascertainment bias is likely to have led to an underestimation of the rate of autism in the younger cohorts, and that the usually later age of recognition of intellectual disability compared with autism meant that the intellectual disability figures would also be underestimates that provided a misleading picture of a fall over time in rate. In response, Croen and Grether¹⁵³ undertook a reanalysis that focused only on diagnosis by age 4 years. The results showed a marked

rise over time for the diagnosis of autism but no change over time in the rate of intellectual disability. They concluded that their original suggestion that the diagnosis of autism might, in part, represent a diagnostic substitution for intellectual disability could not be upheld.

37. The third study¹⁵⁴ used the same records data but differed in that sub-samples were assessed using standardized diagnostic measures. They, too, found an increase over time in the proportion of children with autism said not to be mentally retarded (50% in the 1983-85 group vs 78% in the 1993-95 group) but IQs were not measured. There was no change over time in the proportion showing developmental regression (28% vs 34%). Because there was no change over time in the level of agreement between the administrative diagnosis and the standardized research diagnosis, it was concluded that the rise over time was not due to changes in diagnostic practice. Unfortunately, the findings are severely constrained by the fact that the response rate was so extremely low (10% to 24% according to subgroups).

38. The Brick Township study¹⁴⁵ showed a high rate of autism (40 per 10,000) as compared with earlier reports. Because the rate of autism was higher than that of other autism spectrum disorders (27 per 10,000), it has been inferred by some reviewers that it was unlikely that the rise in rate over time was a result of a broadening of the diagnostic concept. However, that is not a justifiable inference for four separate reasons. First, as the authors noted, the measures used were not satisfactory for the differentiation between autism and other ASD (thus, for example, there was no standardized parental interview such as the ADI-R). Second, the sampling will have omitted higher functioning children with autism spectrum disorders who were not receiving special education – again a point emphasized in the paper. Third, despite this omission, half the children with ASD (including autism) had an IQ in the normal range - a much higher rate than that found in the early epidemiological studies. Fourth, the sample was deliberately selective, in order to focus on a small geographical area thought to have a particularly high rate of autism. It should be added that the rate of developmental regression found (24%) was well within

the range reported in earlier studies - a finding that provides no support for suggestions that so-called 'regressive autism' is the variety of ASD that is particularly increasing.

39. Other epidemiological studies have also shown that the proportion of cases associated with normal nonverbal intelligence is probably substantially higher than that found in the earlier studies. In Lotter's²⁵ survey (the first systematic epidemiological study), two thirds of the children with an ASD had an IQ below 55. Similarly, in Gillberg's Swedish population studies in the late 1970s/early 1980s^{155,156,157}, the majority had an IQ under 50. His further study in 1988¹⁵⁸ showed an increased rate of autism (11.6 per 10,000) compared with the first two surveys (4.0 per 10,000 in 1980; 7.5 per 10,000 in 1984). He commented that there had been a parallel rise in the proportion of children with autism spectrum disorder who had a normal, or near normal, IQ (precise figures were not given but, in the 1988 survey, 18% had an IQ above 70). By contrast, the modern studies have all shown a much higher proportion of children with a normal IQ and a lower proportion with severe retardation. Thus, in the Baird *et al* study¹⁴¹ the proportion with an IQ in the normal range was estimated to be 60%. Similarly, in the British nationwide survey of child mental health¹⁵⁹, only about half had intellectual disability (although it needs to be noted that the assessments of intellectual level were rather crude). Chakrabarti & Fombonne¹³⁹ in their well conducted prevalence study in children aged 22 to 62 years found that of the 26 children with autism 31% had an IQ in the normal range; of the 53 with some other unspecified type of autism spectrum disorder the proportion was 92%; and all (100%) of the 12 with Asperger syndrome had an IQ within the normal range. A more recent repeat survey of a new preschool population produced broadly comparable findings¹⁶⁰. In summary, although there appears to have been a rise in all varieties of ASD, the rise seems to have been greatest in those with a nonverbal IQ in the normal range. This implies a broadening of the diagnostic criteria.
40. The evidence is far too fragmentary for there to be any quantitative estimate of the size of this effect but there can be no doubt from the evidence considered as a whole that a substantial part of the rise in the rate of autism as diagnosed reflects a combination of better ascertainment and a broadening of the diagnostic concept. What is impossible to

determine, however, is whether or not these account for the whole of the increase over time in the rate of diagnosed autism. When this issue was discussed at a Novartis symposium in 2002 bringing together international experts in the field²⁴ there was a consensus that no firm conclusions were possible. Although much of the apparent rise is undoubtedly a function of better ascertainment and broadening of the diagnostic concept, it remains possible that there has, in addition, been a true rise that is not simply a consequence of changes in methodology. The evidence on the effects of removing Thimerosal from vaccines (see paragraphs 83-88) does not support the suggestion that the rise was due to Thimerosal but the evidence does not rule out the possibility that there may have been a true rise associated with some other as yet to be identified environmental risk factor.

Developmental regression in ASD

41. Despite the fact that regression has long been recognized as a feature of the development of autism in about a third of cases^{25,139,161,163,164,165,166,167,168,169,170,171} it has been subjected to very little systematic study until recently. The reports are consistent in showing that the phenomenon occurs in some 20-40% of children with ASD and there is no indication that this proportion has either increased or decreased over time. As indicated by the definition used by Tuchman and Rapin¹⁶², the phenomenon may involve the loss of no more than a vocabulary of three words. On the other hand, it may involve a more marked loss of language skills after the acquisition of a higher level of language competence. In some cases the loss involves only language but, more commonly, there is an accompanying deterioration in nonverbal communication and in play skills, and there may be a worsening in other aspects of behavior and, occasionally, a cognitive decline. According to parental reports, about a third of the children appear to have been entirely normal before the regression¹⁷⁰ but, in the majority of cases, there have been some pre-existing autistic features that have worsened. Regression typically takes place between 1 and 3 years of age, but most commonly between 18 and 24 months.
42. The few systematic studies of regression have employed several rather different strategies. First, Werner and Dawson¹⁷² used home videos to test the validity of

regression as reported by parents. The findings showed that a sizeable subgroup of individuals with autism appeared behaviorally unremarkable during the first year of life but then regressed in ways that could be documented on the home videos. The small size of the sample and nature of the data did not allow a firm quantification of the frequency of this valid regression but, clearly, it was not rare. Second, there have been a small number of prospective research assessments of individuals who are regarded as high risk because of an older sibling diagnosed as having autism^{173,174}. The findings showed that the great majority of the infants were unremarkable in the first year of life but many showed regression during the second year of life. Again, the number is too small to allow quantitative estimates, but the findings do suggest that substantial regression is a relatively common feature, rather than a rare one.

43. The third approach is represented by the multisite study undertaken by Richler *et al*¹⁷⁰. The data concerned 1,592 children diagnosed with ASD across the multiple sites. There was a focus on the children reported to have shown regression and a detailed telephone interview was undertaken to provide more detailed information. The findings indicated that just over 70% of the children who showed developmental regression had already exhibited autistic-like abnormalities before the onset of regression. On the other hand, there were nearly 30% of children whose functioning prior to the regression was in the normal range. The implication is that regression following a period of normal development is quite a common feature, and not a rare one. A variety of comparisons were made to compare the regression and no-regression groups. Taken as a whole, the findings indicated that children with regression, like children without regression, were rather heterogeneous, with varying trajectories of development. Although some differences between the regression and no-regression groups were found, the results did not suggest a characteristic regression syndrome. The findings were necessarily limited by the need to rely on parents' retrospective recall regarding regression. Chaworska *et al*¹⁷⁵ similarly compared children with ASD according to both presence and absence of regression and the age of the child when the parents recognized the problem. Again, no distinguishing features for regressive autism were found.

44. The fourth approach, represented by Pickles (personal communication), Baird (personal communication) and their colleagues addressed the question as to whether developmental regression is particularly associated with autism or whether it is found in a broad range of non-autistic developmental disorders. The findings showed that developmental regression was, indeed, much more frequent in children with autism than in those with other developmental disorders. However, the data also showed that it was probably misleading to see regression in categorical terms because regression in the autism group varied enormously in its extent, as also noted in the other research designs.
45. Although not well documented by systematic research, it is likely that the phenomenon of developmental regression covers several somewhat different clinical circumstances. To begin with, particularly when the loss of language involves just the loss of usage of a vocabulary of only 3 or 4 words, or an apparent diminution in communicative intent (i.e. vocalizations or words come to be less used in a manner that is directed to another person with the apparent intent of conveying some meaningful message - either with respect to social interest or request for something), it may well be that the change is in parental perception and recognition, rather than any real change in the child's capacity. It is normal for children's development (in all its features and not just in language) to have its ups and downs and plateaus without any of these indexing any fundamental change. It is entirely unknown what proportion of apparent cases of developmental regression fall into this category of measurement artifact but it seems likely that some do.
46. Second, there are undoubted cases in which there has been a true loss of language skills, generally associated with a loss of communicative intent, but usually from an initial low starting point of language competence. None of the evidence available so far (but the amount of evidence is meager and its quality limited) has shown any difference between autism with and without this type of developmental regression. At first sight, it is tempting to suppose that it ought to be due to some new stress or trauma but there is no evidence that this is in fact the case. It is much more likely that it reflects changes in the pattern of language skills (in which the role of understanding in language is becoming increasingly important) and/or the brain systems underlying their development. For

example, it is well established that the vocalizations of deaf children are near normal during the first six months of life but, during the second six months of life, become increasingly distorted and abnormal in quality^{176,177}. This is likely to come about because it is only at this age that vocal productions become influenced by auditory input. In much the same way, children all over the world show much the same range of ability to make phonological discriminations (meaning discriminations among different sounds) but that, during the second half of the first year, this changes according to the language environment in which the children are being reared^{178,179}. In other words, in parallel fashion to the changes that occur with deaf children, normal children's phonological discriminations begin as independent of language input but, increasingly, become influenced by such input. In the same way, it may well be that the language usage of children with ASD becomes impaired as reliance on language comprehension becomes increasingly important. There is also evidence from animal studies that various cognitive skills are under the influence of different brain systems in early infancy from those that sub-serve the same function at a somewhat later age¹⁸⁰. We do not know whether or not these considerations apply to the phenomenon of developmental regression as seen in individuals with ASD but it is quite likely that they do. Certainly, they indicate that it would be most unsafe to assume that developmental regression has to constitute a response to some external stress or trauma. Moreover, considered as a whole, the evidence suggests that regression is a common (not rare) feature in autism, and that it varies greatly in degree making it more appropriate to consider it as a dimensional feature and not a categorical one.

47. In addition to these apparently milder varieties of developmental regression, there are two more severe varieties, both of which are substantially rarer. First, there is a neurodegenerative disorder first identified by Rett which first became widely known as a result of a paper by Hagberg *et al* (see paragraph 8). This occurs almost exclusively in girls and is now known to be due in most instances to an abnormal mutated gene on the X chromosome^{181,182}. In its early phases it often includes social impairments that have some similarities with autism but it differs markedly in its subsequent course. It is associated with a loss of purposive hand movements and with progressive neurological impairments. Rarely, the condition seems to run in families but usually it develops in

sporadic fashion. Despite slight surface similarities with some autistic features in the early stages, it probably has no basic connection with autism and the available evidence suggests that mutations in the coding region of MeCP2 (i.e. those associated with Rett syndrome) do not play a major role in autism susceptibility. Thus, the molecular genetic study by Beyer *et al*¹⁸³ of 152 patients with autism found only 3 mutations. These were not localized within functional domains of MeCP2 and were of unknown significance. As is the case with other genes, there are many variants other than the one known to cause the handicapping conditions and little is known on whether these unusual variants are just normal variations that have no clinical implications or whether they have functional consequences that have not yet been determined. It is not possible entirely to exclude the role of such variants in autism, but on the evidence to date, it seems unlikely that they play a causal role.

48. In addition, there is an ill-defined condition now usually termed a 'disintegrative disorder' although it has received several other diagnostic labels in the past. It is distinctive in terms of having been preceded by normal development up to the age of about 2 years of age and by the fact that the regression is much more widespread in its effects than is the case with those types ordinarily associated with ASD and with a worse prognosis^{184,185,186,187,188,189}. The condition is much rarer than autism and it remains quite unknown whether, in most instances, it represents a variant of autism or something quite different. In the great majority of instances, investigations have failed to show any identifiable neurological, or other medical, condition that might be responsible for the deterioration. However, new cases of neurological disorder have very occasionally been found¹⁹⁰.

The CHAT (Checklist for Autism in Toddlers) study

49. The results of this study showed that a substantial number of children were not detected as showing autism at the age of 18 months, when a particular, highly focused, screening instrument was used, but were subsequently diagnosed as having the syndrome of autism. However, that does not necessarily mean either that the children were developmentally normal at 18 months or that autism subsequently developed in relation

to regression. Thus, in relation to the first point, Baird *et al*¹⁴¹ noted that many parents, when interviewed when their children were aged 3 to 7 years, reported abnormalities that had been present at 18 months, although they had not reported it at that age contemporaneously. In part, this seems to have arisen because parents misinterpreted the meaning of some of the questionnaire items, and in part, because the items required quite subtle discriminations. Such discriminations were particularly difficult for parents when it was not evident that their children had a handicapping disorder, the significance becoming much more apparent at a later age when it was clear that the children had an autism spectrum disorder. So far as regression is concerned, it is noteworthy that only 9 of the 31 false negatives (i.e. those children who passed the 18-month screening but subsequently were diagnosed as showing autism) were reported on contemporaneous clinical records as having had a period of regression after the 18-month screen. This proportion is almost exactly the same as that found in all previous studies of autism. Moreover, because the study concerned only a single birth cohort of children, the findings present no evidence one way or the other, on whether the early recognition of autism or the recurrence of regression had changed over time.

The age of the children when first concerns regarding ASD are raised

50. The age of first parental concern has been assessed in several different ways. Thus, the Autism Diagnostic Interview¹⁵ asks parents whose children have a recognized autism spectrum disorder to report retrospectively on the age at which they first became concerned over their children's development. Because retrospective recall is likely to be influenced by later knowledge, one must expect that the reported age at first parental concern may well be earlier than was actually the case at the time. Nevertheless, in spite of that, the data reported by Fombonne and Chakrabarti¹⁶¹ on three separate samples all reported a mean age of about 19 months.

51. Howlin and Moore¹⁴³ used a questionnaire survey of nearly 1,300 parent members of autistic societies in the UK (likely to be an unusually well-informed group). They found that parents first became aware of a problem in their children's development at a mean age of 1.7 years, with only 16% aware by the age of 12 months. On average, parents

tended to wait a further 6 or 7 months before actively seeking professional help and a formal diagnosis was not usually made until the age of 6 years. However, there had been a dramatic fall over time in the age of diagnosis from about 10 years for children currently aged over 15 years to 2.7 years for those currently aged under 5 years. Apart from the report by Gillberg *et al*¹⁹¹ (based on a small sample that was unusual in that all children attended the clinic before the age of 3 years, and half did so before age 2) that most parents identify problems before 12 months, the evidence is consistent that it is usual for parents to first become concerned around the age of 19-20 months. The range is quite wide but a concern as early as 12 months of age is decidedly unusual.

52. An alternative approach is provided by a focus on case identification using infant screening tests. Johnson *et al*¹⁹² found that children with learning disabilities showed a sharp increase in reported abnormalities in four main categories at 12 months of age but, by sharp contrast, the children with autism had a selective increase only in social abnormalities as assessed at 18 months of age. In other words, the infant tests were not picking up autism at 12 months, although they did pick up learning disabilities.

53. Surveys of parents show the same pattern. Thus, parents are understandably and appropriately concerned with the delay between the time when they first realize something is the matter with their child and the time when professionals diagnose autism but the usual age at which they first become concerned is mid-way through the second year of life^{143,193}. In summary, the evidence is consistent in showing that although subtle abnormalities in social behavior are often evident by 12 months of age, it is decidedly unusual for parents to be aware of these abnormalities at that age. Moreover, infant tests usually fail to identify autism at 12 months and, as the CHAT study showed, they even miss many cases of autism at 18 months. The early manifestations of autism are quite subtle and it is usual for them to be detected only at some point during the second year of life.

Early manifestations of ASD

54. Several studies using home video tapes or movies have provided suggestive evidence that, in many cases, children with ASD do show features of behavior that are abnormal even at or occasionally before 12 months of age^{194,195,196,197,198,199,200,201}. Most of these studies were severely limited by the fact that many, perhaps most, of the children with ASD are also intellectually impaired and it may be that some of the differences derive from the intellectual impairment rather than the ASD. Moreover, several points need to be made about most of these studies and the inferences based on them. To begin with, most of the studies do not specify when the parents first felt concern about their children's development. Many of the children with autism also had intellectual disability (leaving it quite uncertain whether the early manifestations were of intellectual impairment or of autism) and it was often unclear whether or not the observers who made the ratings from the videotapes knew which group each child was in. Particularly with the early studies, the measures were not particularly rigorous.
55. However, none of these criticisms apply to the study by Osterling, Dawson and Munson²⁰¹ and, therefore, more detailed attention needs to be paid to their findings. Of the 20 children with an ASD that they studied, one third (7) were considered by their parents to have shown no abnormalities of behavior at 12 months but did have a later regression. Accordingly, the findings indicated that, as with other research findings, a regression in the second year of life is found in about a third of cases. The 20 children with an ASD were compared with 14 children with intellectual disability and 20 typically developing children. Both the first two groups had a mean IQ in the mentally retarded range but particular attention was paid to the six children with ASD whose IQ was in the normal range. Out of the seven behaviors investigated, only two differentiated the ASD group from the children with intellectual disability and the normal children. These items concerned orienting to the child's name being called and looking at people. The differences in these two measures of social attention were not found for the seven children with later onset autism. The author's note that it would be very difficult in a clinical assessment to assess the amount of time the child spends looking at other people and the differences on this item, although statistically significant, were relatively small.

Orienting to call by name was a much more satisfactory indicator of autism. When five items were combined in a statistical analysis (a discriminant function analysis) designed to assess the predictive accuracy of the observations, it was found that three of the 13 children with ASD were wrongly classified as not having a disorder and four of the 34 children without an ASD were classified as having such a disorder. Because, in the general population, normality is much more common than autism, this misclassification rate means that the great majority of children designated on a videotape as having an ASD would, in fact, not have such a disorder. Thus, if autism occurs in one child in 500, this means that, of the 499 without autism, about 59 would be wrongly designated by videotape evidence as having autism, compared with less than one child who truly had autism.

56. In that connection, an earlier report from the same research group^{194,199} noted that a pediatrician viewing videotapes of the children at 8- to 10-months could not differentiate the groups at a better than chance level and even on the tapes at 1-year she wrongly identified half the normal children as showing autism – in other words, a very marked tendency towards over-diagnosis.
57. An earlier, more impressionistic study by Lösche¹⁹⁸ showed that the main differences between normal children and children with autism became apparent from early in the second year of life. The findings from Adrien *et al*^{196,197} also showed that more differences were evident in the second year of life than in the first. It is said that the home movies were taken before the parents themselves recognized developmental problems but no details are given with respect to the time relationship between parental recognition and abnormalities as assessed by experts on observation of the movies.
58. It may be concluded that although experts are often able to identify features of autism on videotapes taken at the age of 1-year, in most cases the parents had not recognized problems at that time, and experienced professionals, too, were not very good at differentiating autism from normality. From a theoretical point of view, it is undoubtedly important that subtle social abnormalities are evident in many cases by 12-months of age,

but the findings certainly do not indicate that the diagnosis can be made readily at that time on the basis of ordinary clinical assessment. In summary, there needs to be caution in using home videotapes for diagnostic assessment. Similarly, there needs to be caution with respect to any conclusion that development was fully normal up to some specified age. That is because, inevitably, videotapes are selective (having been taken for family purposes rather than diagnosis) with respect to abnormalities missed. On the other hand, when home videos show clear positive evidence of developmental abnormalities, this can and should be used to rule out fully normal development prior to the specified age. That is relevant in assessing regression.

Consistency of the clinical pattern of ASD

59. All studies of all kinds over the years have been entirely consistent in showing the very wide variation in the manifestations of autism spectrum disorders. This is perhaps most strikingly shown in the amazing variation even within monozygotic (identical) twin pairs when both twins have autism⁹⁹. Not only did the twins often differ markedly in the details, pattern, and severity of autistic features but they also varied greatly in their level of measured intelligence. In one pair, the two twins differed by over 50 points of IQ. Family studies show the same in indicating that when two members of the same family both show autism, the particular features show surprisingly little similarity. Epidemiological studies show comparable variability in patterns of symptomatology and levels of associated intellectual impairment¹³⁸. Follow-up studies, too, have shown a remarkable variation in the outcome in adult life even within a group of individuals with an autism spectrum disorder who have a normal level of nonverbal intelligence²⁴.

60. It is important to emphasize that this variability in clinical manifestation is a feature of many medical conditions and it is in no way specific to autism. Moreover, the variation is apparent even in conditions due to a single mutant gene that causes a medical condition without the need for any environmental risk factor. For example, that is apparent with both tuberous sclerosis²⁰² and Rett syndrome^{42,181}. Thus, with tuberous sclerosis the condition may be shown just by subtle skin features that can be detected only by an expert but, equally, may take the form of a grossly handicapping disorder

involving severe epilepsy and severe intellectual disability. Accordingly, because of this extreme variation in clinical features, it is very difficult, if not impossible, to draw firm conclusions about a possible new cause of autism solely on the basis of a supposed atypicality in behavioral manifestation. So far as the postulated association between Thimerosal and autism is concerned, none of the reports indicate whether there is anything distinctive about the pattern of symptoms when the ASD has supposedly been provoked by exposure to Thimerosal. Whenever a postulated new syndrome is reported in the research literature, it is standard practice to expect a systematic account of the clinical features so that other investigators may undertake studies to see if they can confirm the original reports of an apparent new syndrome. It was just those detailed clinical descriptions that meant that Kanner's original description of 11 cases³⁷, made at a time before anyone had recognized the possibility of a syndrome of autism, became accepted all over the world. The same followed the similarly detailed description of Rett syndrome⁴². There is no systematic account of the autism alleged to be associated with Thimerosal.

The Alleged Links Between Thimerosal and Autism Spectrum Disorders (ASD)

61. As judged by the evidence given to the Institute of Medicine²⁰³ for its immunization safety review with respect to vaccines and autism, claims that Thimerosal may cause autism largely derive from five main bodies of evidence:

- 1) Observed neurotoxic effects of mercury; 2) comparison of outcomes between Thimerosal-containing and Thimerosal-free vaccines; 3) time trends analyses; 4) associations between mercury levels and autism and 5) chelation studies.

Neurotoxic effects of mercury

62. First, numerous studies have documented the serious neurotoxic effects of methylmercury poisoning²⁰⁴. Thus, there are studies of an epidemic of metal mercury poisoning in Iraq of a result of the ingestion of home made bread prepared with wheat treated with a methylmercury fungicide^{205,206}. Harada²⁰⁷ reported findings of methylmercury poisoning in Japan caused by environmental pollution. There are also

clinical case histories of ethylmercury poisoning that indicate that ethylmercury can be severely toxic and can lead to death. The overall pattern of findings is reasonably clear-cut in showing that very high doses of mercury (in any of its various forms) have serious neurotoxic effects. However, it is important to note that the consequences of mercury poisoning involve gastrointestinal reactions, kidney damage, disturbances of balance and sensory disturbances. None of the reports of studies of large numbers of children with autism spectrum disorders mention any association with kidney problems.

63. Bernard *et al*⁵⁰ have put forward the proposal that the features of mercury poisoning and of autism are closely similar, but the claim is singularly unconvincing (see Nelson and Bauman²⁰⁸). Bernard *et al* start by comparing the traits associated with autism and those known to arise from mercury poisoning. They list 32 traits, many of which are not in the least bit specific to autism (e.g. temper tantrums, depression, poor concentration, ADHD traits, agitation, sleep difficulties and anorexia). Some traits are ones that are decidedly uncommon in autism. For example, Bernard *et al* drew attention to the slow slurred word production reported with mercury poisoning⁵⁰. In contrast, difficulties in pronunciation were found to be less common in individuals with autism than in children with developmental language disorders²⁰⁹. Similarly, Bernard *et al* claimed that clumsiness or lack of coordination is found in both ASD and mercury poisoning⁵⁰. Although clumsiness has been associated with Asperger syndrome, most accounts of individuals with autism have commented on their good balance and coordination. Bernard *et al* noted the excessive response to pain in babies with mercury poisoning but the usual finding in autism has been a diminished response to pain. Bernard *et al* then went on to make comparisons on biological abnormalities. In this connection, it is important to note that the neurochemical features in autism have been highly inconsistent across studies. Nevertheless, there are some striking differences between autism and mercury poisoning. Thus, Bernard *et al*⁵⁰ noted that microcephaly (reduced brain size) is a feature of mercury poisoning whereas macrocephaly (increased brain size) is more usual in autism⁶⁴.

64. Throughout the range of clinical studies of individuals with mercury poisoning, what is striking is the absence of any indication of the occurrence of autism. Of course, the studies were not designed to detect autism, but this would apply to a range of other studies of conditions involving organic brain dysfunction, which have reported the occurrences of autism. For example, an early report noted the frequency of autism in individuals with brain damage of various kinds²¹⁰. Autism has been noted to be an unusual occurrence in the individuals with Down syndrome but, nevertheless, there are several reports of autism in young people with Down syndrome^{107,108}. Nelson and Bauman²⁰⁸ drew attention to these and other differences and concluded: 'The clinical picture of mercurism - from any known form, dose, duration, or age of exposure - does not mimic that of autism. No case history has been encountered in which the differential diagnosis of these 2 disorders was a problem' (p 678). In summary, it is indisputable that mercury poisoning damages the brain. What is much more doubtful is whether or not this leads to autism.
65. The next question concerns whether or not there are comparable neurotoxic effects associated with elevated levels of mercury that are well below those leading to a clear-cut clinical picture of mercury poisoning. The main evidence on this point derives from a study in the Seychelles^{211,212}, and a different study in the Faroe Islands²¹³ where the high mercury levels were a consequence of a high intake of ocean fish containing methylmercury. The available findings suggest that the dosage of methylmercury was much higher in the Faroe Islands than in the Seychelles. On the whole, the findings in the Seychelles were negative with respect to adverse sequelae in children^{211,212}. The study from the Faroe Islands indicated slight neuropsychological deficits in some children at aged 7 years²¹³ but the effects of mercury were small and the individual variation large. The same applied to studies with a sub-group of the population in New Zealand with an unusually high fish consumption^{214,215}. It would be reasonable to conclude from the evidence from these studies considered as a whole that probably (but not certainly) markedly raised mercury levels that are short of clear-cut poisoning have slight effects on neuropsychological functioning in some children. The effects are small and, as with the studies of overt poisoning, it is striking that none of the

studies make any mention of autism spectrum disorders as a consequence of the raised mercury levels. With respect to extrapolations to Thimerosal, it is important to note that all of these studies deal with chronic elevation of mercury levels that are well above those associated with Thimerosal-containing vaccines.

66. There is one study in infant monkeys that compared blood and brain mercury levels after exposure to oral injection of methylmercury and similarly after intramuscular injection of vaccines containing Thimerosal²¹⁶. Brain concentrations of mercury were three times as high in the methylmercury group as in the Thimerosal group but the proportion in the form of inorganic mercury was higher. The study raises important questions but does not as yet provide answers on the toxicokinetics or developmental toxicity of Thimerosal. Baskin *et al*²¹⁷ used a quite different approach by studying Thimerosal toxicity in cultured human brain tissue and fibroblasts. Toxic effects were found but the dosage used was high. The authors appropriately drew attention to the value of the approach rather than deriving conclusions on Thimerosal effects in life. Somewhat similarly, Herdman *et al*²¹⁸ studied Thimerosal neurotoxicity in cell cultures, with evidence that there were effects on specific signaling pathways. Again, extrapolation to real life effects with ordinary dose levels of Thimerosal remains uncertain. I conclude that experimental approaches are likely to be very useful in studying Thimerosal effects but findings are not yet at a stage where conclusions are justified. It should be noted that I am commenting here only on causal inferences rather than the details of the toxicology, on which I do not have the relevant expertise.

Comparison of adverse outcomes between Thimerosal-containing and Thimerosal-free vaccines for diphtheria, tetanus and pertussis.

67. Geier and Geier^{219,220,221} made use of the Vaccine Adverse Events Reporting System (VAERS) to make this comparison. The methodology involved a comparison between a time period during the 1990s when the vaccines contained Thimerosal and a later time period in 1997-2000 when Thimerosal-free vaccines were available. The relative risk following Thimerosal was calculated by dividing the incidence rate of a particular adverse event following a Thimerosal containing vaccine by the incidence rate of the

same event following a Thimerosal-free vaccine. They reported a relative risk of about 6 for autism and intellectual disability and relative risk of about 2 for speech disorders. As discussed in the Institute of Medicine²⁰³ report and in the paper by Parker *et al*²²² this research has numerous methodological problems. To begin with, VAERS is a passive reporting system so that, necessarily, there is reliance on what adverse events people choose to report. Second, it is not clear how individuals were allotted into exposed and unexposed groups on the basis of the information in the data set that was used. VAERS reports include the vaccine type and manufacturers for the visit associated with the report adverse events, and within 4 weeks before that date, but they do not indicate whether a child received vaccines with or without Thimerosal on previous visits. Accordingly, the designation of Thimerosal exposure involves considerable uncertainty. A third fundamental concern is that it is not possible from the reports to determine what denominator should be used. That is to say, the database gives information on the number of adverse events, but there is substantial uncertainty as to the number of children who received the vaccines and therefore were eligible to have an adverse effect. Additional problems concern the fact that the comparisons involve different time periods and, therefore, meant that the groups varied in the time period over which diagnosis could be made. That is an important limitation because the children in the later age period (when there were Thimerosal-free vaccines) will have been quite young when the diagnosis had to be made. Indeed, it is noteworthy that the mean age of diagnosis of autism even in the Thimerosal group, was only 1.7 years, which is below the age when there can be confidence in the diagnosis of autism. Yet another problem derives from the uncertainty over the time period receipt of the vaccine and the development of the autism. Both the Institute of Medicine²⁰³ and Parker *et al*²²² concluded that these methodological problems were of sufficient severity to make it impossible to draw conclusions about any possible causal effect of Thimerosal on the development of autism. The most that could be said is that the findings raise the possibility of an association that ought to be investigated using more appropriate epidemiological methods, but even that suggestion is an uncertain one.

68. Geier and Geier^{219,220,223} also used VAERS data to compare the estimated mercury doses from Thimerosal containing child vaccines and the prevalence of autism from the late 1980s through the mid 1990s. The results showed an extremely strong association between the two measures, with a linear regression co-efficient between the two of 0.89. This implies that Thimerosal was likely to have been the main influence on the rise in the rate of diagnosed autism (because the association was so strong). The analyses have all the same problems as those already discussed in relation to comparison of Thimerosal-containing and Thimerosal-free vaccines. In addition, however, the data concern correlations between aggregated data. These provide a most inadequate basis for extrapolations to causation at an individual level. All that can be concluded is that the time trends over this period for both Thimerosal and the diagnosis of autism followed much the same trajectory. Blaxill *et al*²²⁴ noted the same parallels. Without any opportunity from the data used to examine what happens to that trajectory when Thimerosal is removed from vaccines, no causal influence is justified. As the Institute of Medicine²⁰³ concluded, the evidence (because of its methodological problems) is essentially non-contributory to the question of whether or not Thimerosal exerts any causal influence on the development of autism.
69. Rather similar concerns apply to the ecological study undertaken by Palmer *et al*²²⁵ showing a statistically significant association between variations in environmentally released mercury in particular school districts and variations in the rate of autism among the districts. There are no data on how environmental release of mercury actually gets into the body and hence there is no way of telling whether the mercury effect should be considered likely to be restricted to the county within which the industrial output existed. Similarly, there is no evidence on whether or not this environmental release from industrial outputs had any effect on mercury levels in individual children and, if it did, what levels it produced. No causal inferences are justified on the basis of this study. The same limitation applies to the Wyndham *et al*.²²⁶ study relating the distribution of hazardous air pollutants in the San Francisco Bay area and the rate of autism spectrum disorders. No causal inferences are possible from either study.

Associations between mercury levels and ASD

70. Holmes *et al*²²⁷ made a comparison of mercury levels in head hair samples from 94 children with autism and 45 control children, the median age at sampling being just under 18 months in both cases. The findings are described as based on a "first baby hair cut" but, as the Institute of Medicine noted, this has an uncertain relationship to prenatal mercury exposure²⁰³. The findings also were based on a clinic sample derived in a way that focused on people with an interest in the role of vaccines in autism. In the event, the results showed that the mercury levels in the children with autism were significantly lower than those in the controls and, moreover, that the mercury levels decreased in relation to increases in the autism severity score (this being based on clinical assessment rather than a standardized measure). The authors interpreted the findings as indicating that children with autism do not excrete mercury into their hair, with the apparent suggestion that, for reasons unspecified, the mercury is instead deposited in the brain. No direct evidence in support of this hypothesis is presented in the paper.

71. More importantly, the basic findings with respect to a lower level of mercury in the hair of children with autism have not been confirmed in a study from Hong Kong²²⁸. A cross sectional study of both hair and blood mercury levels of 82 children with an ASD and a mean age of about 7 years were compared with a control group of normal children. No differences were found between either the blood or hair mercury levels of the two groups. The evidence runs counter to the suggestion of a causal relationship between mercury and ASD and the fact that the two groups were so similar on both hair and blood measures is incompatible with the Holmes *et al* hypothesis²²⁴. Adams *et al*²²⁹ found no difference in the level of mercury in the hair of older children with autism as compared with controls - in line with the Hong Kong study. A further study by the same group²³⁰ used mercury in baby teeth (at about 6-7 years) as an index of body burden of mercury during several years of prenatal/infant development. The rate of mercury was about twice as high in the autism group but the sample size was small (16 in the autism group versus 11 controls) and it was a volunteer sample. Obviously, it would be desirable to have further studies in order to resolve the contradiction between these two investigations. But, in the meantime, the conclusion has to be that there is no good evidence of either an

increase or a decrease of mercury levels in children with autism. The fact that the ages of the children are so different in the two studies could possibly account for the difference in findings but there is no direct evidence to indicate that that is so. Again, as in Paragraph 66, I am commenting on the causal inference and not on the details of the toxicology.

Chelation Studies

72. Bradstreet *et al*²³¹ compared the results of chelation in 221 children with autism and 18 controls. The findings showed that the children with autism excreted significantly more mercury in the urine following chelation challenge than did the controls, but the range was very great. Unfortunately, the study does not report levels of mercury in either hair or blood prior to chelation. The implication is that there is likely to have been a higher level of mercury in the bodies of autistic children but that is an inference rather than a direct observation in the absence of pre-chelation levels. Most crucially, the findings are entirely non-contributory with respect to the Holmes *et al* hypothesis²²⁷. The hypothesis could have been tested with the chelation study because the hypothesis necessarily predicts that there should be a raised excretion level of mercury in the urine despite an usually low level of mercury in the hair prior to chelation. It has to be said that the hypothesis seems an implausible one, but the opportunity to test it was lost in the Bradstreet *et al.* study²³¹.

Summary of evidence on mercury levels and ASD.

73. The hypothesis that low levels of mercury might cause brain impairment leading to ASD clearly has a basic biological plausibility because of the incontrovertible evidence that mercury in very high dosage is a serious neurotoxin. Nevertheless, despite the demonstrated neurotoxicity of mercury, the causal suggestion is greatly weakened by the failure of the reports in the literature on either mercury poisoning or raised levels of mercury below the severe poisoning threshold to mention autism as one of the consequences that has been observed. Given the striking characteristics of autism, it is improbable that such conditions would not have been mentioned if they had occurred. As discussed, the claimed parallels between mercury poisoning and autism are far fetched

and unconvincing. The direct studies of Thimerosal using the VAERS database have so many serious methodological problems that they do not warrant any conclusion about an association, let alone a causal influence. The same applies to the ecological correlations between environmental mercury and ASD. The findings on mercury levels in autistic children, as compared with controls, are contradictory and therefore not interpretable until the reasons for the contradictions have been demonstrated which they have not been at the moment. The chelation findings are compatible with an increased body burden of mercury in children with autism but, because of the lack of data on mercury levels before chelation, they are uninformative about the causal model proposed by Holmes *et al*²²⁷. Also, even if the mercury levels were raised in the sample studied, the findings do not show whether or not such levels were a consequence of Thimerosal exposure. In short, I conclude that the evidence put forward to suggest that low levels of mercury might cause autism is entirely unconvincing. However, more systematic epidemiological studies dealing specifically with Thimerosal are available and those are considered below.

Epidemiological studies

Risks of ASD according to dose of Thimerosal received

74. Ordinarily, studies with prospective longitudinal data constitute the best means of examining the consequences of any postulated risk-variable. The rationale is that a direct comparison at an individual level can be made between individuals who were exposed to the risk factor and individuals who were not. Because the findings are based on a prospective longitudinal study, the incidence of any specified outcome variable can be examined. In this way, there can be a direct comparison of the incidence in the presence, as compared with the absence, of the risk factor and this can be examined in relation to various ages. Thus, it would be possible to examine the incidence of ASD as diagnosed by some specified age such as 5 years or 7 years. Unfortunately, that is not possible in the case of Thimerosal and there are no such studies published. That is because, at any one point in time the vaccines either do or do not contain Thimerosal and virtually all children receive vaccines. Accordingly, there is no appreciable number of children who are not exposed to Thimerosal in a single time period during which Thimerosal-containing vaccines were used. Moreover, with respect to the tiny number of children

who do not ever get vaccinated, there has to be a major query as to why they did not receive the vaccination. It is extremely unlikely that this will be a matter of chance. Rather it is likely that it indexes or (reflects) a range of other adversities²³².

75. The consequence is that there has to be recourse to cohort studies in which the total dose of Thimerosal, by specified ages, is related to the rate of ASD (or any other specified adverse outcome). The strength of the strategy relies on the fact that the associations are at an individual, rather than a group, level. There are two main limitations to this approach, with respect to its application to Thimerosal. First, because most children are vaccinated according to a generally agreed schedule of vaccines at specified ages, there is relatively little variation in the total amount of Thimerosal received. The main variation concerns the age at which the total dose was received (the variation being according to the proportion of children who were late in receiving booster doses or additional vaccines). Second, there is uncertainty on the reasons why some children were vaccinated according to the agreed schedule and some were not. Inevitably, this introduces a potential source of bias.

76. The only prospective cohort study is that undertaken by Heron *et al*²³³ in the United Kingdom, based on the Avon longitudinal study of parents and children (ALSPAC). The findings are based on 13,000 children (out of an original enrolment of 14,541). The age at which doses of Thimerosal-containing vaccines were administered was recorded and measures of mercury exposure by 3, 4 and 6 months of age were calculated and compared with a range of measures on childhood cognitive and behavioral development covering the period from 6 to 91 months of age. The study has the very considerable strength of good population coverage and comparable measurement of all subjects. In addition, the study had the important advantage of good measurement of a wide range of possibly confounding variables that could be taken into account in multivariate analyses. On the other hand, it has the considerable limitation that there was no measurement of ASD. The sample was too small for this to be an appropriate objective.

77. With respect to the possibility of autism, the most relevant outcome measure was whether or not the child had special educational needs (as judged by the mother) and whether this had been recognized by the local authority in terms of an official 'statement'. The former applied to 8.4 per cent of the population, and the latter to 2.9 per cent of the population. Various speech outcomes (including speech therapy that was given to 11.2 per cent of the population) might also be relevant. The general pattern of results was of very little difference according to the level of Thimerosal exposure but with a tendency to Thimerosal having an apparently protective, rather than risk effect. The only risk effect found was for poor social behavior at 47 months. In other words, where there were significant effects they appeared to indicate benefits of Thimerosal, rather than risks from it. The main conclusion concerns the lack of substantial effects and it is rather unlikely that the supposed protective affect is real. It should be noted however, that the total dosage in this U.K. study was substantially less than the dosages that would be received at that time in the United States and there was very little variation within the sample. So thus, for example, of those children who had only 2 doses by 4 months of age, nearly four-fifths, had received their third vaccine by the end of the fifth month. The findings provide no evidence of risk from Thimerosal but conclusions have to be limited by the important constraint on the very limited variation in dosage.
78. Andrews *et al*²³⁴ also provided data on a U.K. sample, but this time using the very large general practice research database. The total sample was very large (over 100,000) and quite big enough to detect any Thimerosal effect on ASD. The rate of autism in the database was approximately 10 per 10,000, and the rate of general developmental disorders was about 2 per cent. An apparently protective effect from Thimerosal was found for general developmental disorders and no effect on ASD was evident. The only significant risk for an adverse outcome was that associated with tics. It is noteworthy, however, that this was not found in the Heron *et al* study and, hence, it may well be a chance finding. The findings provide no indication that Thimerosal increases the risk of autism but there is the same problem with this study as there was with the Heron *et al* study - in terms of the very limited variation in Thimerosal dosage. The authors noted that the dosage of Thimerosal by 4 months of age in the U.K. was similar to that in the

U.S. of the same age but the U.K. situation was different in that there was not the same increase in exposure in the months that followed. A further disadvantage is that the database did not allow proper examination of possible confounding factors, although the Heron *et al* study suggests that this would not have made much difference²³³.

79. The most extensive U.S. study is that reported by Verstraeten *et al.*²³⁵, based on the data from 3 health-maintenance organization databases on a total of over 140,000 children receiving vaccines. The same kind of data analytic procedure was followed - namely comparing risks according to the dose of Thimerosal - both before and after taking account of a range of confounding variables. Only 1 of the 3 organizations had a sufficient sample size to examine the rates of autism and in this organization, no association was found. An additional sub-analysis on the data from this organization, comparing children exposed to Thimerosal-containing vaccines and those receiving only Thimerosal-free vaccines, showed no risk effects from Thimerosal (but an apparently protective effect in relation to attention deficit disorder). As with the first two studies described above, the findings provide no evidence of a risk from Thimerosal for the development of ASD. Data were thoroughly analyzed in an appropriate way but there are three possible concerns. Although this is the only published report of the study in a peer-reviewed scientific journal, there had been two earlier presentations of preliminary findings and it is noteworthy that these differed somewhat from the final findings. That, in itself, is not in the least bit remarkable because that is often the case with studies requiring multivariate analyses. Moreover as judged by the account given in the published paper, the right approaches were followed in the analyses undertaken. Nevertheless, it is not possible from the published report to understand adequately what accounted for the differences between the preliminary findings and the final findings. Second, the findings differed to some extent among the three health-maintenance organizations and it is not evident why that was so. The third concern is that the first author was employed by a pharmaceutical company making vaccines during the period when the further analyses were undertaken. It is stated that he played no part in the further analyses but it is not clear from the paper whether he had adequate access to the analyses to accept responsibility for them and, it is not clear why the published paper

makes no acknowledgement of his link with the pharmaceutical company, although this was later made explicit²³⁶.

80. The largest scale study is that undertaken by Fombonne *et al.*²³⁷. The strategy here involved relating ethyl mercury exposure to ASD over a time-span when exposure will have changed as a result of alterations in the immunization schedule. The prevalence rate of autism in this Canadian sample was 65 per 10,000 – showing the significant linear increase in ASD over the study time period between 1987 and 1998. The prevalence in Thimerosal-free birth cohorts was significantly higher than that in Thimerosal-exposed cohorts, providing no indication that Thimerosal was associated with an increased risk of ASD. Variations over time in Thimerosal dosage were sufficiently high to provide useful variation. The main limitation, however, was that individual Thimerosal exposure data were not available. Also, although exposure data were precisely calculated for each birth cohort, the method of estimation of the birth cohort was somewhat indirect. It seems unlikely that these important limitations could have created an artifactually negative finding.

81. The fourth Thimerosal dosage study is that by Hviid *et al.*²³⁸, reporting findings using the Danish central registers. The study has the major strength of a very large population sample and systematic diagnostic coverage of the total population. No risks effects of Thimerosal were found. A major potential strength of this study, as compared with the U.K. and U.S. studies, is that Thimerosal ceased to be used for vaccines after 1992 (the sample covered children born between 1990 and 1996). Accordingly, there was quite a substantial proportion of the population who did not receive Thimerosal and this was entirely a consequence of a decision to remove Thimerosal from the vaccines, rather than from a failure to follow an agreed vaccine procedure. Moreover, in order to avoid immunization take-up biases, it was possible to compare ASD rates in those receiving a Thimerosal-containing vaccine and those receiving a Thimerosal-free formulation of the same vaccine. Within those receiving the former, risk effects could be related to the number of doses received. Because the registration of ASD changed in 1994/5 to include outpatients, a time trends analysis was conducted for the 1995-2000 period when

registration followed the same rules across the entire time period. The rate of ASD continued to rise despite Thimerosal having been eliminated three years earlier.

82. Summary of dosage variation findings

In summary, all four of the dosage variation studies of Thimerosal on ASD showed no significant effects on the overall rate of ASD. For the reasons given above, each of the studies has limitations, but there is no reason to expect that the limitations were likely to have led to a misleading negative finding. Certainly, the findings provide no convincing evidence that Thimerosal has effect on the overall rate of ASD.

Effects on rate of ASD associated with removal of Thimerosal from vaccines

83. The study of time trends provides an entirely different strategy for studying causal mechanisms. A key part of the claims that Thimerosal contributed to the causation of autism concerned the postulate that it was the use of Thimerosal that was responsible for the rising rate of diagnosed autism. If that hypothesis was true, it should follow that the removal of Thimerosal from vaccines should result in a fall in the rate of autism. As noted above, causal inferences rest on a most uncertain foundation when based only on two time trends that seem to be running in parallel. The real test comes when there is a reversal with respect to the risk factor, giving the opportunity to see whether or not it has an effect on the outcome that is supposed to have been caused by that risk factor.
84. In the case of Thimerosal, the natural experiment arose as a result of the fact that Scandinavia stopped using Thimerosal as a preservative in vaccines at a time when it was extensively used in the United States. The key test, then, is whether the trajectory of the rate of diagnosed autism changed with the discontinuation of Thimerosal, and whether or not there was a similar change of trajectory in the United States where Thimerosal use continued. The design has one extremely important strength and that is that the exposure to the postulated risk factor (namely Thimerosal) would be unaffected by individual choice. That is because the discontinuation of Thimerosal was a decision taken at a national level and it was not left up to individual families to decide for themselves. The importance of this is that it immediately removes the most serious of

confounding variables - namely the reasons why people choose, or do not choose, to follow vaccination procedures^{11,232}. The strategy relies on the availability of systematic data over time on the rate of autism, the comparability of such measures within the country being examined, and the comparability across the countries to be contrasted.

85. With respect to Thimerosal, it is therefore pertinent that the increase in the rate of autism as found in the United States was closely paralleled by comparable rises in other countries. The other assumption that is necessary is that there was minimal usage of outdated vaccines (which would contain Thimerosal) after the time Thimerosal was discontinued. That assumption seems likely to be correct but it would be prudent to pay more attention to time trends, say, a year after discontinuation of Thimerosal than on what happened in the months immediately following discontinuation. The main disadvantage of time trends analyses is that, necessarily, they concern large groups of people, rather than what is happening at an individual level. That makes them a weak research strategy if the hypothesis (or claim) is that the risk factor was responsible only for a small number of cases, or if the risk factor applies only to a portion of a population, and if there were many other risk factors that might have been changing over the same period of time. None of those concerns apply in the case of Thimerosal. The claim is that Thimerosal was responsible for the population rise in autism and the risk factor is one that applied to almost all the population at one point in time and to none at some other point in time. That makes it an usually strong strategy in this particular instance. There are two studies that are informative in this connection.

86. The first used the Danish psychiatric central research register^{239,240}. Vaccinations are provided free and the acceptance of vaccinations in Denmark has always been very high (with population coverage exceeding 90 per cent). The removal of Thimerosal from vaccines took place in 1992 and it is possible therefore to compare rates in the era when Thimerosal was in vaccines and the era when it was not. The results showed that the discontinuation of Thimerosal-containing vaccines in 1992 was followed by a continuing increase in the rate of autism, rather than the decrease that was expected if Thimerosal was a risk factor. Unfortunately, the situation is complicated by the fact that up to 1995

the psychiatric registration dealt with in-patients only, whereas from 1995 onwards it also included outpatients. In their papers, the authors^{239,240} report that the trends were similar when the analysis was confined to in-patients, but no data on this point were provided. The other limitation is that, although the rate of autism is reported according to different age groups of children, it is not reported in terms of the diagnoses having been made by a standard age. The consequence is that the later born children will have had a reduced period of time for the diagnosis of autism and, on that basis, were likely to show an artifactually low rate of autism as compared with the earlier cohorts whom the diagnoses could be made up to a later age. The published data do not provide what is needed in order to examine this effect. However, it should be noted that this bias means that the rate of autism in the Thimerosal-free period is likely to be misleadingly low. Accordingly, this difference between the two time periods is probably greater than that reported in the paper. In other words, the negative finding from Thimerosal's supposed responsibility for the rise in autism is likely to have been even more negative than reported. Moreover, a later report from Denmark²⁴¹ similarly showed that the rate of ASD continued to increase during the Thimerosal-free years. This later study by Atladóttir *et al* had three main strengths: i) it used the register data only after it included both in-patients and out-patients; ii) it examined cumulative incidence by each age of diagnosis; and iii) it compared time trends for ASD and three other diagnoses. It is noteworthy and relevant that there were similar rises over time in the rate of both hyperkinetic disorders and Tourette syndrome. As already noted (paragraph 81) Hviid *et al*'s²³⁸ findings showed the same.

87. Stehr-Green *et al*²⁴² took the time trends research strategy further because they compared the rate of autism in California with the rates in Sweden and Denmark. The natural experiment arises from the fact that exposure to Thimerosal was eliminated in Sweden and Denmark in the early 1990s whereas the average dose from vaccines increased in the United States throughout the 1990s - i.e. the time periods to be compared. The data on the diagnosis of autism in Sweden were available at a national level in terms of children diagnosed in in-patient settings between the ages of 2 and 10 years during the time period 1987-1989. The Danish data were the same as those published by Madsen *et al*²⁴⁰ and

Lauritsen *et al*²³⁹. The Californian data on the diagnosis of autism were based on records of the California department developmental services. What the findings showed is that the rate of autism in California rose steadily from the mid 1980s to the mid 1990s and that the cumulative mercury exposure through childhood vaccines rose in parallel over the same time period. The Swedish data also showed an increase in the rate of autism from the mid 1980s onwards, but the gradient of the increase was less than in the United States. Most crucially, however, there was no change in the gradient of the increase in the years following the discontinuation of Thimerosal. These figures have the disadvantage of being based only on in-patients, but they have the advantage, as compared with the Danish data, of using the same measure throughout the whole time period. The Danish data showed the pattern already noted in Lauritsen *et al*²³⁹ and Madsen *et al*²⁴⁰. The Swedish data, based on in-patient registrations, differed from the Californian ones based on children seeking special educational services. Accordingly, it would be invalid and inappropriate to compare the absolute rates. It is the comparability in gradients that makes the comparison justifiable and it is the difference in the exposure to Thimerosal across the time periods that makes the comparison meaningful. It is relevant that these data use the same method (i.e. with respect to time trends) put forward in the claim that Thimerosal causes autism¹⁵². The main important disadvantage of these analyses based on group trends is that it is not possible to examine hypotheses that concern individual differences in effects (such as with respect to the timing of the exposure or the role of predisposing factors). Hence, the data provide strong evidence against the hypothesis that Thimerosal was responsible for the rise over time in the rate of diagnosed autism but they cannot be used to examine the quite different hypothesis of an unusual susceptibility to Thimerosal in a subgroup of children.

88. A very recent study²⁴³ using California Department of Developmental Services (DDS) data has shown that the prevalence of ASD continued to rise up to 2007 despite the exclusion of more than trace levels of Thimerosal from nearly all childhood vaccines. The DDS rates rely on administrative data, with all the limitations that those involve, but they do provide a reasonable guide on time trends. The findings do not support the hypothesis that exposure to Thimerosal is a cause of ASD in the population as a whole.

89. Ecological analyses have several important limitations with respect to testing hypotheses on individual risk^{244,245}. In particular, there is the inability to control for confounding variables at the individual level and the impossibility of investigating specific moderators (such as time of exposure, lags in effects, and interplay with genetic predisposition). Nevertheless, it is noteworthy that a time trends analysis constituted a major element in the claim that Thimerosal had led to an epidemic of autism. The findings that discontinuation in the use of Thimerosal did not lead to a fall in the rate of ASD constitutes an important refutation of the Thimerosal-induced epidemic hypothesis. The Danish analyses^{239,240} were severely constrained by the coincidental change in diagnostic registration procedures but that concern did not apply to the well-conducted Stehr-Green *et al*²⁴² study. The one relevant consideration is that the average cumulative ethylmercury dose in Sweden (never above 4 µg between 1980 and 1988 when Thimerosal usage was phased out over the subsequent four years) was always far below the average cumulative dose in the USA in the mid 1990s (up to above 15 µg). Accordingly, the Scandinavian ecological data cannot exclude the possibility that levels in excess of 4 µg might have adverse effects. On the other hand, the Californian data make even that possibility extremely unlikely.

Overview of epidemiological findings

90. In that connection, it is necessary to bring these data together with the Thimerosal dosage findings (see paragraphs 74-82), with special reference to the findings in the USA²³⁵. The Verstraeten *et al* paper²³⁵ provides the relevant data and, despite the queries raised over a few specific features (see paragraph 79), it is a large scale, well analyzed study. No significant effects on ASD rates by variations in Thimerosal dosage were found. The same applies to Fombonne *et al*'s²³⁷ Canadian study because Canadian levels of Thimerosal exposure are more similar to the USA than to the lower levels in Europe. Taken together with the ecological analyses (see paragraph 83-89), it is clear that the epidemiological findings provide no indication that Thimerosal has led to an epidemic of autism. Accordingly, I conclude that that claim can be regarded as without research support.

Hypothesized Unusual Individual Susceptibility to Mercury

Regression as a possible marker of individual susceptibility

91. It remains to consider the possibility that, although Thimerosal is likely not to increase the risk of ASD in most children, it could still do so in a small unusually vulnerable subgroup. That possibility needs to be examined in two rather different ways. First, Sander Greenland, in his 13 August 2007 report, argued that Thimerosal might be specifically associated with a regressive form of autism. A similar suggestion had been made earlier (by others) that this also applied to the effects of the measles-mumps-rubella (MMR) vaccine on the risk for ASD. Epidemiological studies were consistent in showing that the rise in ASD was not particularly associated with ASD involving regression^{246,247}. Similar analyses have yet to be conducted in relation to the Thimerosal hypothesis but the crucial points (see paragraphs 41 to 46) are: 1) throughout the entire period of time over which ASD have been studied, regression has been found to be very common - applying to some 25 to 40% of cases; 2) regression is probably a feature that is particularly characteristic of ASD overall (as compared with other neurodevelopmental disorders); and 3) regression is probably a dimensional and not a categorical feature. That is, children with ASD vary in the extent to which they exhibit regression but it cannot validly be considered a feature that is either present or absent. Several studies have sought to compare cases according to whether or not regression was preceded by apparently normal development but this differentiation has (at least so far) not proved particularly meaningful. I conclude that there is no good evidence to support the speculative suggestion that Thimerosal results in a form of ASD characterized by regression. If this claim is to be taken seriously, it needs to be supported by research findings and those are singularly lacking.

92. It would have been possible to test the regression hypothesis in a vigorous way but that has not been done. For example, cases involving regression and those apparently without regression could be compared (blind to knowledge on regression) on the presence of multiple congenital physical anomalies (which will have to have arisen prenatally). The advantage of such an approach is that it would not be reliant on anyone's recognition of

subtle behavioral changes in the first year of life. The same might also apply to the characteristic increase in head size first evident in that first year. The problem throughout is that the regression hypothesis has been put forward in a speculative fashion without thinking through how it could be tested.

Biological measures that might index susceptibility

93. A quite different approach is to seek some biomarker that could represent a measure of an unusual individual susceptibility to Thimerosal. For example, James *et al*²⁴⁸, in a very small case-control study of children with ASD reported that those with a regressive onset were found to have a significant decrease in methionine levels. A further study²⁴⁹ compared metabolic profiles and genetic allele frequencies between individuals with an ASD and controls. Eight significant (plus several nearly significant) differences in allelic frequencies were found together with seven significant gene-gene interactions. Many significant differences between ASD individuals and controls in trans-methylation and trans-sulfuration metabolites were evident. However, the genetic findings were not examined for associations with the metabolic differences. The authors rightly cautioned that the findings were preliminary and required confirmation in larger population-based studies. Nevertheless, they hypothesized that both might be associated with oxidative stress, and that the allelic variations might be indexing an increased vulnerability to oxidative stress.

94. A somewhat comparable approach was adopted by Woods *et al*²⁵⁰ who, in a study of dentists, reported that some 15% of those had an atypical porphyrinogenic response (a greatly higher excretion of porphyrins in the urine) which they linked conceptually to the dentists' occupational low level of exposure to mercury. They went on to demonstrate that a polymorphism in the human coproporphyrinogen oxidase gene was associated with this atypical response. I have been unable to find any independent replication of this genetic finding. However, there are two other reports of increased porphyrins in the urine of individuals with autism^{251,252}. It is also notable that both the biological marker and the genetic allelic variation differ from those in the James *et al*^{248,249} study.

95. Others²⁵³ have taken up James *et al*'s theme of oxidative stress and have argued that hyperbaric oxygen therapy might constitute a useful therapeutic intervention, but no additional empirical evidence was provided. The review notes, however, that oxidative stress has been implicated in a wide range of disorders. Ming *et al*²⁵⁴ reported that two oxidative stress biomarkers were increased in children with autism, but there was no association with regression.
96. A report of a mouse study by Hornig *et al*²⁵⁵ has investigated the possibility that an auto immune propensity influences sensitivity to Thimerosal effects. In brief, a comparison was made between genetic strains of mice that differed in their susceptibility resistance to autoimmunity. The rationale was that it has been hypothesized that ASD may be associated with an autoimmune tendency, although the supporting evidence for this view is weak¹². Infant mice were given either Thimerosal containing injections or control injections at an age that was judged comparable to that used for vaccine schedules in humans. Various behavioral tests were undertaken at specified later time periods. Some mice were sacrificed and their brains were examined microscopically. The genetic strains of mice with an autoimmune susceptibility showed growth retardation following the Thimerosal injections. They also exhibited reduced activity levels and reduced stereotyped behavior; however there was no effect on spatial learning or on motor coordination. Examination of the brain showed enlargement of the hippocampus and densely packed neurons. This was a well-conducted study with interesting findings but there are four reasons why its relevance for ASD following Thimerosal exposure remains questionable. First, the genes implicated in autoimmunity have not been found to be associated with ASD in humans. Second, the evidence pointing to autoimmunity in ASD is weak and inconclusive^{19,256}. Moreover, in one recent study²⁵⁷, when antibody differences between children with ASD and controls were found, the pattern associated with ASD did not differ from that associated with neurodevelopmental disorders other than ASD. Accordingly, the autoimmune model is of uncertain relevance. Third, the parallels between the behavioral effects in mice and ASD are not at all close. Fourth, the only replication study that I have been able to find²⁵⁸ did not confirm Hornig's main finding that SSL mice showed abnormal somatic growth or altered the structure of the

hippocampus. Equally, no Thimerosal-induced changes in social interaction, sensory gating or anxiety were found. Moreover, the limited locomotor effect found applied only to female mice. The study appears well conducted but is limited by not including a comparison with other genetic strains of mice. It should also be noted that the biological mechanisms proposed by Hornig *et al*²⁵⁵ differ from those in the James *et al*^{248,249} and Woods *et al*²⁵⁰ models. In summary, the research literature provides some useful leads to follow but none come even close to evidence that biomarkers (metabolic or genetic) have been found that could identify an unusual individual susceptibility to mercury.

97. I do not have the necessary technical expertise to evaluate the metabolic findings, but I can comment on the suggestion that there might be a genetic index of an unusual vulnerability to Thimerosal. The biological reality and importance of gene-environment interaction (G x E) in relation to medical diseases has been well demonstrated¹⁰, although to date they have not been shown for ASD. For the hypothesis of a genetic index of an unusual vulnerability to Thimerosal to have validity, certain requirements must be met. First, the reality of a true environmentally-mediated risk effect must have been established^{259,260}. As already discussed, this has not been done. Second, crucial methodological hazards (such as artifactual G x E resulting from scaling features)^{261,262} must have been dealt with satisfactorily, and there is no indication in the published paper that that has been done. Third, independent replications must have been achieved. I have been unable to locate any such replication. Fourth, there should be evidence that the alleged genetic marker influences the hypothesized biological mechanism (in this case oxidative stress). Finally, there needs to be evidence of a causal connection with the hypothesized disease outcome (*ie*, ASD in this case). At the moment, all that can be said is that it constitutes an interesting possibility that warrants further study but, so far, there is no evidence that the possibility actually operates in relation to the effects of Thimerosal.

Epigenetics, programming and other possible mediating mechanisms

98. The suggestions about possible genetic influences on variations in individual susceptibility to mercury mainly focus on G x E and the suggestions on metabolic

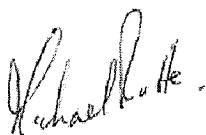
biomarkers focus on a supposedly characteristic short-term response. It is necessary finally to mention two potentially relevant biological mediating mechanisms that have received attention as possible explanations for long-term consequences of time-limited exposure to environmental hazards of any kind. First, it is well established that genetic influences are crucially dependent on the expression of these genes, and that the influences on expression include both other genetic elements and environmental influences^{8,263,264,265}. Moreover, there is some evidence that epigenetic effects become greater as individuals grow older²⁶⁶. The reality of the phenomenon is not in doubt and potentially it could provide a key mechanism for long-term environmental effects. It is crucial to appreciate, however, that very little is known on which environmental effects do operate via this mechanism. It is striking, for example, in the work of Michael Meaney's research group dealing with early nurturing effects in rodents (see⁸ for a brief review) that it was confined to experiences in the first week of life and only then for particular forms of nurturance. The mechanism is almost certainly much more widespread than that, but the point is that we know very little about their generality. It is also relevant that gene expression is tissue-specific (meaning that it requires study of the relevant tissue - in this case the brain). That means that there are major practical difficulties measuring gene expression in living humans. In summary, as a research topic it is likely to prove very important, but at the moment invoking it in relation to Thimerosal is entirely speculative.

99. The second possible mechanism (which could turn out to involve epigenesis) is provided by biological programming^{267,268}. Animal experiments have provided sound evidence that, in certain circumstances, experiences that occur during a critical period in brain development can seriously interfere with the normal structural and functional development of the brain. Accordingly, it is possible that the effects of mercury poisoning might operate through influences on developmental programming of the brain rather than through damage to the brain as such. Once more, however, it is entirely speculative to consider the issue in relation to Thimerosal effects on the liability to autism because these effects have not been demonstrated.

Overall Conclusion

100. There is no doubt that mercury is neurotoxic and that mercury poisoning results in severe neuropsychiatric disorders. The claim that these mimic autism is not supported by the research evidence. Epidemiological studies provide tentative evidence that high levels of ingested mercury may possibly cause subtle neurocognitive impairments even when there is not any overt poisoning. However, these do not seem to include ASD. The epidemiological findings provide no support for the claim that the use of Thimerosal has led to an 'epidemic' of autism indicated by the marked rise over time in the rate of diagnosed ASD. It is biologically plausible that, despite a lack of any general effect of Thimerosal on the liability to autism, there might be an unusual idiosyncratic response in a small subgroup of individuals. Nevertheless, although biologically possible, there is no evidence that Thimerosal actually causes ASD in a subgroup of children. The claims that the unusual susceptibility may be indexed by the presence of regression are not supported by research findings. The same lack of evidence applies to parallel claims on possible indexing by either metabolic responses or particular genetic allelic variations. I conclude that I find no support for the claim that Thimerosal causes ASD. The available epidemiological evidence runs counter to the claimed causal effect.

I confirm that insofar as the facts stated in my report are within my own knowledge I have made clear which they are and I believe them to be true, and that the opinions I have expressed represent my true and complete professional opinion.



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