

The increase in allergic disease: environment and susceptibility. Proceedings of a Symposium held at the Royal Society of Edinburgh, 4th June 2002

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Introduction

This symposium 'The increase in allergic disease: environment and susceptibility' continued the 219-year-old tradition of the Royal Society of Edinburgh of bringing experts together from around the world to discuss topical problems. The aim of the symposium was to provide insight into known and as yet unknown factors underlying the recent increase in allergic disease and to identify factors that could potentially be modified for the purpose of either preventing or treating allergic disease.

The problem

The increase in asthma and atopy. Professor Peter Burney

Studies designed to detect changes in the prevalence of asthma and atopy should be conducted in the same population using the same methods on more than one occasion. In order to reliably detect small time trends in prevalence, repeated studies need to be several years apart (exactly how far apart will depend on many factors including the rate of change and the size of the sample). Morrison Smith probably conducted the first studies demonstrating a changing prevalence of asthma during the 1960s and 1970s, in schoolchildren living in Birmingham, UK [1, 2]. Widespread interest in the increase in asthma and atopic disease began in the mid-1980s, and by the mid-1990s a number of studies had fulfilled the general precepts of sound study design [3]. It is clear from these studies that between the mid-1960s and the mid-1990s there was an increase in asthma prevalence of approximately 5% per year. Although the rates of increase in asthma between studies are similar, the baseline from which the increase started varied. Several methodological

considerations account for this. Geographical differences in the prevalence of disease make it necessary to study the same geographical population. Differences in the measured parameter of asthma prevalence are important; studies quantifying wheeze report higher prevalence rates than studies quantifying diagnosed asthma.

There has been considerable discussion surrounding these studies and the possibility that subtle methodological limitations may have contributed to the observed increase in asthma prevalence. Firstly, there is the issue of migration and population change. One criticism of Morrison Smith's original work is that there were rapid population changes during the period of his studies such that the population studied at the end differed considerably from that at the start [4]. Secondly, despite best efforts there have been some methodological variations within repeated studies [5]. Perhaps the most problematic methodological criticism is the concept that there have been changes in symptom perception and/or tolerance of symptoms over time; this concept is almost impossible to adjust for and to test in retrospect. Several studies have attempted to investigate this by measuring asthma severity. A recent UK study has demonstrated similar increases in the number of mild and severe asthma attacks between 1990 and 1998, suggesting that there has not been a simple increase in the reporting of minor disease; rather there appear to have been increases in all grades of asthma severity [6]. The relatively few studies quantifying changes in objective measures of asthma are not influenced by symptom perception. Burr, for instance, reported not only an increase in asthma prevalence in 12-year-old schoolchildren but also an increase in exercise-induced bronchoconstriction [7]. In one study of adults in Australia, Woolcock demonstrated that, although there was an increase in symptoms, if anything, there appeared to have been a reduction in bronchial hyper-responsiveness; however, interpretation of this study is complicated because there were clear changes in the population during the study period [8]. It is important to emphasize, nevertheless, that these limitations are not sufficient to detract from the conclusion that there has been an increase in asthma prevalence.

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It is well established that asthma is closely related to atopy, raising the question as to whether the increase in asthma merely reflects an increase in the prevalence of atopy. In general there is less evidence to suggest an increase in atopy. Aberg has demonstrated an increase in eczema, allergic rhinitis and asthma in Swedish schoolchildren [9] and, in Aberdeen, Russell and colleagues have also demonstrated increases in eczema and hayfever over a 25-year period [10–12]. These studies suggest that where there has been an increase in asthma there has also been an increase in atopy. The interpretation of these studies is subject to the same caveats as the studies of asthma. Most studies of eczema and hayfever have relied on parental reporting and it is possible that the reporting threshold for these conditions may have changed. The limited amount of objective data has, in general, demonstrated increases in specific IgE over time, supporting the notion that there has been an increase in atopy [13–15] although, in a Swiss study, the increase in specific IgE appears to have plateaued since the mid-1980s [16]. It should also be noted that IgE sensitization to all allergens has increased, suggesting that the increase in sensitization has not been limited to indoor allergens such as house dust mite.

Although there has been an increase in atopy it is not possible to attribute the increase in asthma solely to this increase. Woolcock demonstrated an increase in diagnosed asthma, recent wheeze and bronchial hyper-responsiveness in the absence of an increase in atopic sensitization [17]. An analysis of the 1958 and 1970 British Birth Cohorts demonstrated increases in asthma prevalence in both atopic and non-atopic individuals [18]. It would appear that some of the increase in asthma has been independent of the increase in atopy, but the evidence is ambiguous. The recent Midspan study concluded that the difference in asthma between parents and their children was largely due to generational differences in atopy and in the reporting of 'asthma' in non-atopic subjects with wheeze [19].

At present it is difficult to pinpoint the factors behind the increase in asthma and atopy. Several studies have reported positive associations between body mass index and asthma, with a tendency for this association to be stronger in women than in men [20–24]. In the last 30 years there has been a well-documented increase in population obesity and because many of the risk factors for obesity are the same as those for asthma it has been proposed that the increase in asthma is a direct consequence of increased obesity. It is, however, unlikely that the increase in obesity is the factor underlying the increase in asthma, since a study of UK children between 1984 and 1994 demonstrated that the increase in childhood asthma and wheeze was not related to the increase in body mass index. Similar analyses of the 1958 and 1970 British Birth Cohorts has demonstrated that the increase in asthma is not the consequence of changes in birth weight, maternal age, breast feeding, birth order, maternal smoking or social class [25].

Although there is fairly consistent evidence of an increase in asthma and atopy between the 1960s and the mid-1990s some recent data suggest that this increase may have plateaued. GP asthma consultations increased between the mid-1970s and mid-1990s, but since 1993/4 there seems to have been a decline in consultations [26]. These data suggest that the increase in asthma prevalence in the UK may be ending, although better management of asthma and the huge increase in the use of

inhaled corticosteroids will have contributed to this decline. There are now several studies that support the notion that the rate of increase may be tailing off. Braun-Farlander has reported a decrease in wheezing illness and asthma in Swiss schoolchildren that is consistent with time trends in specific IgE, and Ronchetti has reported similar changes in Rome [16, 27].

In conclusion there has been an increase in asthma, partly the consequence of an increase in atopy and partly the consequence of factors unrelated to atopy, but it is difficult to identify the non-atopic factors. There are, however, some signs that the dramatic increase in asthma and allergy witnessed in the last 25 years may be starting to level off.

The hypotheses

The Hygiene Hypothesis. Professor David Strachan

Anecdotal evidence suggests that there have been changes in the profile of population immunology in the last 200 years. It would appear that atopic disease was rare in the 19th century. In 1827, John Bostock, a physician with hayfever himself, took 9 years to find 27 patients with hayfever. The international appeal launched by Phoebus in 1862 for hayfever sufferers seems bizarre by modern standards. Charles Blackley, who first identified grass pollen as the causative agent in hayfever, noted that it was a disease of the urban educated classes and rare in farmers, an observation that appears to have been discounted but actually has resonances in recent data.

There is evidence that the prevalence of hayfever increased in the 20th century. The Swiss have provided some remarkable data; in 1926 Rehsteiner, in a study of 28 000 individuals, noted a 10-fold difference in hayfever between rural (0.1%) and urban (1.2%) areas. In a similar study by Wüthrich in 1985 this difference had disappeared (urban 8.0%, rural 10.5%) [28]. The concept of atopic disease as an urban phenomenon was proposed by Finn in 1992 [29]. He suggested that the emerging allergy epidemic was a consequence of 'chemical damage to the mucous membrane of the nose', and that allergies 'are diseases of civilization and that the current high incidence of allergic diseases might be reduced by control of chemical pollution'. As discussed later, atmospheric pollution has dominated lay thinking and the research agenda, especially in the UK.

The hygiene hypothesis arose from an analysis of the 1958 and 1970 birth cohorts. The prevalence of wheeze and hayfever was greater in the 1970 cohort but within each cohort there was a marked negative association between the prevalence of hayfever and the number of older siblings [30]. There were also associations with social class, the more affluent classes having a greater prevalence of hayfever. The hygiene hypothesis was put forward as a possible explanation for the observed association between hayfever and birth order, proposing that this association could be explained if allergic diseases were prevented by infection in early childhood. These infections would be transmitted by unhygienic contact with older siblings and thus the recent increase in allergy was a consequence of reduced opportunities for cross-infection in young families. The reunification of Germany provided an opportunity to investigate the infection and pollution hypotheses. The prevalence of positive skin prick tests was associated with living in the former West

Germany and with birth order [31,32]. These data do not support the notion that the increase in asthma and allergy were the consequence of increasing levels of 'old fashioned' atmospheric pollution because concentrations of both particulate and sulphur dioxide pollution were greater in the former East Germany [33].

By the mid-1990s the hygiene hypothesis was supported by studies demonstrating associations between allergy and birth order, family size, social class and a western lifestyle. More recent studies have demonstrated a decreased prevalence of atopic disease in farming families and in children attending Steiner Schools [34–38]. Some studies have also suggested that antibiotic use in early life increases the subsequent risk of atopic disease and others have related patterns of microbial colonization of the infant's large bowel with atopic disease [39–41]. These newer data support the broadest concept of the hygiene hypothesis, but interpretation is far from straightforward. For example, several studies have reported less atopic disease in Alpine, Scandinavian and Canadian livestock farms; the explanation for this is far from clear. The farming effect has been attributed to a general increase in dirtiness around the farm, specific animal contact, exposure to bacterial endotoxins, environmental mycobacteria, and a more organic diet. In our Shropshire study, the likelihood of atopic disease is strongly negatively associated with consumption of unpasteurized milk, independent of whether the child lives on a farm. It may be necessary to add a dietary component to the infection/hygiene hypothesis.

A disappointing aspect of the hygiene hypothesis is that after 10 years no specific protective organism has been identified. In general the data relating atopy with infection with measles, mycobacteria, hepatitis and pertussis have been inconsistent [42]. It may be that wild-type infection is more protective than immunization. The general burden of infection may be relevant, or there may be a critical window for the protective effects of infection during early childhood or at the time of first allergen exposure.

In order to clarify any role of infection in the natural history of atopic disease, it is necessary to consider the original observations of associations between atopy and birth order and social class. Family size is more consistently related with markers of atopy, hayfever and eczema than with asthma, possibly because asthma is a heterogeneous condition that does not necessarily have an atopic basis. A negative association between allergy and number of siblings has been consistently demonstrated and it appears that older siblings are more protective than younger. There is a consensus that there is an as yet undetermined factor that explains the family size/birth order effect. Clearly family size is not the factor that directly determines the likelihood of atopic disease; rather it is an indicator for another factor. This explanatory factor must be associated with family size/birth order and be a strong determinant of developing atopy. Ideally it will have varied in the last 30 years and vary from place to place, explaining recent time trends and geographical variation in atopy. Recently, the Avon Longitudinal Study of Parents and Children (ALSPAC) study has demonstrated that birth order is associated with gestational age, birth weight and length, head circumference, spending first night with the mother, all immunizations, febrile cough, pus from ear, stool colour and antibiotic use, all by 6 months. However, none of these variables is itself associated with atopy. Therefore, although these factors are

associated with birth order, they are unlikely to explain the birth order–atopy association.

Future studies of the hygiene hypothesis and the family size effect need to address the questions: 'What biologically plausible risk factors are strongly associated with socioeconomic status, family size and birth order?'; 'do indirect markers of early infection (correlated with birth order) indicate large differences in early microbial exposure?'; and 'is the timing of early infection or immunization predictive of later atopy?'.

The Dietary Hypothesis. Professor Anthony Seaton

As outlined above there have been increases in the prevalence of asthma and atopic disease that appear to be associated with increasing prosperity. Diet appears to be a promising aetiological factor underlying the increase. Human beings have evolved consuming a fairly consistent diet comprising locally grown fruit, vegetables and meat. However, in the 1960s our diet started to change in a dramatic way, so that we now eat food from far afield, much of which has been processed in some way to allow transportation.

In the 1980s and early 1990s a number of reports linked diet and asthma. Burney highlighted an association between regional table salt sales and asthma mortality, but it remains unclear whether salt is directly associated with asthma or is merely a marker of a poor diet [43]. Studies from New Zealand have demonstrated negative associations between asthma and blood levels of selenium and glutathione peroxidase activity [44, 45]. It is known that selenium is a vital cofactor for the antioxidative/anti-inflammatory properties of glutathione peroxidase [46]. The National Health and Nutrition Examination Study (NHANES) study of 9074 US adults demonstrated negative associations between the prevalence of wheeze and serum levels of vitamin C, niacin, zinc and copper [47].

In 1994 we proposed that the recent increase in asthma was a consequence of the population becoming increasingly susceptible, rather than the environment having becoming increasingly toxic and asthmagenic [48]. Theoretically it would only take a small increase in population susceptibility to asthma and atopy for the prevalence of these conditions to increase dramatically. An analysis of National UK Dietary Surveys revealed that there had been marked changes in the UK diet since the 1960s with the consumption of fresh foods decreasing, particularly the consumption of fresh green vegetables. Based on these data, we proposed that the recent increase in asthma and atopy was a consequence of declining dietary intakes of fresh fruit and vegetables and the antioxidants they contain [48]. Moreover, the UK dietary data suggested an increase in fresh food consumption since the mid-1980s and we suggested that children born at this time might be less likely to develop asthma; this suggestion is consistent with the recent plateauing of the increase in asthma discussed above [26]. Since atopic diseases manifest very early in life we also suggested that maternal diet during pregnancy might be important in modulating the susceptibility of children to atopic disease.

Since the dietary hypothesis was postulated, a number of studies have reported associations between diet and asthma and atopy. The US Nurses' Health Study prospectively studied 77 866 female nurses and demonstrated that doctor-diagnosed asthma was negatively associated with consumption of foods containing vitamin E [49]. In a study of Australian children the

consumption of oily fish by children reduced substantially the likelihood of current asthma [50]. This study also demonstrated that early chest infections were associated with a twofold increase in the risk of current asthma. A recent study by Fogarty et al. has also demonstrated a negative association between intake of foods containing vitamin E and serum IgE and allergen skin prick sensitization [51].

In 1997 Black & Sharpe noted the marked changes in dietary intake of fatty acids that had preceded and paralleled the recent increase in asthma and atopic disease [52]. They implicated the biochemical pathway whereby n-6 linoleic acid is metabolized to PGE₂, a Th2 promoting prostaglandin, whereas n-3 PUFA, especially those derived from oily fish, were potent inhibitors of PGE₂ synthesis. On epidemiological and immunological grounds Black & Sharpe suggested that the decreasing dietary intake of n-3 polyunsaturated fatty acid (PUFA) and increasing dietary intake of n-6 PUFA could modulate Th cell differentiation towards the Th2 phenotype and atopic disease. The lipid hypothesis was a modification of the original dietary hypothesis but the emphasis was now on lipids and their interactions with Th cell differentiation.

In our first test of the dietary antioxidant hypothesis in a small nested case control study, bronchial hyper-responsiveness was negatively associated with dietary vitamin C, vitamin E, carbohydrate and fibre intakes [53]. We also demonstrated a positive association between a high fat diet and bronchial hyper-responsiveness. In a second nested case control study, adult onset wheeze was negatively associated with dietary intake of foods containing vitamin E, plasma ascorbate levels and serum α -tocopherol levels after adjustment for serum triglycerides [54]. We have also conducted a study of diet in a non-western country. In general, in Saudi Arabia there has been a huge increase in prosperity in the cities where asthma and atopy are common but in the desert, where people still lead a traditional lifestyle, asthma and atopy are rare. Hijazi developed a dietary questionnaire for an Arabic diet and demonstrated that childhood asthma was negatively associated with dietary intakes of milk, vegetables and foods containing vitamin E, magnesium and sodium [55]. The apparent protection by milk is of interest in view of the comments of Strachan above.

In all our three studies, of subjects of differing ages, with contrasting backgrounds and differing respiratory outcomes, vitamin E appeared to be negatively associated with bronchial hyper-responsiveness, adult onset wheeze and childhood asthma. Consequently our opinion is that foods containing vitamin E provide some protection against the development of asthma and atopy. Our ongoing dietary study is investigating the possibility that maternal dietary intake of antioxidants and lipids during pregnancy influences fetal immune development and the likelihood of childhood asthma and atopic disease. We have recruited 2000 women during early pregnancy, representative of the generality of local pregnant women. The babies born to this cohort of women have now been followed up for two years. Immunological investigations have also been conducted in a subset of neonates born to the cohort. Devereux et al. have demonstrated that *in vitro* cord blood mononuclear cell (CBMC) proliferative responses after stimulation with allergens are positively associated with a family history of atopic disease and maternal smoking. CBMC proliferative responses have also been shown to be negatively associated with birth order and maternal dietary intake of foods containing vitamin E, i.e.

the lowest CBMC proliferative responses were in neonates whose mothers consumed the highest levels of foods containing vitamin E [56]. The one year follow-up of 1500 children has demonstrated that wheeze in the first year of life was positively associated with male gender, maternal atopy and use of antibiotics (independent of whether the antibiotics have been prescribed for respiratory or non-respiratory conditions). Wheezing in the first year of life was negatively associated with breastfeeding and maternal dietary intake of foods containing n-3 PUFA.

It would appear that there is reasonably strong evidence that dietary factors influence the risk of wheezing, bronchial hyper-responsiveness, atopy and *in utero* immune development. As discussed below by Barker, it is also looking increasingly likely that vitamin E and PUFA interact to modulate Th cell responsiveness and influence the risk of developing asthma, atopy and, possibly, autoimmune diseases such as Type 1 diabetes mellitus. There are theoretical reasons why vitamin E, selenium and fatty acids can modulate Th cell differentiation and there is also the issue of bronchial hyper-responsiveness that can be independently influenced by dietary sodium, magnesium, vitamin E and vitamin C. The independent effects of dietary antioxidants on Th cell differentiation and bronchial hyper-responsiveness may partly explain the dissociation between atopy and asthma/bronchial hyper-responsiveness.

The role of antenatal influences. Professor Patrick Holt, Perth

There is increasing interest in the concept that the development of asthma and atopic disease is influenced by antenatal influences that interact with factors that regulate the postnatal maturation of immune function, to influence development of atopic disease [57, 58]. There is a growing body of data suggesting that one of the major determinants influencing the risk of asthma and atopy is the overall rate of postnatal maturation of immune competence and its impact on the capacity to develop protective immunity against allergens and microbial pathogens early in life. There is a large body of data indicating that the key stimulus for the overall maturation of the immune system is postnatal exposure to signals from the microbial environment that are not displayed to the fetal immune system.

Human immunological memory to environmental allergens is like, but not exactly the same as, the Th1/Th2 paradigm described in mice, the issue being not whether these alternate forms of immunity exist in humans but how the human equivalent is programmed into long-term immunological memory. It is clear from cross-sectional and longitudinal studies of immunity to inhalant allergens that the Th1/Th2 pattern is well imprinted in the immune system by the age of 5 years. By that age, atopic children's Th cells secrete a range of Th1 and Th2 cytokines and the discriminating factor between atopic and nonatopic children is the absence of Th2 cytokine secretion such as IL-4 and IL-5 by non-atopic children. Instead in these children there is low-level IFN- γ and IL-10 secretion that appears to limit Th1 differentiation and prevent pathological Th1 responses.

In the early 1990s we hypothesized that the rate of postnatal maturation of general adaptive immune function may be a discriminator of risk of atopic disease. In 200 T cell clones from infants at low risk of atopic disease, after polyclonal stimulation there was a wide range of Th1 (IFN- γ) and Th2

(IL-4) secretion, with some clones secreting exclusively IFN- γ , some exclusively IL-4 and others both IFN- γ and IL-4 [59]. In contrast, in 200 T cell clones from children at high atopic risk there was reduced capacity to secrete both Th1 and Th2 cytokines. It would appear from this study that T cell clones from infants at high atopic risk are relatively Th2-polarized and have a generalized reduced capacity to mount adaptive immune responses that is most marked with Th1 IFN- γ responses. It is clear from a number of similar studies that the cord blood cells of neonates without a family history of atopic disease secrete more IFN- γ and more importantly, mature into the adult pattern more quickly than those from neonates with a positive family history of atopic disease [60–63]. Consequently, CBMC from neonates with a family history of atopic disease have a relative deficiency in their capacity to make Th1 cytokines for a much longer period postnatally.

During the developmental window in which T cell memory to inhalant allergens is being programmed there are major differences in the capacities of infants to mount Th1 responses depending on their family history of atopic disease. This differential delay in maturation may have consequences for immune responses against infectious disease because children who have suffered an intense respiratory syncytial virus (RSV) infection, have retarded postnatal maturation kinetics of Th1 function relative to children with less intense RSV infections [64]. Interactions between infection, maturational delay and atopic sensitization appear important in the development of asthma.

There is also evidence for multiplicative interactions between atopic sensitization to inhalant allergens and respiratory tract infections that increase the likelihood of children developing asthma. There appear to be two pathways that can generate airway inflammation and these two pathways interact to modify the risk of asthma at the age of 6 years [65]. In virus-induced inflammation, children developing bronchiolitis in the first year of life, when airways are actively growing, develop high intensity airway inflammation, reactive airways and occasional wheeze. In contrast, children progressing down the atopic sensitization pathway develop Th2-polarized memory to inhalant allergens, and continuing low grade allergen exposure results in low to moderate airway inflammation, some reactivity, and occasional wheeze. However, if the two forms of inflammation occur in the same child there is a synergistic interaction with magnification of the inflammatory response, reactivity and frequency of wheezing. The low IFN- γ phenotype and maturational delay associated with a positive family history of atopic disease is potentially very important in this model because IFN- γ has important antiviral properties and relative deficiency will increase the risk of intense viral infections and airway inflammation and, potentially, development of asthma.

There is a growing literature arguing that Th1 function is suppressed early in life in order to protect the placenta from the toxic effects of Th1 cytokines. This raises the question as to what happens postnatally when Th1 function is required to protect the infant against the microbial environment. Studies of mucosal immunology going back to the 1960s have virtually resolved this question for every species from the mouse to the cow, showing that the main stimulus driving normal maturation of Th1 function is microbial signals not present *in utero*. This particular set of mechanisms is being reduced to information about specific receptors and their environmental ligands. It

would appear that the CD14 high affinity receptor for bacterial lipopolysaccharide and the TOLL receptors are extremely important in the process of postnatal maturation of the immune system. Recent data report genetic polymorphisms in a number of these important receptor molecules. For example polymorphisms in the CD14 gene have been shown to influence susceptibility to allergic sensitization [66].

We have recently been investigating the molecular mechanisms that are important in down-regulating Th1 function in the fetal environment. We have shown that the regulatory mechanisms for the IFN- γ gene depend on the levels of methylation of CpG groups in the promoter region for the IFN- γ gene, and that one of the mechanisms that the fetus uses to damp down Th1 function is to hypermethylate these CpG sites [67]. Other mechanisms have been described that appear to regulate Th1/Th2 function in the fetal environment. The placental trophoblast secretes a range of immunoregulatory molecules such as IL-4, IL-10, PGE₂, and prostaglandins [68, 69]. *In utero* it would appear that there is a generalized reduction in Th cell secretory cytokine capacity but that inhibition of Th1 cytokine secretion is more profound. It is these regulatory barriers that need to be overcome in the postnatal maturation of the immune system.

We have also been investigating the possibility that the mechanisms regulating the production of Th1 cytokines in the postnatal environment are already expressed *in utero*. We have demonstrated that the detection of IL-4 and IFN- γ cytokines in cord blood at birth is associated with a reduced likelihood of the infant developing doctor-diagnosed asthma, wheezing and positive skin prick tests to house dust mite by the age of 6 years. The association between the reduced IL-4/IFN- γ phenotype and atopic disease is the same as that reported in the earlier cloning studies. These results suggest that the mechanisms of the suppressed IL-4/IFN- γ phenotype and susceptibility to atopic disease are already operative by time of birth. However it is necessary to point out that the main source of these serum cytokines in the fetal environment is the placenta rather than the fetal immune system.

In conclusion, an attenuated ability to secrete Th1/Th2 cytokines is the hallmark of increased risk of atopic disease. The attenuated Th1/Th2 cytokine phenotype is evident *in utero* and this phenotype can be modified by factors present in the maternal environment.

The role of allergen exposure. Professor Tom Platts-Mills, Virginia, USA

There are three types of hypotheses to explain the marked increase in asthma prevalence and its time course. The first type relates the increase in asthma to greater exposure and sensitization to perennial allergens such as house dust mite because of carpet use, warmer houses and less time spent outdoors. This notion is difficult to sustain given the increase in asthma in areas where there has been no increase in, or there are no, house dust mites. The second hypothesis type proposes that the increase in asthma is a consequence of changing immune responsiveness consequent upon changing diet and/or changing infective load, microbial gut flora and use of antibiotics. This hypothesis provides an excellent basis for understanding the increase in hayfever that occurred in the first half of the 20th century but fails to explain the dramatic increase in asthma among allergic individuals that started about 1960. The final

hypothesis proposes the loss of specific factors that protect the lung from asthma; this proposal originates from the well-documented decrease in physical activity and increase in obesity. This hypothesis proposes that these factors can increase the likelihood of wheezing and that this effect is exaggerated in atopic children. It is possible that the severity of the epidemic reflects elements of all these, i.e. high exposure year round in homes; separation from animals; and a progressive decline in physical activity. It is worth noticing that dietary changes could be relevant both to changes in inflammation and obesity. Clearly it is important to ascertain whether there has been an increase in atopy or an increase in asthma in atopic children.

The physical and immunological characteristics of allergens have been known for a long time. The major allergen of dust mite is a potent cysteine proteinase, which becomes airborne on relatively large particles, 10–40 µm. In contrast, none of the described cat allergens have enzymic activity and their particle sizes are smaller at 2–15 µm. Cat and house dust mite allergen behave differently when airborne; cat allergen is always airborne in houses whilst house dust mite is only airborne after disturbance. We have estimated that the number of house dust mite allergen particles inhaled daily is in the order of a few hundred, whereas the inhaled particulate load for cat allergen, both as allergen or number of particles, must be at least 100-fold higher.

The description of Th1 and Th2 subsets in mice has greatly increased the understanding of immunological mechanisms behind atopic sensitization, although in humans this paradigm is not so clear cut [70–72]. The immune responses mounted against common inhalant allergens do not follow the Th1/Th2 classification. Th2 responses are the basis of atopic sensitization, but classical Th1 delayed-type hypersensitivity reactions to common inhalant allergens are extremely rare, if they occur at all. It may be that immune responses to inhalant allergens are either Th2 responses or regulatory Tr cell responses that can be regarded as a form of tolerance. Tr cells appear to play a major role in immune responses to cat allergen. A series of studies has demonstrated early life exposure to cats or dogs to be associated with a decreased risk of allergy and asthma. The European Community Respiratory Health Survey has demonstrated that in Spain the prevalence of cat sensitization is 4.5% and cat ownership is 7.8%, in Sweden cat sensitization is 14% and ownership 19%, whereas in Australia the prevalence of cat sensitization is 10% whilst ownership is 48% [73–77]. It appears that a sizeable proportion of children do not become sensitized to cats despite significant exposure, and effectively there is much less sensitization (approximately 65%) than would be expected from extrapolation of house dust mite data. Immunological characterization of children with high cat exposure reveals that many of them have IgG antibody including IgG₄, which is a marker of a Th2 response because it is IL-4-dependent [78]. We have proposed that the alternative immune response to cat exposure is a modified Th2 response characterized by IL-10 secretion that enhances IgG₄ production and limits IgE production and that this immunophenotype is not associated with an increased risk of asthma. Further evidence suggests that the whole of the immune response to cat is controlled possibly by IL-10 thus preventing damaging Th1 and Th2 responses.

There appear therefore to be two types of allergens, type 1 such as mite and cockroach that have a linear dose-response [79–81] and type 2 such as cat and dog that are associated with

higher exposures, elicit immune tolerogenic responses and have a different dose-response slope [72, 78]. These differences may be related to enzymic activity and/or particle size, or to particle size distribution. The observed increase in allergy cannot be a direct consequence of increased exposure as, for example, in Sweden 80% of cat-sensitized children have never lived in a house with a cat [75, 82]. If the bulk of cat sensitization occurs after exposure to low levels of allergen, increasing exposure would not increase disease prevalence.

It would appear that there were two relatively trivial diseases, wheezing and seasonal hayfever. Unfortunately in the last 40 years these two diseases have combined to produce allergic asthma, which has become both common and sufficiently severe to hospitalize many children. We have to open our minds and consider all the possible influences that have changed in our society, obvious candidates being changes in antibiotic use, diet, housing, and air pollution. Unfortunately, none of these appear to explain the epidemic of asthma. The cleanliness-related hypotheses fail most obviously to explain the prevalence and severity of asthma in the poorest areas of American cities. The highest persistent figures of morbidity and mortality for asthma occur in a population living in crowded conditions often infested with cockroaches and rodents. The one thing that has not been investigated extensively to date is the catastrophic decline in physical activity and increase in obesity. The changes in obesity and physical activity have been dramatic, widespread and associated with affluence; these changes could well provide a major element that explains why wheezing has increased so severely among allergic individuals and has become a disease associated with poverty.

Where do we go from here?

Asthma genetics: recent developments and relationship to the epidemic. Professor Scott Weiss

Genetics by itself is clearly an inadequate explanation for why asthma prevalence has increased. The spontaneous mutation rate in the human genome is 2×10^{-8} per base pair per generation, and this is too infrequent to explain the three- to fourfold increase in asthma within a generation. Furthermore asthma appears not to be associated with an evolutionary advantage. The increase in asthma is also too rapid to be attributed to genetic drift. Heritability for asthma is modest when compared with many other complex traits such as type 2 diabetes, schizophrenia and obesity, and this supports the widely held belief that environmental factors play a major role in its causation. Conceptually, however, asthma appears to be the consequence of gene–environment interactions and it is possible that different genes interacting with differing environmental exposures may be contributing to the increase [83–86]. It would be incorrect therefore, to discount genetics completely when thinking about the epidemic and its causes.

One method used by geneticists to assess possible associations between genes and a phenotype is linkage analysis. By using the phenomenon of linkage disequilibrium, the marker loci may be in linkage disequilibrium or allelic association with putative causative alleles. Linkage analysis identifies the distribution of anonymous DNA markers across the genome within families to determine if a region of the marked genome contains a gene

related to the phenotype. In studies of asthma the phenotype may be, for example, doctor-diagnosed asthma, bronchial hyper-responsiveness to methacholine, or total serum IgE level. Geneticists analyse isolated or outbred populations using either sib pair or pedigree study designs. Within these study designs they relate anonymous DNA markers statistically to the phenotype of interest. To date, nine whole-genome screens have been reported in the literature [87–95]. Most have used an affected sib pair design although a few have used an extended pedigree design. All of these studies have relatively low power to determine genome-wide significance because of relatively small sample sizes. Despite this, a number of studies have independently reported linkages between asthma and a limited number of regions across the human genome. Across the nine studies there is quite good replicability for many of the regions. Seven studies have implicated 6p, six 12q, five 4q, 5q, 11p, 13q, and 21q and four have implicated 12p, 5p, and 7p. Up to the time of this symposium, no genes from these studies have been reported in the literature. There is, however, some evidence that genes have been identified but not reported, and it is likely that in the next year a few genes associated with asthma will be reported.

Advantages of the genome screen technique are that it is comprehensive, no prior knowledge of pathobiology is required and putative unknown important genes will (hopefully) be identified that will open up new fields of asthma biology, research and therapy. This method, however, is technologically intensive and very expensive. It needs to be contrasted with the candidate gene approach that is based on knowledge of asthma pathobiology, is relatively cheap and technologically non-intensive, the importance of a known gene being determined by association studies [96]. This method will however, fail to detect novel unknown genes. Candidate gene studies may use either of two types of association study design, case-control or family-based. The interpretation of a significant positive result from either sort of association study is that it could be due to chance, linkage disequilibrium/allelic association or population stratification/ethnicity. One of the limitations of association studies is the poor replication of findings across different studies. This is probably a consequence of small sample sizes, typically with fewer than 150 cases and controls. Analyses of association studies have tended not to be hypothesis-driven and have performed multiple comparisons. The selection of controls has also been a neglected area and may contribute to the poor replicability. Ideally the results of an association study should be reproduced independently, preferably using a different study design. To date this relatively high standard has not been met in the published literature.

It is becoming apparent that for the majority of the genes that will be described for asthma, interactions with the environment will probably be very important. Interaction between two factors has been defined as their coparticipation in the same causal mechanism of disease development, measured as departure from an additive model. It is important to recognize that when there is an interaction between a gene and an environmental exposure, the gene itself may have no effect on the disease phenotype, but when it interacts with the environmental exposure there is either a markedly increased risk or an element of protection. The fact that the main effect of the gene is not significant does not preclude the possibility of significant biological influences.

A number of genes have been described in at least two studies as showing associations between variants in the gene and particular asthma phenotypes. Not all of the gene effects are interaction effects. The candidate genes implicated in the immunobiology of asthma encode for IL-13 (5q), β_2 AR (5q), CTLA4 (2q), IL-4 R α (16q) and IL-10 (1q). The β_2 -adrenoreceptor (AR) is one of the best-described genes associated with the asthma phenotype [97, 98]. It is encoded on chromosome 5q; this region has been identified as being of potential importance in five of the nine genome screens and contains a large number of candidate genes such as IL-4, IL-13, IL-5, CDC25, β_2 -AR and many others. The β_2 -AR is a 7 spanning G-protein coupled receptor that is found in the cell membranes of smooth muscle cells. Thirteen gene variants have been identified; the two that will be briefly discussed here are the polymorphisms at positions 16 and 27 that result in a glycine for arginine substitution and a glutamate for glutamine substitution, respectively. *In vitro* studies have demonstrated that gly16 homozygotes are down-regulated relative to the arg phenotype and that the glu27 mutation results in resistance to down-regulation. There is linkage disequilibrium between these two loci and, in terms of pharmacological effect, it appears that gly16 predominates over the glu27-associated response to β_2 -agonists. A number of case-control studies have reported that genotypic variation is associated with variation in asthma phenotypes, particularly those relating to asthma severity [99]. We have recently conducted a case-control association study in China; cases were characterized by a physician diagnosis of asthma, > 12% bronchodilator response and PD₂₀ < 1.68 mg methacholine, and were compared with non-asthmatic, non-symptomatic controls who had normal lung function, no bronchodilator response and no methacholine-induced bronchial hyper-responsiveness. This study found that there was an interaction between β_2 -AR polymorphisms at position 16, smoking status and the likelihood of asthma. Those individuals who were arg/arg homozygous at position 16 of the β_2 -AR and who smoked 30 cigarettes a day had an odds ratio for having asthma that approached 20. This is an example of a minor gene that appears influential when it interacts with an environmental exposure, namely cigarette smoking. Potentially this gene may be useful in identifying smokers who may develop asthma and/or chronic obstructive pulmonary disease (COPD).

In conclusion, genes alone cannot explain the recent increase in asthma. It is envisaged that asthma genetics will identify novel genes (linkage/association) and novel gene/environment interactions (association studies). The genetics of asthma is still in its infancy and, to date, a small number of genes of relatively small effect have been identified while a few as yet unnamed genes with significant effect have been identified.

Immunology: Paradigm lost. Dr Robert Barker

In recent years the Th1/Th2 paradigm and the notion that atopic disease is caused by an immune system skewed towards the generation of Th2 responses has dominated immunologists' thinking about atopic diseases. The practical consequence of this has been a focus on the possibility of treating or preventing atopic diseases by promoting Th1 responses. Recent epidemiological and immunological evidence, however, shows that the relationship between Th cell immunity and atopic disease is more complex than the simple Th1/Th2 paradigm.

It is well established that Th cells can differentiate into subsets that secrete different patterns of cytokines and that these Th cell subsets have differing functions [70, 100]. It is also accepted that Th2 cells, characterized by the secretion of cytokines such as IL-4, IL-5 and IL-13 are central to the pathogenesis of atopic diseases because of their abilities to switch B cells to make IgE and to influence eosinophilic inflammation [101, 102]. However, there are some difficulties with the concepts relating to the regulation of the Th1- and Th2-based responses, in particular the notion that regulation is achieved primarily by mutual antagonism between Th1 cells and Th2 cells. This notion originally arose from *in vitro* work, and has developed into the paradigm that Th2-associated diseases such as asthma are caused by a lack of Th1 responses. By extrapolating from the experimental observations of mutual Th1/Th2 cellular antagonism, it has become popularly believed that individuals with atopic diseases have an immune system biased towards Th2 and that individuals with autoimmune diseases such as rheumatoid arthritis and Type 1 diabetes have an immune system biased towards Th1 responses. A further extrapolation has been the proposal that the increase in atopic disease reflects a generalized bias of the immune system towards Th2 responsiveness associated with westernization. There is a widespread belief that the neonatal immune system is inherently biased towards Th2 at birth, and that bacterial and viral infections are required early in life to induce Th1 immunity and to 'correct' this neonatal Th2 bias [61]. The conclusion from this way of thinking has been that therapeutic or public health measures to treat/prevent atopic disease should aim to promote Th1-based immunity.

There are three lines of evidence that cast doubt on the notions that immune regulation is primarily based on Th1/Th2 antagonism, and that the recent increase in atopic disease is a consequence of a general bias of the immune system towards Th2. Epidemiological studies have demonstrated that autoimmune diseases characterized by Th1 differentiation such as rheumatoid arthritis and type 1 diabetes mellitus have become more prevalent in parallel to the increase in atopic disease [103–105]. The simultaneous increases in both Th1- and Th2-based diseases would not be expected if there had been a generalized population bias towards Th2 immunity and atopic disease. One possible explanation for the increase in both Th1 and Th2 diseases is that they could be occurring in different population groups that are exposed to different environmental factors. However, this appears not to be the case, because within individuals there is a positive correlation between Th1- and Th2-mediated diseases, such that patients with an autoimmune disease are at greater risk of developing atopic diseases and vice versa [106]. Thus, it is now very difficult to argue that Th1- and Th2-based diseases are mutually exclusive; rather they are increasing in parallel in the same groups of patients. The second line of evidence casting doubt on the relevance of a simplistic Th1/Th2 imbalance to asthma derives from work on a mouse model of eosinophilic pulmonary inflammation [107]. In these studies, mice are sensitized to ovalbumin and then challenged by inhalation, with the consequent development of eosinophilic lung inflammation. A series of experiments with this model has demonstrated that, in order for eosinophilic lung inflammation to develop, both Th2 and Th1 cells are necessary; furthermore the inflammation can be made more severe by increasing the number of Th1 cells. These data argue against the relevance of

mutual regulation by Th1 and Th2 cells, since it would appear that there is co-operation between the two subsets in causing pathology, rather than antagonism. Finally, studies of human Th cells have demonstrated that Th1 and Th2 responses are not mutually exclusive. The *in vitro* profiles of cytokines secreted by human T cells suggest that there is not a simple polarization towards Th1 or Th2. Rather, the majority of human Th cells are of the Th0-type and secrete cytokines from both categories and, in atopic individuals, Th cell responses to allergen are characterized by both Th1 and Th2 cytokines [108, 109]. We have demonstrated in atopic adults and in cord blood that there is a strong positive correlation between Th cell secretion of Th1 and Th2 cytokines after allergenic stimulation. Thus, although the Th cells from atopic individuals and neonates may secrete elevated levels of the Th2 cytokine IL-4, there is a commensurate increase in the secretion of Th1 IFN- γ . These data demonstrate that, rather than being reciprocal, there can be positive associations between Th1 and Th2 responses in human subjects. The notion that neonatal Th cell responses are inherently biased towards the Th2 phenotype is now also being challenged, because several studies have demonstrated that typical human cord blood Th cell responses are characterized by the secretion of both Th1 and Th2 cytokines. Our own work has demonstrated that cord blood mononuclear cell responses are heterogeneous, some individuals being polarized towards Th1, some towards Th2, but the majority being Th0 in character, with the ability to produce both Th1 and Th2 cytokines [110]. This work on human cord blood has also demonstrated that the established risk factors for atopic disease, family history and birth order, do not correlate with overall Th2 bias; instead there appear to be positive associations with both IFN- γ Th1 and IL-4 Th2 responses [56]. Thus, CBMC IL-4 responses after stimulation by allergen and other agents tend to be elevated in neonates whose mothers have atopic disease, and for some stimuli this increase is statistically significant. However, there are stronger associations with the Th1 cytokine IFN- γ , the cord blood mononuclear cells of neonates whose mothers have atopic diseases demonstrating statistically higher IFN- γ responses after a number of stimuli. These results differ from studies demonstrating Th2 polarization at birth, but they are consistent with the concept that atopic disease is associated with a generalized up-regulation of both Th1 and Th2 immune responses at birth.

Taken together, these lines of evidence lead us to propose an alternative hypothesis to replace the concept that the development of atopic disease is a consequence of lack of regulation of Th2 responses by Th1 immunity. The alternative is that a key factor in predisposition to atopy is the effectiveness of immune regulation by T cells other than the Th1 subset. Recent work has highlighted T cell subsets that have important regulatory influences on both Th1 and Th2 responses and a number of such regulatory T (Tr) cells have now been described. It appears, particularly from studies in the field of autoimmunity and inflammation, that Tr cells play important roles in preventing, or possibly reversing, the pathological processes that ultimately lead to disease [111–115]. For example, a Tr cell subset that secretes predominantly TGF- β (Th3 cells) can control some of experimental models of autoimmune diseases and in some circumstances is responsible for the induction of oral tolerance. Tr1 cells are characterized by the secretion of IL-10, and this subset is a potent regulator of inflammatory diseases in animal

models. There is also great interest in a Tr cell subset identified by the CD25 antigen, which in many examples appears to exert its regulatory function by cytokine-independent mechanisms. Although the interrelationships between such Tr subsets remains to be established, it is increasingly accepted that they play key roles in protection and recovery from experimental models of immune mediated diseases. In contrast to the large and rapidly growing body of evidence that Tr cells are important in animal models of autoimmune and inflammatory diseases, the study of these cells in atopy and in humans has lagged behind. Nevertheless, crucial experiments have established the relevance of IL-10 secreting Tr cells to protection from airway eosinophilia in mice [113]. Tr cells are now being identified in humans [114–116], and our own work has demonstrated that Th1 response to an autoantigen on human red cells from patients with autoimmune haemolytic anaemia can be attenuated *in vitro* by stimulating IL-10-secreting T cells, and exaggerated by inhibiting IL-10 with antibodies against IL-10. As described above by Platts-Mills, there are also some human data demonstrating that lack of susceptibility to cat allergen sensitization is associated with IL-10 secretory responses [78].

In conclusion, irrespective of the exact mechanisms of action and the relationships between Tr subsets, it is clear that such cells have profound effects. They can inhibit Th1 cell responses and there have been some studies suggesting that they can also suppress Th2 responses. It is important that future work should focus on the roles of Tr cell subsets in atopic disease, rather than on the simple Th1/Th2 antagonism model. This change in emphasis should not be taken to mean that Th2 cytokines do not drive atopy, but rather that their production is controlled primarily by Tr, and not Th1, subsets. There are early data demonstrating an involvement of these Tr cell subsets and of the cytokines they produce in the immunopathogenesis of atopic disease. Thus, there is now evidence to support the hypothesis that an important factor in predisposition to atopy is the effectiveness of immune regulation by T cell subsets other than Th1/Th2. It is likely that the function of these Tr cells will be modulated by the factors known to influence the development of atopic disease, namely; genetic and environmental factors including diet and infection by agents such as mycobacteria [113] and viruses [116]. An attractive feature of this regulatory hypothesis is that, for the first time, it is possible to unite Th1-mediated and Th2-mediated diseases and to start to look for shared epidemiological and genetic factors predisposing to both types of disease.

Why is asthma on the increase? The views of people with asthma. Ms Marjory O'Donnell

The National Asthma Campaign is very interested in knowing why asthma has increased. From studies conducted by the Campaign it is clear that people with asthma patients consider atmospheric pollution to be the major cause of asthma. In the Needs of People with Asthma Study, the consistent overwhelming opinion of people with asthma was that the increase in asthma is the consequence of general air pollution (84%) or more specifically traffic fumes (30%). When asked what they wanted to be done about the increase in asthma, 67% wanted a decrease in air pollution, 34% a reduction in traffic emissions, 16% a ban on smoking, 16% mentioned healthier lifestyles, 13% expressed a wish for more research and 12% felt that nothing

could be done. Interestingly, although the majority of people with asthma felt that air pollution was the cause of the increase of asthma, in general only 34% felt that air pollution triggered exacerbations of their own asthma.

When asked what caused asthma, 42% mentioned genetics, 23% pollution, 22% allergies, 12% unhealthy lifestyles and 27% had no idea. When asked why their asthma had improved, 23% mentioned better medications and only 6.2% mentioned improved air quality. 32% said they had grown out of it. What these data tell us is that people with asthma regard pollution as the most important factor in the increase in asthma, but that this is less pronounced as people talk about their own lifetime experience of asthma. These findings may have implications for the concept of the 'expert patient'; people with asthma are experts in the field of their own asthma but not in the field of asthma. This is to be expected because they base their opinions on their asthma on their own experiences but base their opinions on asthma in general on what they read, hear and see on the television. Here they tend to be selective in what they understand and what suits them.

The Campaign's recommendations for the direction of future research are that there should be a better definition of asthma phenotypes, that more longitudinal studies are needed, and that there is an urgent need for the testing of current hypotheses relating to the increase in asthma in order to conclude the current theorizing.

Industry's concerns. Mr Michael Hardiman

The disparity between the scientific community's and the general public's understanding of the factors behind the increase in asthma and allergy has been highlighted by the work of the National Asthma Campaign. The disparate views of the general public and the scientific community are not merely of academic interest because there are real financial implications for certain industries and the workers they employ. The practical consequences of the popular misconceptions surrounding asthma and allergy are well illustrated by their effects on the carpet industry.

In the last 10 years there has been a generalized trend for the removal of carpet in the home and public places. This has been widely recommended and has occurred despite the absence of studies demonstrating beneficial effects of isolated carpet removal. Indeed, in Sweden between 1973 and 1990 there has been a 3-fold increase in asthma and allergy despite an 80% decline in carpeting and the substitution of wood and laminate flooring.

Therefore, there is a pressing need for the current scientific understanding of the recent increase in asthma and allergy to be widely disseminated, so that people can make better informed decisions about their lifestyle.

Commentary. Professor Anthony Seaton

It has been said that to every complex problem there is a simple answer that is wrong. Nowhere has this been better demonstrated than in the case of explaining the rise in asthma and allergies. Two superficially attractive ideas (they were never more than speculations), that the answer lay in increased air pollution and/or mite populations in houses, attracted the attention of the media and are now widely believed by lay people to be the truth. Those who promote such speculations as truth may not always consider the consequences, the major one of

which is a potential waste of resources in tackling the wrong target. Few involved scientists believe that reducing air pollution further will reduce the incidence of asthma. But another important consequence is that on industry. Are we really justified in recommending the removal of carpets or the wholesale killing of mites? Or cats? Where is the evidence?

This symposium has highlighted some of the real complexities of this issue and pointed to fascinating new leads. Perhaps most striking have been the paradoxical findings of epidemiological research; these all need to be comprehended in any new hypotheses. While some researchers seek to reduce allergen exposure, allergies and asthma are much less frequent among those with high exposures on farms. While some air pollutants increase the risk of asthma attacks, asthma has increased as pollution has reduced, and prevalence is as high among those living in unpolluted parts of Britain such as the Scottish Highlands as in those living in cities. Early infection has been shown to increase risks of early life wheeze, but some form of microbial stimulus is thought to be necessary for 'normal' immune development. While some believe that asthma has increased because of a population shift to the Th2 phenotype, Th1 diseases such as diabetes have also increased and even occur more frequently than would be predicted on the basis of chance in the same individuals.

So much for some of the difficulties! The patient with asthma, as we have heard, understandably wants answers. What do we know and what are we likely to find out in the short term? Two exciting lines of inquiry seem most likely to be profitable, and both focus on early immune development, setting the scene for later development of illness by increasing population susceptibility. Interestingly, both depend on examination of what is taken in through the mouth rather than inhaled. The hygiene hypothesis emphasizes the early months of postnatal life, and is now focusing particularly on immune processing by gut bacteria. These commensals can be altered by a number of the factors known to show epidemiological associations with risk of allergy, including early administration of antibiotics. If this hypothesis is correct, there is a real prospect of prevention by such measures as administration of *Lactobacilli* and by development of improved vaccines against common childhood illnesses. The dietary hypothesis also emphasizes early immune development, but raises the possibility that *in utero* nutrition may be important (in keeping with observations on head size at birth being a risk factor for allergy). In addition, dietary factors, notably foods containing vitamin E, have been shown in many studies to reduce risks of asthma both in childhood and adult life.

There is no doubt that allergies and asthma increase in association with increasing national prosperity. A more 'natural' existence for our children, with exposure to dust, dirt, animals, unprocessed fresh food, fewer antibiotics and more exercise would be likely to be associated with a substantial fall in allergies and asthma. It is possible that it would also reduce risks of juvenile diabetes, another serious and increasing problem. The challenge is to identify the key factors that would contribute to this without producing any accompanying significant disadvantages to health. Appropriate intervention studies in cohorts will be expensive and take some years to produce results, but require to be done. In the meantime, little will be lost if the word gets round to parents and doctors that fresh foods, yoghurt and exercise are good for children and that antibiotics should not be insisted upon for every runny nose.

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