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Allergic Reactions to Inhaled Environmental Dusts

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Abstract
Extrinsic allergic alveolitides (EAA) are a group of hypersensitivity lung diseases caused by organic dusts of diverse origins. Despite the similar clinical, radiological, histological, serological and pulmonary function findings, the causative allergens in the dusts do not belong to a common molecular species. All EAA dusts have particles within the respirable range (i.e. up to 5 μm) some allergens are wholly soluble, some partly soluble and others associated with insoluble particles. EAA is not due to endotoxin in dusts, and the presence of specific antibodies and sensitized lymphocytes against the allergens provides a pointer to the hypersensitivity pathways that are activated to produce disease episodes. However, some EAA dusts can activate these pathways per se at high dust levels. Exposure to EAA dusts does not always lead to sensitization, yet some people respond to extremely low levels. Heredity has little influence on EAA, and although smoking reduces the immune response to EAA dusts, it does not prevent EAA. Prevention is dependent on reducing or abolishing exposure, and several techniques are outlined.

Farmer's Lung and Related Diseases

Farmer's lung was the first described member of the extrinsic allergic alveolitis (EAA) group of allergic pulmonary diseases [1]. EAA diseases are characterized by having a similar pattern of exposure, sensitization and response to exposure, differing from the more frequently encountered hay fever/asthmatic response to inhaled allergens (table 1).

EAA has two phases, acute and chronic [2]. The acute phase is typified by a 'flu'-like episode occurring some 4–13 h after inhaling the offending allergen in susceptible subjects and resolving, when exposure is low, by 24 h. Under conditions of heavy exposure an episode may not resolve for some days or even weeks, and the occasional fatal acute case has been reported [3]. Whilst the 'flu'-like symptoms (pyrexia, malaise, arthralgia, myalgia) and accompanying lung function and radiographic changes represent the full-blown disease, not all these features may be present on every occasion [4]. Several acute episodes, or sometimes a severe acute episode, may lead to a chronic stage where lung fibrosis is observed, accompanied by a permanent loss in lung function and a contributory cause to mortality [5].

Whilst there are many sources of materials that can induce EAA (table 2), only three diseases in the group have been studied in depth, and from these much of our current understanding of EAA has been derived.

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Table 1. EAA and asthma: characteristic findings

	EAA	Asthma
Time of onset	4–13 h after inhalation	Response within minutes after inhalation; a late reaction at 4–6 h follows in some people
Symptoms	'Flu'-like and/or dyspnoea on exertion	Tight chest, wheeze, cough
X-ray	Acute: fine miliary spotting Chronic: fibrosis	No characteristic appearance
Histology	Acute: diffuse interstitial pneumonitis with non-caseating epithelioid cell granulomata Chronic: fibrosis	Epithelial inflammation Intimal muscle thickening Mucous plugs in airways with cells, predominantly eosinophils
Immune response	Mediated by T cells and/or IgG	Mediated by IgE
Skin test	Negative to skin prick test	Wheal and flare in 10 min to skin prick test
Heredity	No genetic linkage	Genetic predisposition in extrinsic asthma
Typical allergens	Thermophiles, fungi, avian dropping dust, amoebal products	House dust mites, pollens, furs, feathers, dander
Particles	Usually < 5 μm	Typically 15–75 μm

Table 2. Sources of allergens causing EAA

Source	Allergens	Disease
Avian (e.g. pigeons, budgies, turkeys, chickens)	Mainly IgA from droppings but also IgA in bloom	Pigeon breeder's lung Bird handler's lung Bird breeder's disease
Thermophiles		
In mouldy cereal (e.g. hay, grain, mushroom compost)	*Micropolyspora faeni* *Thermoactinomyces vulgaris* *Thermoactinomyces thalpophilus* *Saccharomonospora viridis*	Farmer's lung Mushroom handler's lung
In mouldy sugar cane	*Thermoactinomyces sacchari*	Bagassosis
Amoebal contamination	Derived from amoebal development	Humidifier fever
Fungal moulding	*Aspergillus clavatus* *Aspergillus versicolor* *Aspergillus fumigatus/niger* *Cryptostroma corticale*	Maltworker's lung Dog house disease Citric acid workers lung Coniosporiosis
Insects	*Sitophilus granarius*	Wheat weevil allergy
Chemicals	Toluene di-isocyanate	Toluene di-isocyanate

Farmer's Lung –
Caused by Inhaling Mouldy Hay Dust

Although undoubtedly a problem in agrarian societies from the earliest times, an account of grain-dust-induced disease first appeared in the early eighteenth century [6]. The first modern account was by Campbell in 1932 [1], and for the next 30 years farmer's lung was variously described as an infection by fungi [7], spores swelling in the airways [8] or simply a reaction to inhaled dust [9]. With the realization that immune reactions, particularly in the lung, were not always protective [10], the suggestion that farmer's lung was a hypersensitivity reaction in the lung was established by Williams in 1962 [11], who challenged subjects with sterile extracts of mouldy hay dust (MHD) and caused an acute episode only in those previously experiencing symptoms of farmer's lung.

The concomitant finding of antibodies in the serum of farmer's lung subjects against extracts of MHD [12] allowed the development of the allergens responsible for the disease to be followed [13] and has greatly contributed to our understanding of the disease process.

The critical step for farmer's lung takes place several months before the disease peaks in the farming community, i.e. with the harvesting and baling of damp hay that leads to a high-temperature moulding process [14]. Below about 30% water content, hay moulds with fungi as the predominant micro-organisms. Above 35–40% total water content the initial fungal development is superseded by thermophilic actinomycetes. In particular *Micropolyspora faeni* [4] and to a lesser extent *Thermoactinomyces vulgaris* and *T. thalpophilus* are responsible for producing soluble allergens and allergenic spores in vast numbers in the moulded hay. The temperature of the hay may exceed 50 °C [14] for several days allowing only the development of the thermophiles. Eventually, nutrient sources are used up, water content decreases [14] and drier, more brittle hay, with its allergenic mass, cools to ambient temperature. Usually, in the winter time, when such hay is fed to cattle, mechanical disruption of the moulded bale leads to the release of vast numbers of spores and allergens; over 10^9 spores/m^3 have been recorded [15]. Inhalation results in sensitization, and with a mean diameter of 1 μm [16], their site of deposition in the lung is largely alveolar. The ensuing immune response between allergens and the immune system is presumed to be in the alveoli, hence the term EAA, although, as Seal points out, inflammation of the bronchioles is often a feature in farmer's lung [5].

Pigeon Breeder's Lung –
Caused by Inhaling Pigeon Dropping Dust

The unravelling of much of the disease process in farmer's lung [12–16] allowed more rapid progress to be made when the next member of the EAA group, pigeon breeder's lung, was described by Reed et al. in 1965 [17], although reports of a similar condition had been made earlier in poultry workers [18]. A larger study by Hargreaves et al. in 1966 [19] showed the similarity between pigeon breeder's lung and farmer's lung in terms of clinical symptoms, lung function, radiology and serodiagnosis. The offending agent in pigeon breeder's lung was linked to pigeon droppings (PD), but sera from cases reacted not only with extracts of PD but with other pigeon materials, e.g. pigeon egg and serum [20]. Whereas a reaction to PD might be expected, that against serum and egg was unusual in that inhalational exposure to these materials would only occur in exceptional circumstances. The matter was resolved when it was determined that trace amounts of serum proteins are present in freshly voided PD [21] and that the major allergen in PD is pigeon IgA [22], which has allergenic components in common with serum and egg IgG. The common occurrence of IgG, IgA and albumin in pigeon materials allowed many pigeon components to react when tested [23].

Humidifier Fever –
Caused by Inhaling Allergens from Contaminated Humidifier Water

The HMSO factories inspectorate report in 1969 [24] describes fever in workers in print processing, although the first scientific account was from Banaszak et al. in 1970 [25], who described an illness resembling farmer's lung in workers exposed to humidified air-conditioning where the water used in humidification had become contaminated. The isolation of the thermophile *T. vulgaris* (a minor source of allergen in farmer's lung) suggested this (erroneously) to be the source of the problem [25].

Since then outbreaks of humidifier fever have been associated with contaminated water used in humidification/ventilation systems that are necessary for a variety of industrial processes or simply to control temperature and humidity for worker comfort [26–28].

In general the following sequence of events occurs. The control of temperature and humidity is required to facilitate the handling and processing of materials, e.g. to minimize paper shrinkage and distortion in stationery/printing industry, to keep correct tension in yarns such as rayon or nylon, to keep tobacco rolling at best. Air from the working environment is taken into the ventilation

plant, mixed with a variable amount of external air, humidified and returned to the working environment. Although primary filtration occurs, organic material, e.g. cellulose, nylon or tobacco, finds its way into the humidifier which usually involves a spray system and recirculates unused water for economy.

The organic material is used as nutrients by microbes, and an extremely complex ecosystem evolves, depending on the amount and nature of the organic material taken into the humidifier. As spray systems atomize the water, allergens produced by the microbes are disseminated into the working environment with ensuing sensitization and response as in farmer's lung.

In the four outbreaks of humidifier fever we have investigated [29, 30], the simplest ecosystem was one bacterial, one fungal species and amoebae [30]. Other systems involved several bacteria, fungi, amoebae, other protozoa, nematodes and even aquatic mites. We do not consider *T. vulgaris* to be the source of allergens since the running temperature of mains-supplied humidifiers is at least 20 °C below the minimum temperature of development for this organism. Our findings of amoebae in all humidifier-fever-inducing systems, the natural development of amoebae in such systems and the reaction of extracts of amoebae with the sera of affected subjects [31] makes this a more logical candidate for the production of allergens associated with the disease.

Understanding of EAA from Studies on Farmer's Lung, Pigeon Breeder's Lung and Humidifier Fever

Nature of the Allergens Involved

Detection of Allergens in EAA Systems. If the disease is caused by microbes, then detecting their presence by culture is a pointer to their involvement in the allergic response. However, the isolation of one possible source should not exclude investigations into other sources. The assumption by Banaszak et al. [25] that the isolation of *T. vulgaris* from a humidifier fever source was the cause, is an example of this. As a general rule serological reactions are more accurate. Soluble antigens such as in MHD or PD may be extracted and tested by gel diffusion techniques [32] using sera from disease cases and comparing results with non-exposed subjects' sera. For humidifier fever, the humidifier reservoir water has to be concentrated 500- to 1,000-fold for gel diffusion techniques. However, more modern techniques, now in use in our laboratory, negate the necessity for a concentration step and can accurately follow development of soluble humidifier fever antigens and the effects of remedial measures on the responsible microbes.

Physicochemical Characteristics. Since there is such a high degree of similarity between each disease in the EAA group, it was considered that a common thread might lie with the nature of the inhaled allergens. Two diseases were studied in depth, farmer's lung and pigeon breeder's lung. Any differences in the nature of the allergens might be expected to be maximal comparing allergens from the bacterium *M. faeni* with allergens of avian origin. Eight allergens from *M. faeni* were isolated and characterized [33] and compared with five allergens from pigeon serum and PD [34]. Amino acid and sugar analysis showed the *M. faeni* and avian allergens to differ markedly: from classical proteins, e.g. pigeon IgG and albumin, to glycopeptides, e.g. *M. faeni* antigen 1. There was also a wide range of molecular weights and other physicochemical characteristics, e.g. heat stability. The conclusion is that no one type of molecular species is responsible for EAA.

Particle Size. Micrometre-sized particles are aerodynamically suited for penetration to the alveoli, and for the EAA disease where bacterial and fungal spores/conidia carry the allergens there is a fair degree of homogeneity in particle size. The smaller spores, e.g. *M. faeni* and *T. vulgaris,* are approximately 1 μm in diameter, the larger fungal spores causing EAA, e.g. *Aspergillus fumigatus* or *Cryptostroma corticale,* are 3 and 6 μm, respectively [35].

For non-bacterial/fungal sources there tends to be a wider range of particles, and for pigeon breeder's lung the size range and number of particles generated from voided and dried PD is governed by the litter, i.e. dehydrating agent upon which the voided PD falls [36]. The situation is complicated by the interaction between PD and some litters, producing large numbers of small (micrometre-sized) particles with some, e.g. sepiolite, but lower numbers with others, e.g. woodchip, compared with PD not voided onto litter agents [36]. The effect of PD litter interactions on respirable particle and airbone allergen burden is shown in table 3.

In humidifier fever the larger droplets of water generated by the spray systems are eliminated by baffle plates, and allergens are associated with submicrometre-sized particles. Whilst such particles have different deposition characteristics from micrometre-sized particles [37], sufficient numbers are retained in the lung to trigger an attack.

Solubility Characteristics. The allergens in the respirable range may be (1) initially soluble as in humidifier fever and rendered respirable by atomization and dehydration as water droplets change to the vapour phase, (2) soluble

Table 3. Effect of loft litters on respirable particles and allergens

Litter agent	Respirable allergens	Respirable particles
Sand	54	77
Sand/lime	42	52
Woodchip	25	43
Woodchip/lime	25	24
Hay	58	152
Sepiolite	116	204

Results expressed as a percentage of respirable allergens or respirable particles (0.5–5.0 μm) compared with those derived from equivalent amounts of PD alone. Correlation between allergens and particles $r = 0.928$, $p < 0.001$ (by Spearman's rank testing).

but associated with dust particles as in pigeon breeder's lung (3) mainly insoluble in association with microbial spores/conidia as in farmer's lung. This was demonstrated by extracting respirable (micrometre-sized) fractions of MHD and using the extract for inhalation challenge when no reaction was elicited in farmer's lung subjects [11]. It is of interest that episodes of humidifier fever resolve more rapidly than those of pigeon breeder's lung and farmer's lung as do laboratory-induced episodes using minimal amounts of soluble *M. faeni* or avian allergens [38, 39]. Also, no chronic stage of humidifier fever has been described despite multiple episodes of disease in many subjects. Possibly the soluble nature of the allergens allows a more rapid assimilation and/or elimination than the particulate allergens of pigeon breeder's and farmer's lung under conditions of natural exposure.

Nature of the Response in EAA

Endotoxin versus Immunological Reactions. Endotoxins from Gram-negative organisms are widespread and have been considered as aetiological agents in some dust diseases [40]. However, there are several pieces of evidence to show that EAA is not endotoxin mediated:

(i) Endotoxin introduced into the lungs of the experimental animal failed to produce a pyrexial episode even at 30 times the dose needed to induce pyrexia when introduced intravenously [Edwards J.H., unpubl. observations].

(ii) The amounts of allergen producing an episode of EAA in susceptible subjects did not induce pyrexia even when introduced intravenously into the experimental animal.

Immunological and Non-Immunological Pathways Involved in EAA. Our knowledge of the mechanisms involved in EAA has been derived from human, animal and in vitro studies. A résumé is that several immunological and non-immunological mechanisms capable of inducing features of EAA have been described.

The main tissue-damaging mechanisms involving immune components are considered to be: (i) antibodies capable of forming immune complexes with allergen and activating complement; complement activation then leads to the release of tissue-damaging enzymes and other components from polymorphs [41] and macrophages [42]; (ii) sensitized T lymphocytes capable of cytokine release in contact with allergen [43].

Since both complement-fixing antibodies and sensitized T cells have been found in the broncho-alveolar lavage of subjects with EAA [44, 45], both mechanisms can come into play within the alveolar spaces. Translation to disease, e.g. to a pyrexial 'flu'-like episode, could involve cytokines from macrophages, e.g. interleukin-1 and/or lymphocytes e.g. interleukin-2, tumour necrosis factor or γ-interferon. However, EAA dusts can activate these mechanisms non-immunologically. Particles in MHD can activate complement by the alternative pathway [46], whilst soluble material in MHD activates complement directly at the C1 level [47]. Respirable fractions of MHD can cause hydrolase release directly from macrophages [48]. Enzymes with proteinase activity are found in MHD [47] and PD [49]. In particular components with papain-like activity found in MHD can cause emphysema [50], a feature of the chronic stage of EAA. Thus to ascribe EAA only one mechanism/pathway would deny much of the evidence relating to the availability of other immunological components and the potential of each dust per se to initiate pathways leading to disease. The importance of the latter is borne out by recent work where a disease all but indistinguishable from farmer's lung has been described in farmers after heavy exposure to moulded hay/grain without evidence of sensitization. The disease now termed organic dust toxic syndrome [51] was described by us in 1974 as possibly due to complement activation by inhaled dust [52]. Thus each disease episode is more likely to be dependent on several factors that include the type of EAA dust, amount inhaled, type and degree of sensitization of the subject and recent exposure pattern.

Whilst the acute phase can be attributed to the allergic and/or non-immunological mechanisms described, the chronic stage is due to damage to the lung architecture. The most likely candidates for this are enzymes derived from polymorphs and macrophages released after im-

mune and/or non-immune complement-activated stimulation. The role of inherent enzyme function in EAA dusts is less well documented. Since the chronic stage may occur after a single heavy exposure to MHD but has not been observed in humidifier fever (associated with soluble allergens), the continued presence of biologically active material probably exacerbates this stage.

Time Required to Sensitize Subjects. It is difficult to determine this in farmers since exposure to allergens is usually documented only when disease occurs and an unknown degree of exposure from childhood often precedes. However, most cases occur between 40 and 50 years of age [53], suggesting that the normal course of events is a gradual build-up of sensitization. For pigeon breeder's lung and humidifier fever, contact with allergens can be more readily defined when people start their hobby or their work in a humidifier fever environment. The earliest recorded development time for both is a few months before episodes commence. In our detailed study on pigeon breeder's lung, the median exposure time was 10 years (range 2–30 years) [45]. Thus, in general some time elapses before EAA develops in the majority of the population. However, some exquisitely sensitive subjects become rapidly sensitized, and at the other end of the spectrum some exposed subjects do not exhibit symptoms even when exposed for years or even almost indefinitely.

Heredity. Studies on the relative frequency of EAA and HLA types in pigeon breeder's lung and farmer's lung have been undertaken as well as serum immunoglobulin marker allotypes (Gm and Am) [54]. Results fail to show a definite connection, although there is a suggestion that pigeon breeders carrying the P1 erythrocyte antigen are less responsive to pigeon antigens [55]. The influence of heredity was probably summed up by our local study on a set of identical twins who kept pigeons. Despite equivalent exposures and serological evidence of response, only one twin developed pigeon breeder's lung [White J., unpubl. observations].

Smoking. Several studies have shown that smoking reduces the response to EAA dusts with lower serum titres of antibodies in exposed smokers [56, 57].

Our recent study showed that antibodies of all three classes, IgG, IgM and IgA, were reduced in the bronchoalveolar lavage fluid of smokers compared with non-smokers exposed to pigeon allergens [45]. Interestingly ex-smokers had antibody levels equivalent to non-smokers, suggesting that the effects of smoking were transitory. Smoking did not prevent subjects with EAA responding to inhalation challenge with avian allergens [45].

Prevention of EAA

Farmer's lung is rarely seen nowadays due to changes in farming practices enforced by the awareness of the dangers in inhaling MHD.

Hay is still produced in bales with emphasis on baling under dry conditions or may even be dried in an airstream. Otherwise hay may be 'clamped', i.e. kept tightly under plastic sheeting or stored in a silo. The effect of these procedures is to reduce oxygen tension, thereby reducing thermophile metabolism (*M. faeni* is an oxidative deaminator). The overall reduction in metabolism and temperature generation does not favour thermophile development.

However, once opened, hay in clamps can permit thermophile development, and the top layers on silos often produce vast clouds of spores when disturbed that may lead to organic dust toxic syndrome when inhaled. Wearing of face masks is always recommended in dusty environments, especially for sensitized subjects. The nature of farming is such that it is extremely difficult to prevent moulding totally so that allergic material is almost always present to some degree on farms producing cereals.

For pigeon keepers, exposure can be regulated by utilizing loft cleaning practices that generate least dust. Cleaning is the time of greatest exposure [36] and certain loft litter materials produce more dust than others. Quantitative studies on airborne pigeon allergens have shown most respirable (< 5 μm) allergens to be associated with sepiolite, less with woodchip plus lime as loft litters [36], although daily cleaning of freshly voided PD produced the least dust (however, this was on daily basis compared with 1- or 2-week intervals with loft litters).

After an extensive study by Trotman in 1987 [49] the following measures were recommended: (1) use of a face mask; (2) wearing of overalls exlusively for use in the loft; (3) good ventilation with avoidance of dust-adherent surfaces such as glass or plastic in the loft; (4) use of extraction fans; (5) avoidance of litter materials.

Humidifier fever allergens are associated with a submicrometre particle size and adhere naturally to other dusts and surfaces. In one study we found dust on ceiling tiles and other surfaces to carry extractible allergens [29]. The initial source is invariably the spray system of the humidifier and can be reduced by: (1) reducing incoming nutrients e.g. filtering incoming air effectively; (2) diluting e.g. running water to waste or continuously topping up water in the humidifier reservoir; (3) thorough cleaning of all surfaces within the humidifier. However, in practical terms all these procedures do not reduce soluble allergen in the humidifier water to zero. Levels will fluctuate

depending on the remedial measure undertaken. In one study, cleaning and sterilizing with hypochlorite only served to increase soluble allergen. Such a seeming paradox is resolved when it is realized that dead microbes and/or microbial products are as potent immunologically as their viable counterparts. Thus, the cleaning/sterilizing procedure served to release trapped pockets of allergens causing an overall increase. Sterilizing did not affect existing allergens but did reduce viable organisms responsible for future allergen development. After filling and 'dumping' reservoir water several times, the allergens were diluted out.

Finally, the problem can be solved by using steam humidification. However, at least for the UK, the ambient humidity is such that it need not be regulated with such engineering enthusiasm, or perhaps the use of systems without a reservoir (e.g. spinning cones/tops/discs) should be more thoroughly investigated despite their lack of engineering 'neatness'.

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