FACTORS INFLUENCING THE DEVELOPMENT, INVESTIGATION AND RESOLUTION OF HUMIDIFIER-RELATED DISEASES

by

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"...this most excellent canopy, the air, look you, this brave o'erhanging firmament, this majestical roof fretted with golden fire, why, it appears no other thing to me but a foul and pestilent congregation of vapours." **Hamlet**

Dedication: for Ruth, Lucy and Jack, the sources of many delightful interruptions.

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SUMMARY

Factors influencing the development, investigation and resolution of humidifier-related diseases

The development of lung disorders of environmental origin depends on a host response and the circumstances of exposure to noxious inhalable material. Climate, geography, culture, level of physical activity and the quality of indoor and outdoor air are important extrinsic variables. Host susceptibility is influenced by variable mechanisms which comprise pulmonary defence. Few of these mechanisms are fully understood and most require more precise definition.

Contaminated air conditioning systems are known to facilitate the transmission of infectious disease and also disorders which are presumed to be of immunological origin - humidifier fever, humidifier-related extrinsic allergic alveolitis and asthma. This thesis presents and discusses the findings of investigations performed as a result of humidifier-related disease which developed

in two factories, in four separate outbreaks of humidifier fever and a case of humidifier-related extrinsic allergic alveolitis.

Symptoms and serology were assessed in 88 subjects in the factories by questionnaire and immunological techniques. Serum precipitins to humidifier water were present in workers exposed to these units (and absent in 260 control sera from three other groups - 60 healthy non-exposed donors, 160 factory workers from various sites with symptoms, but not serology, of legionellosis and 40 Pontiac fever cases). Although symptoms were more common in the presence of antibody, the relationship was not strong ($p=0.041$). Cigarette smoking did not significantly influence the presence or absence of antibody. Specific antibody (measured by enzyme immune assay with antigen extracted from humidifier water) was quantified by spectrophotometry and found to correlate significantly with the presence of preciptins ($p<0.001$) and total IgG ($p<0.001$). Antibody was also significantly correlated with duration of service ($p=0.001$) and significantly lower in females. IgG subclass analysis showed that the predominant response was IgG_1. An environmental pseudomonad and Aureobasidium pullulans were isolated from the microprocessor factory humidifier but neither organism was found to

have antigenic activity. No organism was isolated from the printing factory humidifier. Serological analysis was repeated from each factory population after 9 months (microprocessor) and 18 months (printers) when significant reductions in specific and total IgG were found after the alteration of the humidification systems. The presence of symptoms in a large group of seronegative subjects raises the question of a different disease subgroup resembling pulmonary mycotoxicosis, perhaps as a result of the release of a large quantity of material from a humidifier which had been dormant for a prolonged period. The reported symptom pattern was unusual - of ill-defined periodicity - unlike the more usual "Monday fever" classically associated with humidifier fever. Likewise the illness occurred in summer and not in winter. Climatic recordings of unseasonably low nocturnal temperatures were closely associated with the onset of disease. The humidifiers (water spray and spinning disc) operated to maintain a relative humidity of 45% controlled by humidistats and usually at temperatures below 10°C. By implication a non-classical summer form of humidifier fever was present which may have been more common in night shift workers. Symptoms entirely resolved but only after replacement of the contaminated humidifiers with steam humidifiers in the microprocessor factory and water-

atomizers in the printers.

Despite previous observations of a suppressive effect of smoking on the development of humidifier-related diseases, no effect was noted in this group. Serum thiocyanates verified the reliability of smoking history in other groups of 460 subjects but appeared less accurate in the humidifier group - either because of over-estimation of the amount smoked or because of a reluctance to admit to smoking after the introduction of a non-smoking policy (for general health reasons) within the microprocessor factory. The other major confounding variable was the influence of mistaken beliefs within each factory - a component which was more difficult to quantify. After the circulation of an explanatory document in industry, 27 humidifiers were sampled (in ten factory sites) with one steam humidifier found contaminated with antigen (by enzyme inhibition assay) which cross reacted with the near-identical antigen found in the microprocessor and printing factories. A further sampling of 22 other sources within the refinery complex containing the contaminated humidifier was unproductive, suggesting that the antigen is not ubiquitous in the water supply. Cross-reaction with other water samples from humidifiers which caused

humidifier fever elsewhere in the U.K. suggests that the antigen source is neither unique nor specific to each outbreak of humidifier disease.

The studies reported in this thesis contribute to the understanding of the development and control of humidifier diseases and suggest that the presence of these disorders is often unrecognised. Four of the seven reported outbreaks of humidifier-related disease which have occurred in Scotland are reported in this thesis. In total, of 927 individuals exposed to these contaminated water containing units, symptoms were identified by the various investigators in 10.3%. Despite this prevalence, the true nature of humidifier fever and the relationship with either extrinsic allergic alveolitis or pulmonary mycotoxicosis remains unclear and would form a basis for future work.

DECLARATION

The original work reported in this thesis was completed through my own efforts and instigation.

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The work of the thesis would not have been possible without the goodwill and support of industry of whom British Petroleum and I.C.I. deserve special mention.

CHAPTER 1

EXTRINSIC AND INTRINSIC DETERMINANTS OF THE HOST RESPONSE IN ENVIRONMENTAL LUNG DISEASE

1.1 INTRODUCTION

Lung disorders which occur as a result of the inhalation of substances contaminating air arise when certain circumstances induce a pathological response within the airways, lung parenchyma or pleura. These circumstances are determined by a diverse and apparently unrelated array of interactions which comprise the variability of human activity, the exposure to potentially pathogenic substances and the pathological responses which may develop in certain individuals. The incidence of several diseases of environmental or occupational aetiology have been modified by the study of these mechanisms, some of which have resulted in legal statute of major consequence such as the Clean Air Act of 1956 which followed the London smog of 1952 (1).

The complete list of substances which provoke specific lung injury is too large to summarise within the limitations of this text and these are more completely considered elsewhere (2,3,4).

In this chapter the factors which might influence the development of environmental lung disease are discussed. These factors can be divided into two major groups which are internal and external to the host. Extrinsic factors would include climate (5), geography (6), the exposure to noxious substances and the presence of other materials which might interact with a substance outwith the host or perhaps modify the internal or intrinsic response which, in turn, defines the susceptibility of the host to a particular substance. This intrinsic response is further complicated by genetic, anatomical, physiological, biochemical, cellular, and immunological mechanisms some of which have evolved as the pulmonary response to inhaled fumes, particles or infection.

1.2 Extrinsic factors influencing the host response

The circumstances of exposure are the most important determinants of variability of the human response (7) and may be influenced by socioeconomic group, culture, climate, exercise, and smoking habit. While epidemics of chest illness may occur as a result of exposure to uncontrolled release of airborne allergen (8,9,10), government legislation and changing industrial practices since the middle of this century have generally resulted in an improvement in the atmosphere, although outdoor

TABLE 1.1 SOURCES OF INDOOR AIR POLLUTION IN THE HOME, OFFICE AND TRANSPORT ENVIRONMENT

ENVIRONMENT	SOURCE AND POLLUTANTS
Home	Tobacco smoking: respirable particles, CO, VOC
	Gas stoves: NO_2, CO
	Woodstoves and fireplaces: respirable particles, CO, PAH
	Building materials: formaldehyde, radon
	Foundation earth: radon
	Furnishings and household products: VOC, FA
	Gas fueled space heaters: NO_2, CO
	Paraffin fueled space heaters: NO_2, CO, SO_2
	Insulation: asbestos
	Moist materials and surfaces: biological agents
Office	Tobacco smoking: respirable particles, CO, VOC
	Building materials: VOC, FA
	Furnishings: VOC, FA
	Copying machines: VOC
	Air conditioning systems: biological agents, vehicle exhaust with combustion emissions containing particles CO and NO_2
Transportation	Tobacco smoking: respirable particles, CO, VOC
	Ambient air: ozone in jet aircraft, lead in cars
	Car air conditioners: biological agents

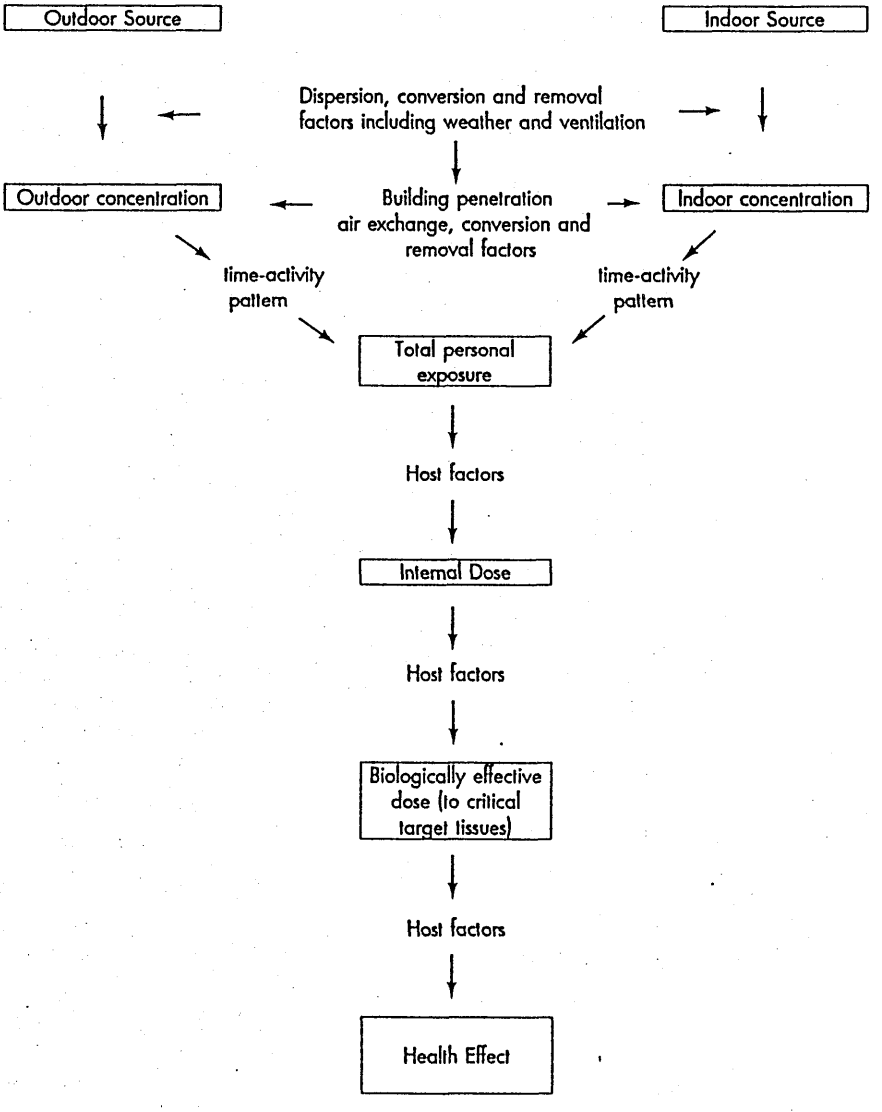
Adapted from Reference 12.

VOC	Volatile organic compounds
PAH	Polyaromatic hydrocarbons
FA	Formaldehyde

air pollution remains suspect as a cause of respiratory disease (11). This change in the degree of outdoor air pollution has stimulated examination of air quality within enclosed environments, particularly those which are artificially ventilated or air-conditioned (12). The relative importance of the indoor environment in comparison with outdoor was emphasised by an intercontinental study of differing cultures which suggested that most human activity occurs indoors (13), either in the home, at work or in transport. Exposure to harmful substances will tend to be concentrated on these locations where investigation of sources (table 1.1) and subsequent exposure conditions (table 1.2) have recently been summarised and discussed by others (14).

The effect of cigarette smoking within the enclosed environment on the quality of indoor air and on the active or passive smoking subjects has been extensively studied (15,16,17,18) and is increasingly recognised as a confounding variable which separately influences the host response in many forms of lung disease (19). Subsequent chapters of this thesis will detail some of these effects and discuss further factors which were recognised in the disorders described.

TABLE 1.2 POLLUTANTS, EXPOSURES AND HEALTH EFFECTS



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These disorders have, as has been acknowledged elsewhere (3), a tendency towards an immunological aetiology rather than those "occupational" disorders which were initially described in response to a specific substance when, perhaps, the complexity of "environmental" lung disease was yet to be discovered, and the science of immunology developed.

1.3 INTRINSIC FACTORS INFLUENCING THE HOST RESPONSE

1.3.1 PULMONARY DEFENCE MECHANISMS

1.3.2 The distribution of inhaled particles within the lung is dependent primarily upon the aerodynamic characteristics of the particle, the presence of nose or mouth breathing and the frequency and depth of respiration (20). The few particles larger than 10 um which penetrate beyond the nasopharynx and into the trachea settle by impaction by first or second order bronchial divisions (21). Most particles in the range 0.2 to 5 um are deposited by sedimentation in regions of low airflow, either adjacent to the bronchial wall or between the 15th and 23rd order bronchi (22). Particles in the range 1-2 um are most likely to deposit in the alveoli. Particles of 0.5-0.1 um are more likely to be exhaled from the lung while those less than 0.1 um deposit as a result of Brownian motion and diffusion (20).

1.3.3 Physiological reflexes reduce the penetration of dusts beyond the bronchial level by decreasing the calibre of the airways through vagally-mediated smooth muscle contraction (23,24) Secretions or larger particles induce the cough reflex in larger airways which may be reduced in efficiency by the presence of airways disease resulting in retrograde mucus movement within the airway (25).

1.3.4 The mucociliary transport system gathers mucus from the nasal cavity and the nasopharynx, and from the larynx down to the terminal bronchioles for expectoration or swallowing (26). This system is the major route of removal of particles within the lung. The particles are trapped within the gel portion of the mucus which arises from goblet and mucous glands and moves over the sol layer which is thought to arise from Clara cells and Type II pneumocytes (27). An intra bronchial wall circulation for sol as a fluid conservation mechanism and in order to prevent tracheal flooding by mucus has been postulated (27). The gel layer is impermeable to water and contains immunoglobulins, including secretory IgA (see later) lactoferrin, with bacteriostatic activity (28) and lysozyme which is bactericidal (29). Alpha-1-antitrypsin is also present in bronchial secretions and has activity against bacterial and neutrophil

derived enzymes (30) although these effects are more important at the alveolar level where the genetic deficiency of alpha-1-antitrypsin is associated with emphysema (31) in young individuals which is even more rapidly progressive in cigarette smokers (32). Complement is found in airway secretions in small amounts to increase when inflammatory mechanisms are stimulated. However, disease states influenced by complement seem more likely at the alveolar level because of the proximity of the pulmonary vasculature (33). Alpha-2-macroglobulin may have a similar purpose to alpha-1-antitrypsin with a role in the protection of normal lung tissue from cellular metabolites (34).

The solubility of particles in the fluid lining the airway is influenced by the chemical constituents of the fluid which contains more potassium and less sodium and a lower pH than serum (35). Substances may bind to constituents of the mucus for disposal upwards or perhaps bind to constituents and become antigenic (36).

The anatomy, physiology and pathology of cilia has been reviewed recently by Greenstone and Cole (37). Ciliary dysfunction can be induced by smoking (38), 100 per cent oxygen (39), anaesthetic agents (40) and sulphur dioxide (including

other air pollutants) (41,42) although the smoking effect is disputed (43). A beta-2 stimulator (terbutaline) has been shown to increase clearance in the presence of bronchitis (44). Cholinergic stimulation has a similar effect (45) however, atropine slows mucus transport in normal subjects (46). Viruses (47), alcohol (48) and Mycoplasma pneumonia (49) are further acquired causes of impaired ciliary clearance.

1.3.5 Immunological Factors. The secretory immune system within the upper airways of the lung is predominately based upon local secretory IgA antibody and forms another major mechanism to preserve the integrity of pulmonary mucosal surfaces. Secretory IgA is formed in the plasma cells of the lamina propria from two monomers of IgA linked by J chain glycoprotein followed by the addition of the secretory component within the bronchial epithelial cell for transport and ultimate excretion into the bronchial lumen (50). Within the lumen, secretory IgA reduces bacterial adherence to the bronchial wall, promotes bacterial clumping and has antiviral activity before and after infection of the epithelial cell (51,52,53). IgA deficient individuals have a higher prevalence of respiratory tract infection, allergic rhinitis eczema and asthma (54,55), however the effect of IgA deficiency on other disorders of immune origin is unknown.

Bronchial secretions also contain IgG but in smaller concentrations than IgA (56). The amount of IgG however, is higher in the lower bronchial tree than in the nose (57), and approximately in the same ratio to albumin which is present in serum (58). This suggests that transudation from serum is the main mode of entry of IgG into the bronchial secretions, a hypothesis which is further strengthened by similar ratios of IgG subclass proteins in the airway and serum despite variation in the molecular size of the proteins (59). The transuded IgG may be complemented by a small quantity of secreted IgG from lymphocytes in the bronchial submucosa (60). Cigarette smoking increases the IgG/albumin ratio but only in around 20% of subjects suggesting either increased local synthesis or defective lumen clearance because if transudation was increased through smoking-induced increased permeability then the IgG/albumen ratio would remain similar in the bronchial fluid and serum (61). The importance of IgG in prevention of respiratory infection is shown by the increased tendency towards recurrent infection and bronchiectasis in IgG deficient states (62) either caused by acquired deficiency or relative deficiency as a result of an overload of infection as in cystic fibrosis, where fragmented IgG may also impair uptake by the alveolar macrophage despite opsonisation (63).

In comparison with IgA and IgG, IgM antibody is present only in small amounts although IgM may, in part, replace IgA in selective IgA deficiency which explains why few subjects with IgA deficiency develop recurrent infection (64).

IgE is present in even smaller amounts than asymptomatic subjects (65). The role of IgE in lumen secretions is not known, however subjects with combined IgA and IgE deficiency have a propensity to respiratory tract infection (66).

IgD has neither been implicated in lung defense mechanisms nor pathogenic mechanisms and has not been found in lavage fluid (67).

1.3.6 Cellular defence mechanisms within the lung have been the subject of extensive investigation which has resulted in a vast bibliography over the past 20 years. The recurrent theme is that no one cellular group operates in isolation (68). Complex responses develop when antigen passes into the small bronchi and reaches the alveoli. In general, the pathogenesis of immune disorders which develop at the alveolar level remain to be fully elucidated.

The activity of the alveolar macrophage is a major controlling factor in the response to a particular material. These cells originate as monocytes from the bone marrow, although interstitial macrophages may replicate limited within the lung (69,70). The macrophage primarily performs a hygiene role in clearing dust and organisms at the alveolar level (71). Opsonised bacteria are ingested after contact with surface receptors on the macrophage (72). These materials are mainly excluded from contact with the immune system by moving upwards in the mucociliary transport system. Some of the antigen laden macrophages may move to the central lymph nodes (73) or over bronchial associated lymphoid tissue for antigen presentation and the generation of a secretory immune response (74).

The macrophage also secretes substances locally which have activity against infection, in inflammation and immune responses (75). Interleukin-1 is one such substance which is secreted in response to numerous stimuli including endotoxin (76) which also produces a systemic response of pyrexia. The interaction of the alveolar macrophage and T lymphocyte is a crucial component of the immune response. Processed antigen is established on the macrophage surface for display with Dr antigens which are essential for antigen recognition by the T lymphocyte (77). Dr

surface antigen can be induced by gamma-interferon (78) and reduced by cigarette smoke (79) which also has several other effects on alveolar macrophage function (80), structure (81), number (82) and content (83).

Lymphocytes (in subpopulations identified by monoclonal antibodies) function together with the alveolar macrophage either to promote or reduce an immunological response within the lung. In some animals lymphocytes are found concentrated in bronchial associated lymphoid tissue (present mainly at the bifurcation of major airway subdivisions (84) although the tissue is less clearly defined in man) and dispersed within the lung parenchyma (85). Broncho-alveolar lavage fluid from a normal subject contains around 7-12% lymphocytes, alveolar macrophages 85-93%, neutrophils 1-2% and less than 1% eosinophils and basophils (61). 80% of these lymphocytes are T lymphocytes (67) with a helper/suppressor ratio approaching 2, (86) which is similar to the ratio in blood. The presence of T cells within the lung is considered a prerequisite for the development of a local cell-mediated hypersensitivity reaction. Recruitment of antigen-specific T lymphocytes to the lung in response to a particular antigen has been reported (87) however a non-antigen-specific increase in cytotoxic activity may occur after viral infection

(88) and in clearance of tumour cells through the natural killer subset of lymphocytes (89). The T lymphocyte may augment the immune response by the release of lymphokines which attract and restrict movement of alveolar macrophages (90,91), which in turn may release interleukin 1 (92) and induce the release of interleukin 2 from helper T cells (93) which then induces interferon and primes cytotoxic T lymphocytes (94). The B lymphocyte function of IgG secretion is regulated by T cells (95) and interleukin production (96).

Neutrophils are also attracted into the lung by the release of interleukin 1 and also after complement activation. The details of neutrophil activity are too extensive for summary here, however the neutrophil has been noted in disorders of presumed immune origin to have prognostic and diagnostic relevance. Broncho-alveolar lavage fluid which is lymphocyte rich is more likely to respond to corticosteroid therapy, and can suggest sarcoidosis, extrinsic allergic alveolitis or a steroid-responsive variant of idiopathic pulmonary fibrosis (97). Neutrophil rich lavage fluid is usually indicative of steroid resistant interstitial lung disease such as idiopathic pulmonary fibrosis or asbestosis. Likewise an increase in eosinophil numbers also has a poorer prognosis. Both the eosinophil and

neutrophil are implicated in the development of chronic chest disease through the release of cellular contents (98,99,100). Eosinophilic lavage fluid in chronic eosinophilic pneumonia carries a better prognosis, however in asthmatics, alveolar macrophage viability is inversely correlated with eosinophil content (101) and phagocytosis is also impaired. The primary activity of the neutrophil in lung defence is usually more beneficial than is suggested above operating as a principle anti-bacterial response. Likewise, eosinophils have strong antiparasitic activity, however, the similarity of the IgE response in both parasitic and allergic diseases and the adverse effects of eosinophil constituents perhaps questions the value of the eosinophil in human groups where parasitic infection (although not atopy) is rare. Recognition of the importance of the eosinophil in allergic diseases is increasing (102).

1.3.7 Genetic Factors

Traits which are clearly genetic in origin such as alpha-one antitrypsin deficiency are also influenced by environment variables such as concomitant cigarette smoking (33). The relationship between host factors, smoking and the development of other disorders such as atopy or chronic obstructive airways disease is well recognised but less marked (103,104) where only

the minority of subjects develop the condition. The finding that neutrophil elastase is increased in smokers (105) suggests an underlying abnormality which may cause emphysema and since not all smokers are equally susceptible the likely cause is assumed to be varying genetic expression. Genetic predisposition towards obstructive airways disease has been demonstrated (106) although non-genetic factors predominate (107).

Reversible airways obstruction or asthma can be linked with the production of IgE although the relationship is not direct (108).

Parenchymal lung disease was reported more common (109) in asbestos-exposed workers with HLA-B27 although this finding has been disputed (110). Similarly the finding of a histo-compatibility antigen relationship in farmers lung and pigeon breeders lung (111,112,113) has also been doubted by others (114).

The major difficulty in ascribing a predominant genetic or environmental cause and inferring innate susceptibility is that each of the variables involved in any one subject is poorly quantified and may be impossible to assess with certainty.

CHAPTER 2

DISEASES ASSOCIATED WITH BUILDING VENTILATION AND DESIGN

2.1 INTRODUCTION

Human ingenuity, when confronted by a difficulty, usually responds by consideration of the nature of the problem and then adopting measures which most effectively and efficiently allow resolution. Such are the complexities of the environment, that resolution of an encountered difficulty, although initially seeming quite satisfactory, may later unfold unforeseen, and perhaps harmful circumstances. This pattern of events closely reflects the course which preceded disorders now known to develop as a result of inhaling air from contaminated air conditioning systems.

2.2 Air Conditioning systems are of two major types, either designed for cooling or warming air intake (or both) depending on the environmental circumstances and the specification for indoor temperature within a building. Calculated air changes are required according to the population within the building ("comfort" air conditioning) or according to the requirements of an industrial process ("industrial" air conditioning). The

tolerance of a certain industrial process may be narrow in terms of air temperature, air quality and relative humidity, with each of these variables increasing the complexity of the air handling plant required to serve the building. In contrast, humans tolerate less exacting conditions as long as the air is fresh, is of a temperature suitable for the level of activity within the building and a comfortable relative humidity of between 40% and 70%. In general terms "comfort" air conditioning is less complex and requires a relatively simple air handling plant (fig 2.1) in comparison with "industrial" air conditioning (fig 2.2). The need for air conditioning in the United Kingdom has been questioned frequently (115) although certain industries such as textiles, printing, microprocessor manufacture and electronics, bakeries, laboratories, hospitals and most which utilise large computer controlled mechanisms require closer control of air quality and humidity (table 2.1), therefore air conditioning is not dispensable when the demands of an industrial process require satisfaction.

2.3 Climate and geographical location are important determinants of a chosen form of air conditioning. Air conditioning originated in the United States of America and was initially utilised for cooling excessively warm air intake. As

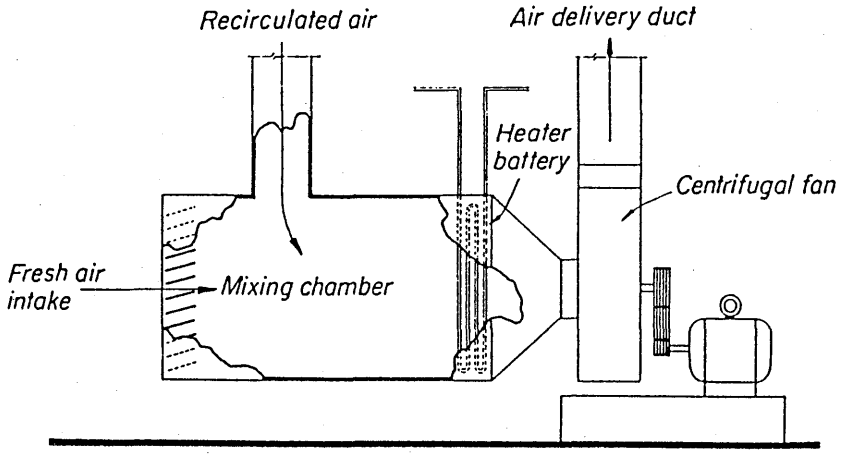

FIGURE 2.1

"Comfort" air conditioning unit, which heats and disperses air through exit ducts without consideration of the moisture content. Recirculation of exhaust air is a heat conservation measure.

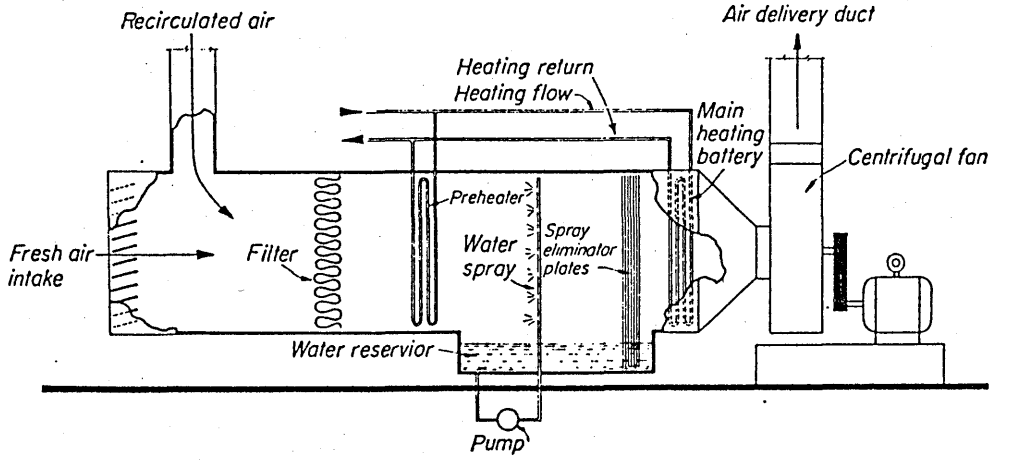

FIGURE 2.2

"Industrial" air conditioning unit. Air may be filtered, cooled, heated or humidified before release into the duct system. A unit of this type served the microprocessor factory described in Chapter 3.

TABLE 2.1 HUMIDITIES* AND INDUSTRIAL PROCESSES

	Relative humidity required
Apple storage	75-85%
Banana ripening	90-95%
Citrus fruit storage	85-95%
Egg storage	75-80%
Mushroom spawning	95-100%
Mushroom growing	60-85%
Potato storage	85-90%
Offices (human comfort)	40-70%
Computer areas	50-55%
Paint spraying	60-80%
Printing	45-60%
Cotton processing	65-80%
Woollen processing	60-70%
Timber storage	45-55%
Woodworking	40-50%
Cigarette production	55-70%

* The relative humidity of air is the ratio of the partial pressure of the water vapour in the moist air at a given temperature, to the saturated vapour at the same temperature. (115)

As a general rule, for every temperature reduction of 10oC the moisture held in the air is reduced by about half.

building design has progressed to compensate for large multistorey development or buildings with sealed windows for energy conservation or security, so air conditioned by air handlers has been increasingly used either warming or cooling intake air perhaps to compensate for solar heat gain. Most of these developments have been encouraged by the fuel crises of the 1970's and the need for fuel conservation. Thus many buildings rely on artificial ventilation for maintenance of a stable internal environment.

In regions of the world affected by excessive cold, the intake air is of extremely low water content. This air is artificially humidified to a relative humidity of 40-70% in order to avoid static electricity generation, excessively dry skin and effects on the less obvious, such as contact lenses (116,117). However, moisture may condense on indoor surfaces and adversely affect the structure of buildings (118), hence lower humidity levels in winter may be of some advantage. Corrosion and mould growth are most likely when relative humidities exceed 70% for prolonged spells (119). Figure 2.3 demonstrates the relationship between temperature and humidity (adapted from 115) clearly showing that although air is fully saturated at temperatures below the dew point, the actual water content of the

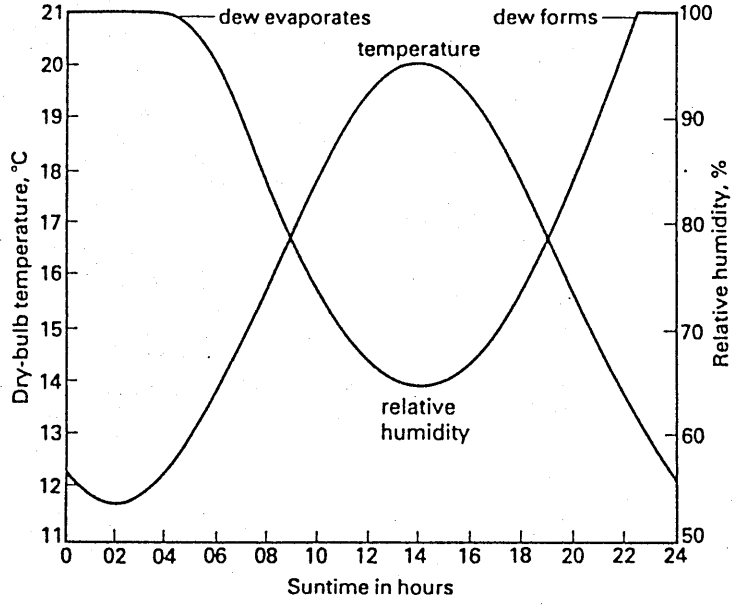

FIGURE 2.3

The climatic relationships of temperature, humidity, and time of day. A calculated example plotted for mean temperature and relative humidity measurements in August showing that the moisture content of air is lowest at low temperatures and (usually) at night. Reproduced with permission of the publishers (Edward Arnold, London) from Reference 115.

air is lower as the ambient temperature falls. Figure 2.4 shows the diurnal range of relative humidity recorded at Abbotsinch as an example to indicate the relationship between temperature (warmest 15.00 hrs., coolest 3.00 hrs.) and humidity encountered over 12 hours (120) throughout an average year. From these figures the deduction is simple that humidity will be at lowest within a building during winter and at night, unless the intake air is artificially moistened.

2.4 Humidifiers incorporated into air conditioning systems are either operated continuously (after the air intake is cooled, it is rewarmed and then precisely moistened to a preset level) or intermittently (usually under the control of a wall-mounted sensor or hygrostat). The humidity is either increased within the air handling unit or outside this unit by isolated humidifiers. Humidifiers are of six major types (figures 2.5 - 2.10) which operate by evaporation, spraying, ultrasonic nebulisation (or water "shattering") or the generation of steam. Biological contamination of this machinery is more likely if water is stored within the humidifier and the water is recirculated (121), and if the machinery is not maintained regularly. The precise cause and method of humidifier contamination is unknown although air taken into the building

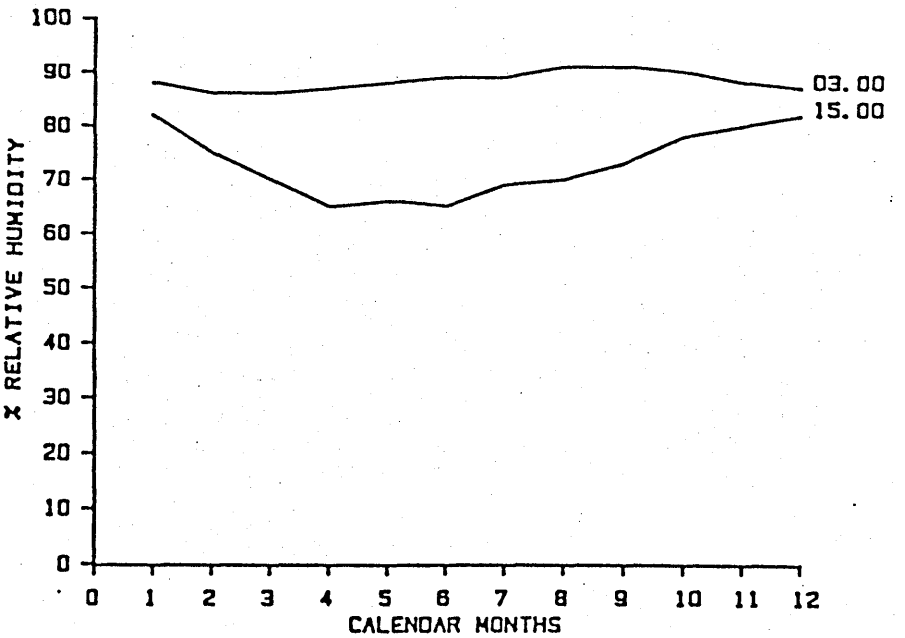

FIGURE 2.4

The diurnal range of relative humidity at Abbotsinch, Glasgow. The results are the mean of measurements over a 10 year period and represent an average year. Data reproduced with permission (as fig. 2.11).

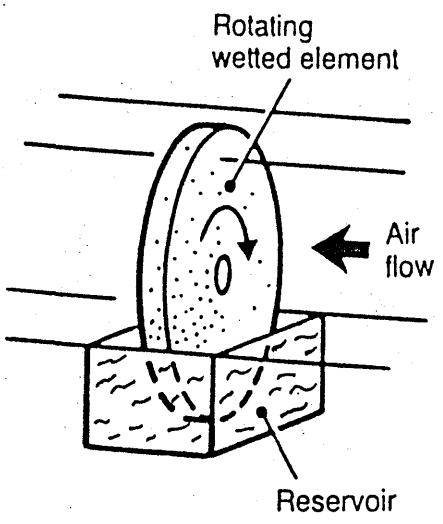

FIGURE 2.5

Cold water evaporator.

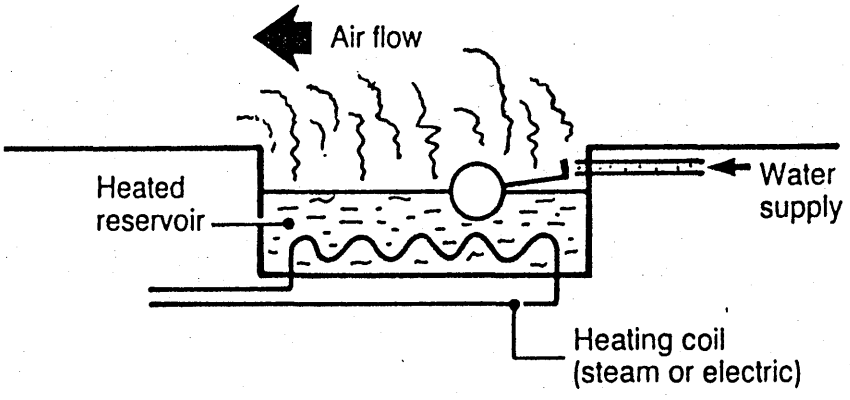

FIGURE 2.6

Open hot evaporator.

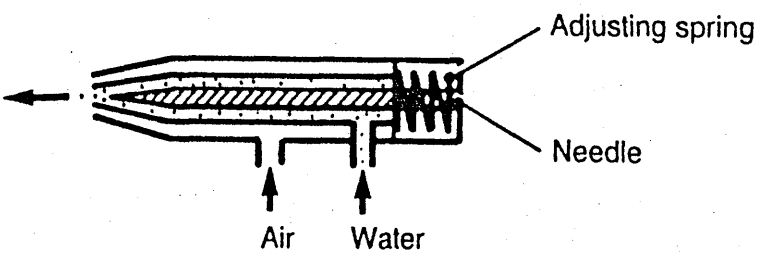

FIGURE 2.7

Compressed air atomiser.

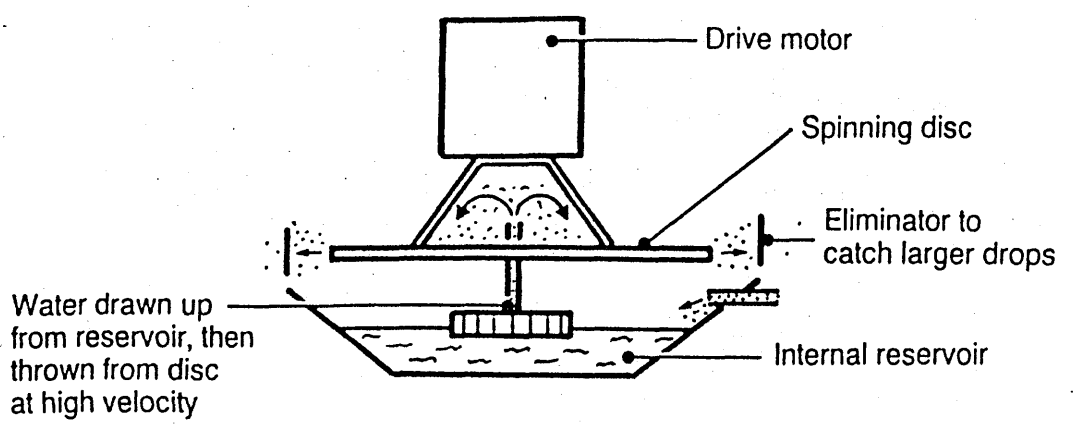

FIGURE 2.8

Spinning-disc humidifier.

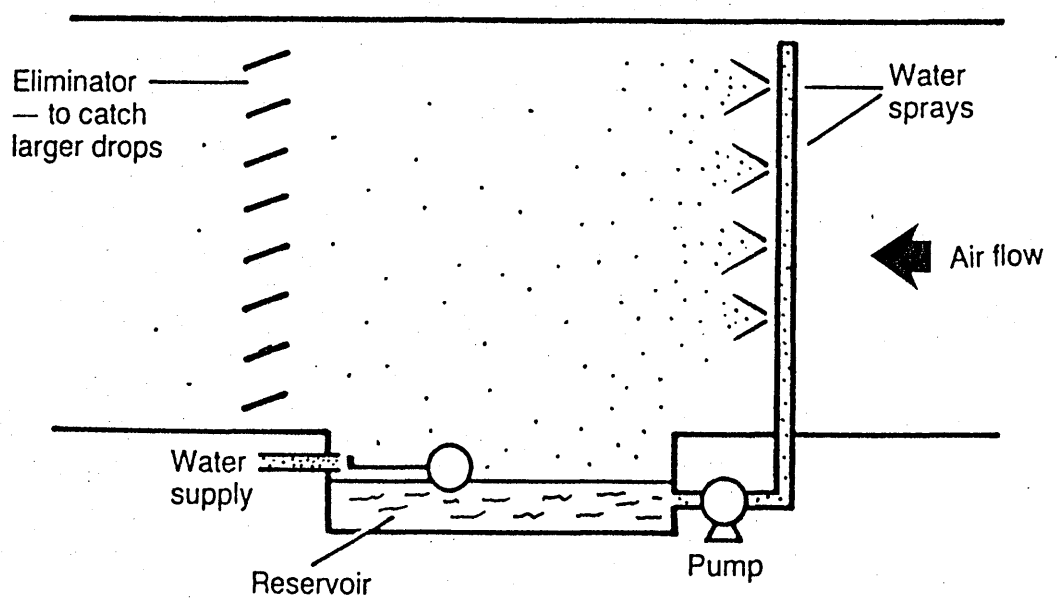

FIGURE 2.9

Spray humidifier (or air washer)

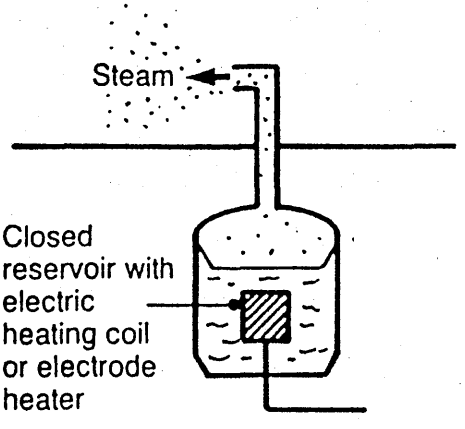

FIGURE 2.10

Steam humidifier. Figs. 2.5-10 are reproduced with permission of the author of reference 121.

will carry airborne material, mains water cannot be considered sterile and air within the building could be contaminated either by the occupants or the industrial environment (122) and subsequently recirculated through the humidifier system. The water, constituents, temperature and pH will determine which organisms will proliferate and may produce a 'growth chain' of one type of organism multiplying after another has reached the maximum population supportable by the fluid (123). Plate 2.1 shows a sample of fluid collected by the author from a contaminated humidifier responsible for humidifier fever in a microprocessor factory (124).

2.5 Humidifier-induced disease in the occupational setting was first recognised by Pestalozzi in 1959 (125) when several carpenters in a workshop developed influenza-like symptoms which settled spontaneously away from work and then reappeared on return. The humidifier in the workshop was found to be heavily contaminated by organic material and the symptoms cleared when the humidifier was cleaned.

Humidifier fever (or "Foreign protein fever") was the subject of a government report of an illness in printers caused by organically contaminated fluid sprayed from spinning disc

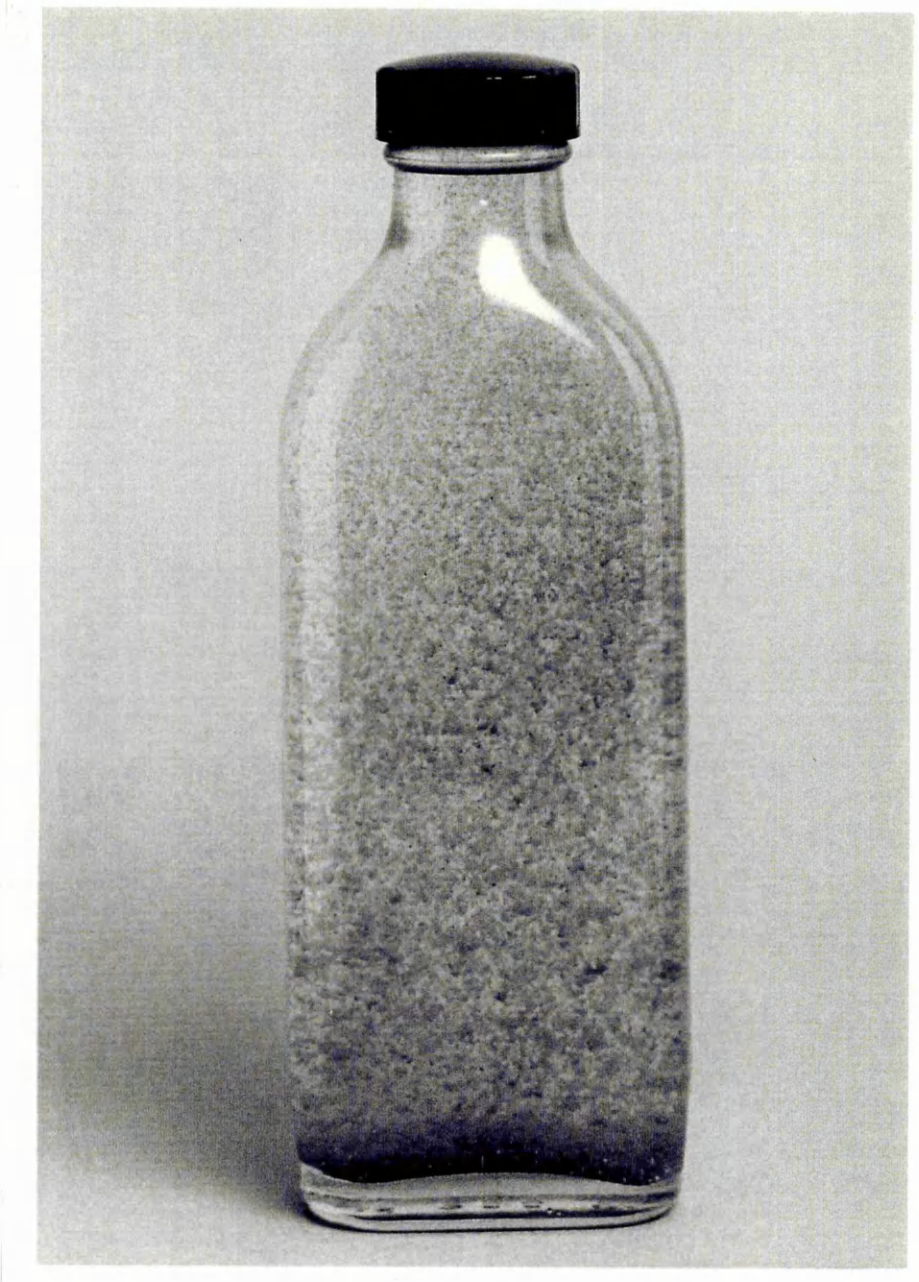

PLATE 2.1

Fluid sample taken from a humidifier unit which was responsible for an outbreak of humidifier fever and a case of extrinsic allergic alveolitis in a microprocessor factory.

humidifiers reported in 1969 (126) where the workers had similar symptoms to those described by Pestalozzi. These symptoms would occur on the first day of return to work after a break and appear 4-8 hours later consisting of tiredness, cough, shivering, muscle aches, headache, shortness of breath and fever. The symptoms usually settled by the following day and did not recur until the next break from work. This "Monday fever" pattern to the symptoms resembles the symptom pattern of byssinosis and was suspected perhaps to be of similar pathogenesis (127).

Over the past twenty years numerous reports of humidifier fever have been published from the U.K. and Europe (128,129,130,131,132,133,134,135,136,137,138,124) where the illness occurred in printing factories, a stationery supplier, offices, a hospital operating theatre, a book binding factory, a textile (rayon) factory, and a microprocessor factory. Spinning disc humidifiers were most often incriminated in these reported outbreaks and installation of this type of humidifier is no longer recommended (121). Steam humidifiers have never been identified as a source of work-related symptoms and now tend to be installed where technology is more advanced, for instance, in computer control centres. These humidifiers are often installed in pairs with one unit operating on demand and the other for

back-up. Although the vapour from the humidifier is likely to be sterilised by boiling, several steam humidifiers examined in an oil-refinery by the author were found to contain debris and corroded metal salts which suggests that air humidified by this method might contain other particulate material (see later). No studies of the effect of steam humidifier on air quality and health have been published, although the possibility of condensate pooling within ducting and sustaining organic growth is a theoretical source of illness.

2.6 The prevalence of humidifier fever is unknown which possibly reflects the use of humidifiers in only a small minority of offices and factories (139) resulting, perhaps, in a relative ignorance of symptoms which are similar to influenza and not considered work-induced. Probable cases have been found during health surveys of offices where the illness was not previously recognised (140).

In enclosed environments where humidifier fever has been found, symptoms are present only in a minority. Seven outbreaks of humidifier fever in 5 different sites have been reported in Scotland (124,129,135,137,Scott J., personal communication,) where of 927 individuals exposed to the output of contaminated

TABLE 2.2 - HUMIDIFIER FEVER OUTBREAKS IN SCOTLAND

Reference	Site/Process	Humidifier	Symptomatic /Exposed Population	A	B	C
1. Anderson (124)	Semiconductor manufacturer	Water spray into air handler	40/250	>45%	+	13
2. Anderson (124)	Printers	2 spinning discs	17/30	>45%	+	18
3. McSharry (138)	Same site as 1.	as 1.	19/50	as 1.	+	12
4. Parrot (135)	Printers	Water spray	7/26	>55%	+	12
5. Friend (129)	Stationery Supplier	Liquid ring vacuum pump exhaust	24/371	not stated	+	27
6. Scott, J Personal communication	Photographic Printers	Drip bank	17/200	>45%	+	10

A - optimum humidity

B - serum precipitins to humidifier extract detected

C - population with serum precipitin to humidifier water/sludge

humidifiers, symptoms were identified in 10.3% (141). Details of each outbreak are shown in table 2.2

2.6.1 Prevalence of humidifier fever and climate

The episodes of humidifier fever numbered 1, 2 and 3 in Table 2.2 will be discussed in more specific detail in a later section. However, some common features were found between each of these episodes. Humidifier fever was primarily described as a winter illness (127) however in three of these Scottish episodes, the illness developed in summer (124,135) when ambient humidity and air temperature might be expected maximal. Climatic details for the semiconductor manufacturers and printers who both experienced summer outbreaks of symptoms are summarised in figures 2.11 and 2.12 when unseasonably low nocturnal temperatures were recorded which correlated precisely with the onset of symptoms. Workers in both factories reported intermittent symptoms of ill-defined periodicity over the preceding 4 months which probably resulted from intermittent activation of the humidifiers during periods of low temperature. Despite previous experience of humidifier disease in the microprocessor factory, these symptoms were missed until the workers returned after the summer holiday when an outbreak of illness developed.

A more classical winter outbreak of humidifier fever

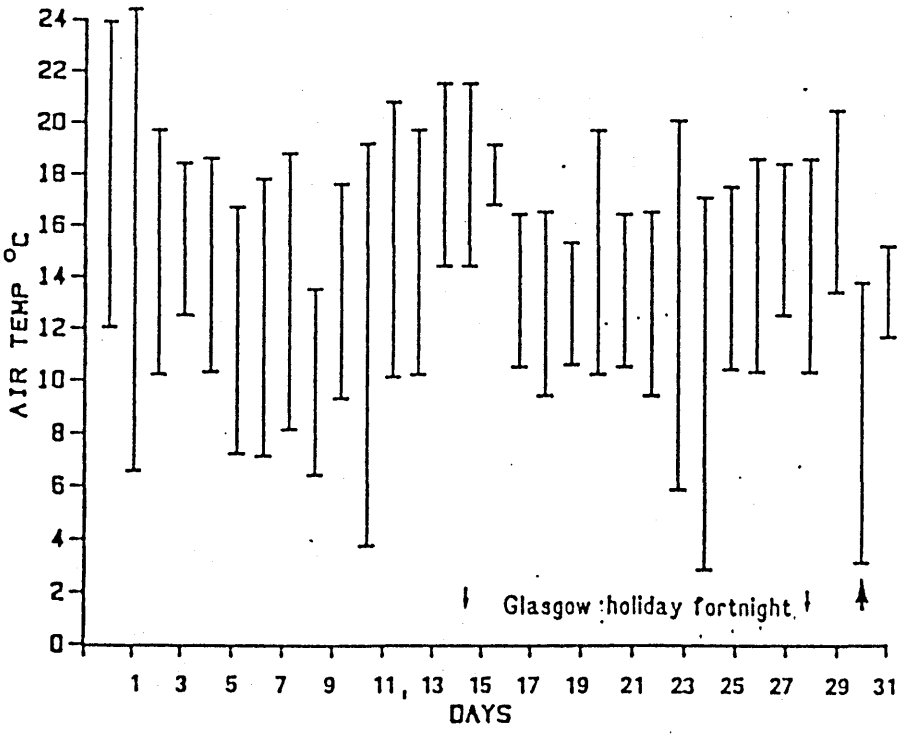

FIGURE 2.11

Minimum and maximum air temperatures recorded in July 1986 during the second outbreak of humidifier fever in the microprocessor factory. The small arrow indicates the date of return to work, large arrow day of onset of symptoms. The humidifiers would tend to operate at temperature below 10°C. This data is reproduced with permission of the Meteorological Office from recordings taken at Abbotsinch, Glasgow.

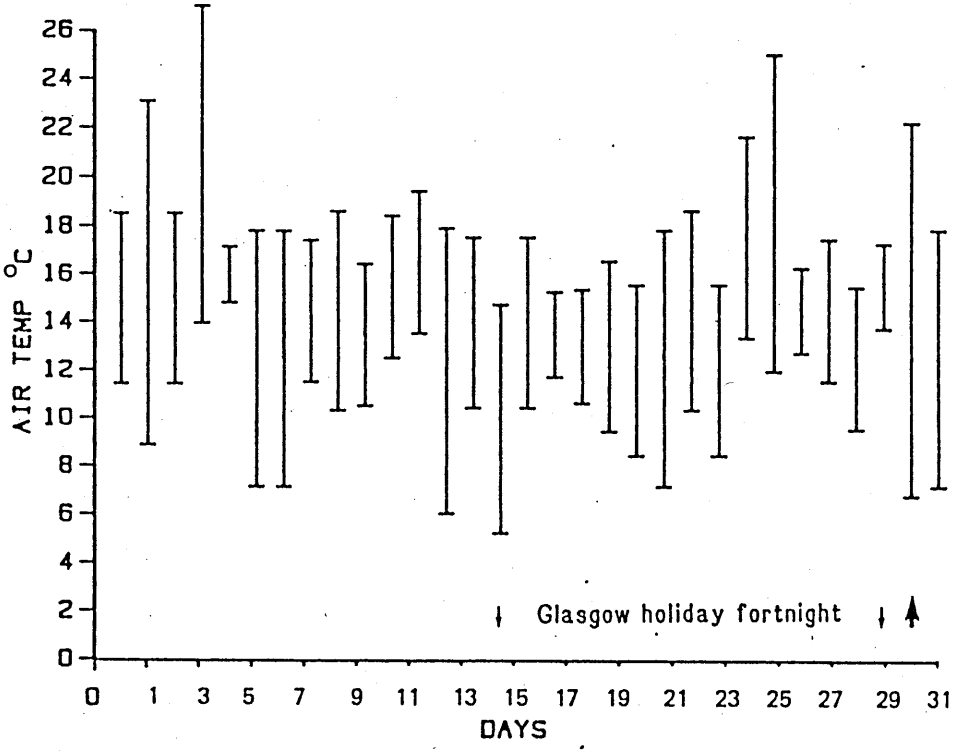

FIGURE 2.12

Minimum and maximum air temperatures recorded in July 1985 during the first outbreak of humidifier fever in the printing factory. Small arrow: day of return from holiday. Large arrow: onset of symptoms. The humidifier would tend to operate at temperatures below 10°C. Data reproduced with permission (as fig. 2.11).

occurred in the printers (figure 2.13) when symptoms developed precisely on the day of return to work producing the "Monday fever" pattern of symptoms. The first outbreak of illness in the microprocessor factory occurred several years before the recurrence and at that time, after 9 months follow up, a small group of individuals had persistent symptoms. All of these workers were working a night shift and the presumption of non-work or stress related symptoms was considered.

Some time later the explanation of this night shift pattern was obtained when the factory engineer described the alterations made to the air handling machinery which was modified to increase fresh water input and run off. The contaminated humidifier had been retained as an emergency unit when maximal demand was required by cool air intakes. These circumstances would occur most often at night thus producing more common and persistent symptoms in the night shift workers. A similar pattern was recognised in the night shift of the printers and in one rotating shift worker who commented that symptoms appeared to be more common at night.

Night shift workers in these environments probably experienced higher antigen exposures however there is no

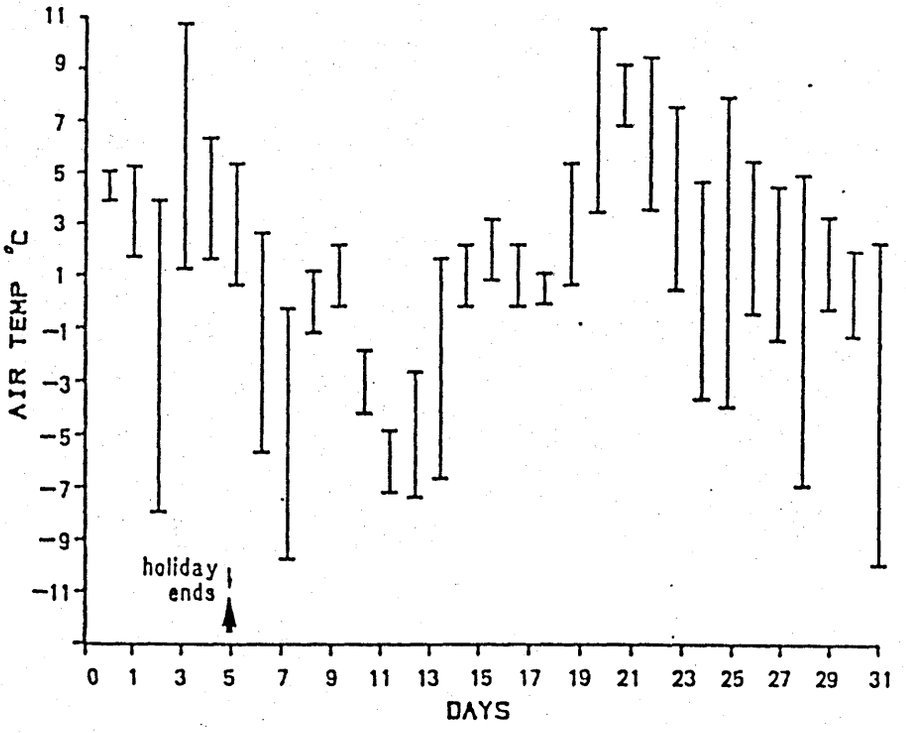

FIGURE 2.13

Minimum and maximum air temperatures recorded in January 1987 during the second outbreak of humidifier fever in the printers. The humidifier would operate continuously during this period. Data reproduced with permission (as fig. 2.11).

published evidence to suggest that this group (the numbers in both factory groups were small) are more likely to develop more severe acute disease or chronic lung disease.

In each outbreak of humidifier fever in table 2.2 the humidifier fluid or sludge obtained from baffles (also known as angled deflector or eliminator plates) cross reacted with serum from some of the symptomatic subjects (59.7%) to produce precipitins on double gel diffusion. Fifty of the 124 symptomatic subjects (40.3%) had no IgG antibody detected by this method. This precipitin negative form of humidifier fever appeared more common in the summer outbreaks and may have a similar aetiology to the organic toxic dust syndrome (142) or pulmonary mycotoxicosis (143) which is most often described in farmers and appears to develop in response to the inhalation of high dust concentrations but without the complex of findings which comprise extrinsic allergic alveolitis. Humidifiers which operate on demand are most likely redundant during the warmer months and may then be more likely to develop organic overgrowth which would be released in quantity when the environmental conditions were suitable. Thus the summer form of humidifier fever may often occur in the absence of precipitins and the presence of precipitins should not be considered a pre-requisite

for the diagnosis of humidifier fever.

The practical aspect to the summer pattern of illness is that humidifiers should be serviced continuously throughout the year in order to avoid organic contamination and should not be assumed dormant during the warmer months. Humidifier fever was present in the factories for some time before recognition and hence the possibility arises that the prevalence of humidifier disease is higher than the number of identified outbreaks reported in the community.

2.7 Humidifier-related extrinsic allergic alveolitis is a much rarer condition in the U.K., where only 4 cases have been described in the literature (144,145). Several cases have also occurred in Europe (146). This form of disease is more often seen in the U.S.A. where thermophilic organisms are consistently implicated in the aetiology. Humidifier lung has been reported in offices (147,148,149,), in the home (150,151,152) a laboratory (153), transport (154) and factories (155). In the United States the disorder produces a syndrome of symptoms, clinical signs, pulmonary function abnormalities and histology which are typical of extrinsic allergic alveolitis (EAA) which can produce chronic disease. These (156,157,158) humidifiers more often

contain warm water which encourages thermophilic proliferation, however, a textile factory outbreak of mixed humidifier fever and lung was reported (155) when a cytophaga species endotoxin was incriminated as a possible cause (159,160).

Several subjects in an office outbreak of humidifier disease (148) also had humidifier fever while other developed more chronic lung disease in the absence of a specific identifiable organism as the cause of the detected precipitins to humidifier water. Solley (153) attributed his case to a Penicillium species.

In the U.K., the reported cases of EAA occurred with cold water humidifiers and in neither humidifier was a specific organism found. Robertson et al (145) reported 3 subjects in a printing factory who all developed EAA suggested by the development of dyspnoea on exercise, cough, weight loss with a background history of humidifier fever in two of the subjects and occupational asthma caused by a contaminated humidifier in the other. It is not stated in this report whether the same contaminated humidifier was the cause of the humidifier fever, asthma and EAA. Each case had impairment of gas transfer and a restrictive defect on pulmonary function assessment, nodular

shadowing on chest X-ray and serum precipitins to extracts of the humidifier water (and also pigeon serum). Lymphocytosis was found in the broncho alveolar lavage of two cases and features were reproduced in two after inhalation of humidifier antigen producing typical inhalation provocation findings of EAA (161) of reduced gas transfer, fever, aches and pains, headache, malaise, and an appearance of subjective illness. The diagnosis was further strengthened by transbronchial biopsy histology in keeping with EAA, and some recovery in 2 subjects who stopped work and the other subject who required treatment with steroids and azathioprine.

Our own group (144) reported a similar illness which developed in an electronics engineer in a microprocessor factory where others in the workforce were affected by humidifier fever. This index case was reported independently from the other patients in the factory because of the chest radiograph which was consistent with EAA (Plate 2.2). The history consisted of six months progressive dyspnoea on mild exertion initially accompanied by a dry cough and subsequently producing mucopurulent sputum. He was a previously fit, 29 year old, non atopic Caucasian. Two or three times a week he complained of feverishness, muscle, joint and backache, and on one occasion a

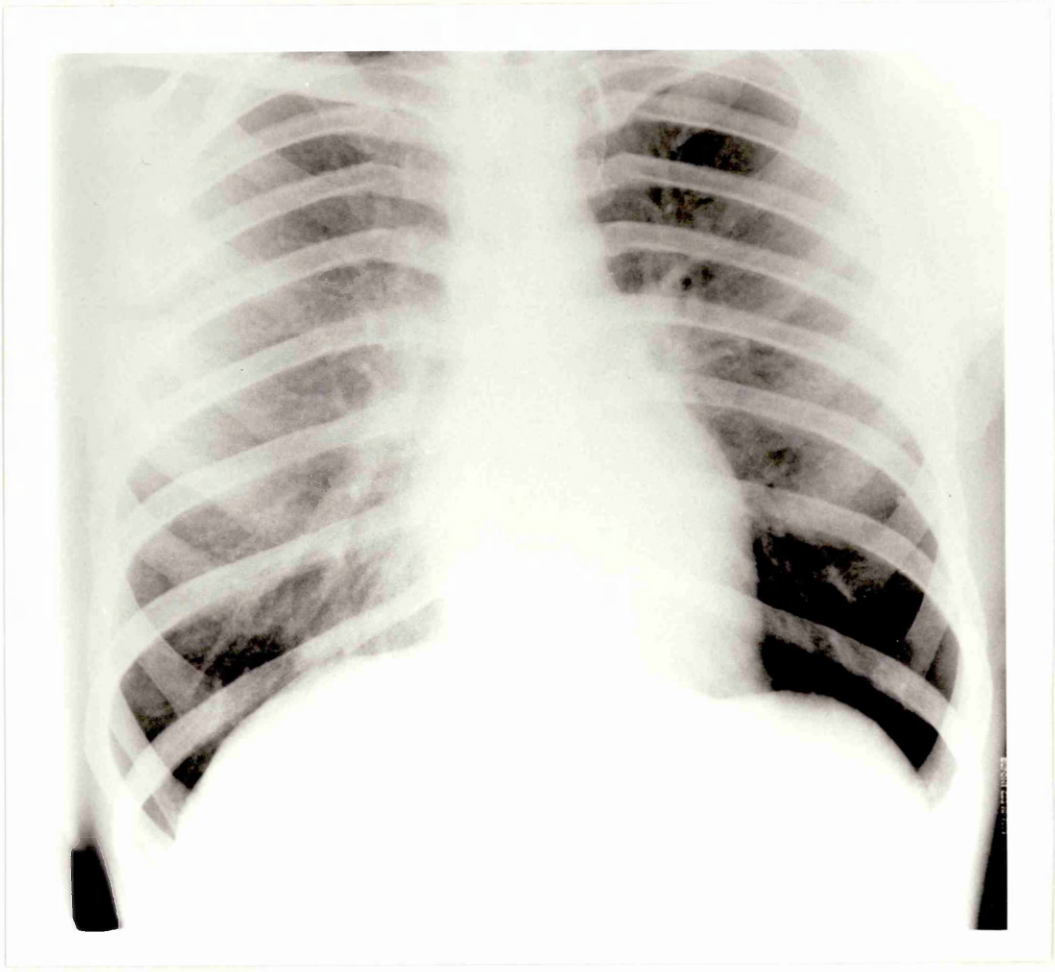

PLATE 2.2

Chest radiograph of a man with extrinsic allergic alveolitis caused by a contaminated humidifier. Bilateral, predominately mid-zonal shadowing is present. Basal crackles were heard on auscultation during deep inspiration at this phase of the illness.

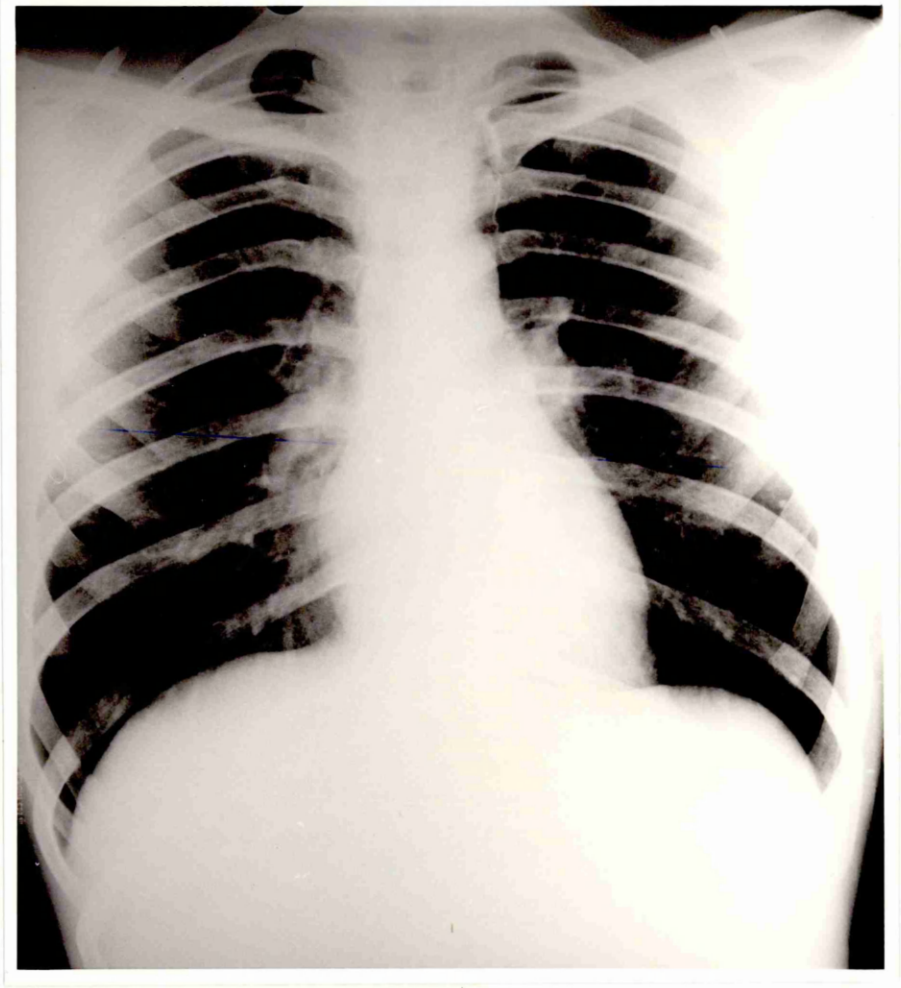

PLATE 2.3

Chest radiograph of the subject shown in plate 2.2 following symptomatic recovery after a two week holiday from work. The crackles previously present had disappeared.

temperature of 38.3°C was recorded. The symptoms developed at the end of a working day and settled overnight. Weight loss of 15kg was noted. Fine crackles were present on auscultation over both lung fields. Neither finger clubbing nor nail-bed splinter haemorrhages were present. Examination was otherwise normal. His symptoms rapidly improved on holiday and the chest X-ray (Plate 2.3) two weeks later showed improvement. Pulmonary function measurements are shown in figure 2.14. Improvement in the restrictive defect and diffusing capacity occurred after the holiday with deterioration again after returning to work when his symptoms recurred. Subsequently he was treated with corticosteroids when his symptoms entirely cleared. His working environment was fully air conditioned with addition of water vapour to the air to maintain a relative humidity of 45% from a constantly recirculated closed circuit supply (see later). Serum was tested against extracts of the humidifier water and found to contain IgG antibody precipitins on gel diffusion analysis, and reported to show evidence of IgG antibody against Naegleria gruberi (Kindly performed by J.H. Edwards, Cardiff) and three extracts from humidifiers associated with previous outbreaks of humidifier fever (table 2.3) with negative results from two others (Kindly performed by Dr. Peter Austwick, Cardiothoracic Institute, London). Serum total IgG was

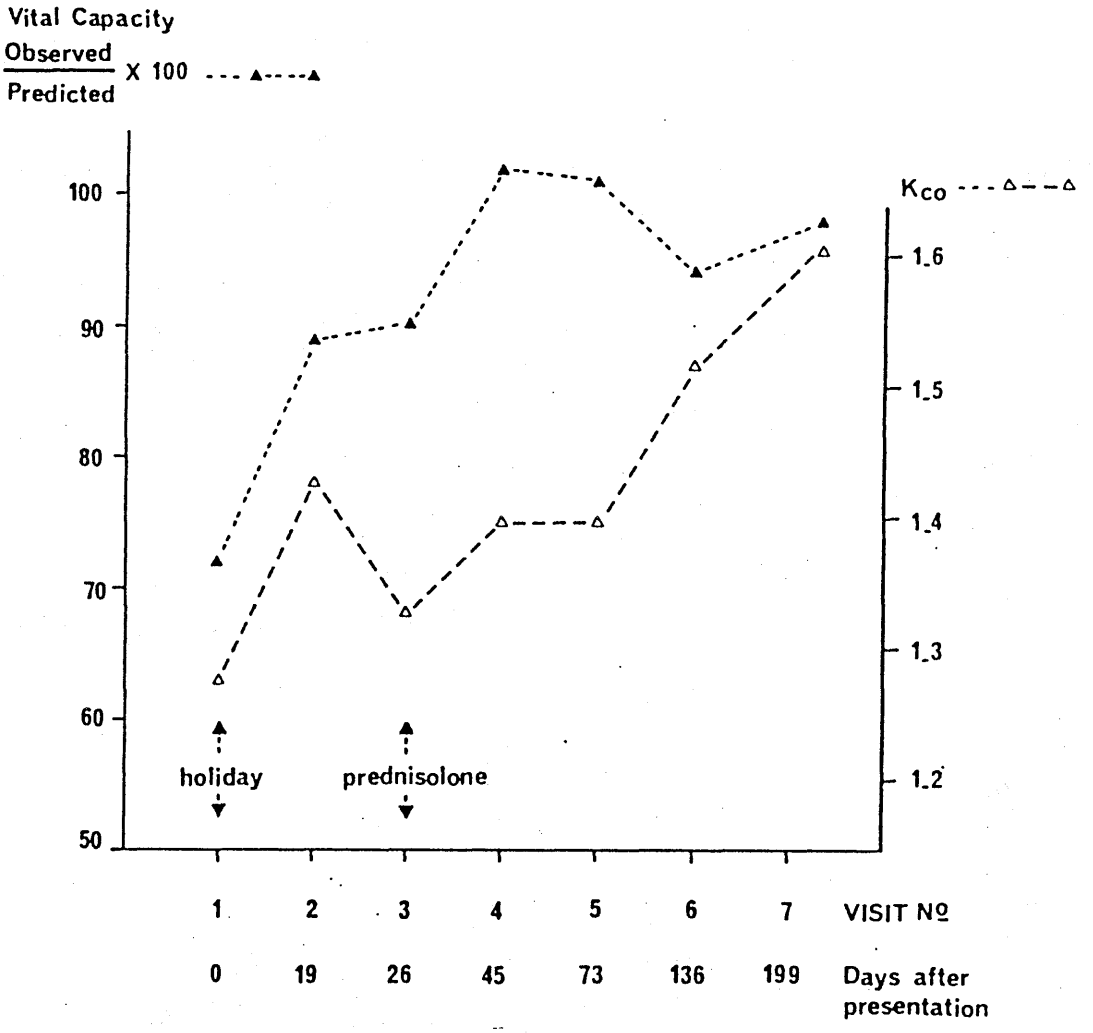

FIGURE 2.14

Pulmonary function measurements [vital capacity (VC) presented as a percentage of observed/predicted value, and diffusion coefficient (KCO)] consistent with the presentation of, and recovery from extrinsic allergic alveolitis. Note the recovery of lung function with the holiday and deterioration after return to work. The chest radiographs for visits 1 and 2 are presented in plates 2.2 and 2.3.

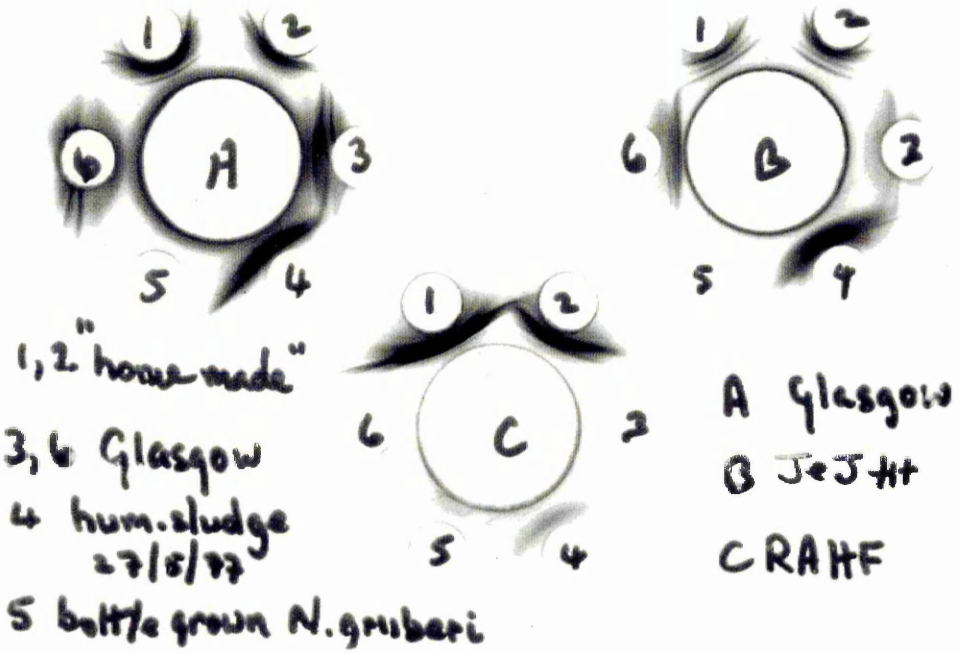

PLATE 2.4

Gel-diffusion plate showing activity of serum from Glasgow (case of extrinsic allergic alveolitis described in 2.7), Cardiff (well B) and a sensitized rabbit (well C) against antigen from Glasgow (wells 3 and 6), Cardiff (well 4), "home made" antigen (wells 1 and 2) and "bottle grown" Naegleria gruberi. Significant cross reactions between Glasgow and Cardiff samples are demonstrated. The absence of precipitins to N. gruberi was explained by laboratory origin which is thought to alter the organism's antigenic activity. (Kindly performed by Dr J Edwards, Cardiff).

TABLE 2.3

Cross reactions of serum from Glasgow with antigen samples from various sites in the U.K. affected previously by humidifier fever, judged by the development of precipitins on gel-diffusion analysis.

		Location
Cross reactivity	:	Chester
		Stockport
		London A
		Cardiff
No cross reactivity	:	Aberdeen
		London B

initially highly elevated and gradually reduced with recovery. No antibody was detected to Micropolyspora faeni, Aspergillus fumigatus or avian protein. No increase in specific IgE directed against common allergens measured by RAST tests or elevation of total IgE was found.

This case resulted in an investigation of the factory workforce where other, milder cases of illness were found (138), consistent with the diagnosis of humidifier fever. Chest radiographs and pulmonary function tests were performed in five other cases with more severe symptom profiles, but were all normal. Chest radiographs in humidifier fever outbreaks reported previously have been normal (162,135,129,) although in one of these outbreaks (129) the authors comment that the illness resembles EAA with a similar response in pulmonary function and symptom development after antigen challenge. The presence of serum precipitin was also similar but the periodicity of symptoms and absence of radiographic changes in their subjects was not typical of EAA. Others (163,127) suggest that solubility of the antigen might explain the lack of radiological change in the U.K. cases and others also accept that radiographic abnormality is unnecessary to diagnose EAA (161) but we presume that the antigen causing EAA in our case was similar to that producing humidifier

fever in others in the factory. The subject who developed EAA probably experienced higher antigenic exposure than others in the factory because he worked in a sealed room with the input of the air conditioning directly above his head, while the others worked in an open plan area. Perhaps also the intensity of the immune response found in the serum of this case reflected a particularly intense response by lung tissue.

2.8 Sick Building syndrome is the term accepted to describe the cause of symptoms of transient or permanent nature which are most often found in sealed, air conditioned buildings (164). Work-related headache, lethargy, mental fatigue, irritation of eye, nose and throat, nausea, dizziness, wheezing, chest tightness, itching, drowsiness and dry skin are included in the reported symptoms (165,164). No specific cause has been found, however several possible sources and mechanisms have been implicated (166). A large epidemiological study has confirmed that symptoms are more common in air conditioned buildings than those which are naturally ventilated (167).

The current view is that these symptoms have many causes, some of which may be present in combination. The major difficulty with the generalization of the term "Sick Building

Syndrome" is that a common aetiology is inferred which cannot be substantiated to non medical personnel who may then question the reliability of the diagnosis (168). Finnegan et al (165) postulated that the diagnosis is often neglected and suggested that longitudinal studies for symptoms in populations who work in naturally ventilated and non naturally ventilated buildings might provide the cause(s) of the syndrome. In the meantime, more dramatic measures may be attempted by some (169) while others would increase fresh air turnover (170).

2.9 Infection through air conditioning is probably not uncommon. Vents within a sealed building may release air recirculated from infected individuals who have released droplet nuclei in areas distant to the primary infectious case (171,172,173). Air intake into a building may also be a source of infection as described by Sarubbi et al (174) when Aspergillus flavus within a hospital increased during a period when construction work was taking place outside. In that air conditioning unit, the filtering mechanism was defective. Similar malfunctions of air handling have resulted in fungal infection in immuno-compromised subjects (174). Some of these cited references are the result of hospital based studies on in-patients where awareness of infection is more acute. The

general office is unlikely to recognize several cases scattered throughout the building and hence air conditioning spread of infection is likely to be ignored unless the infections are of a more serious nature.

2.9.1 Legionellosis is currently the most topical and, perhaps, feared infection which is associated with air conditioning. The media has increased public awareness of the condition although perhaps not knowledge to the same extent, a feature of the condition recognised early during the phase of description of the disease (176). The association between buildings and legionellosis was recognized by the investigators of the Philadelphia outbreak who commented that the hotel lobby appeared to be a common link although some victims had merely walked past the building (177). Subsequently Legionella pneumophilia has been isolated from water supplies in hospitals and public buildings where cases of the pneumonia have occurred (178,179,180). The organism is ubiquitous in water supplies, usually in the absence of infection. Current advice to service engineers contains descriptions of factors which predispose to proliferation of the organism such as hot water cooling systems (fig 2.15), types of plumbing and fixtures which are difficult to disinfect, layout of water systems and

Statement of Collaboration

The work of this thesis was primarily clinical and the author was responsible for the recognition, assessment, investigation, assimilation of the results and direction of the corrective procedures in each factory investigated. The science of the subject matter was discussed in collaboration with laboratory based colleagues in immunology, biochemistry and meteorology on the instigation of the author. Aspects of this component of the work relied on the technical abilities of the laboratory and measurements taken under the direction of the acknowledged collaborators. The author respects that parts of the thesis would have been incomplete without the contribution from the scientific collaborators who were essential for the technical excellence of the investigations but not responsible for the generation or interpretation of, or correlation with the clinical data. The views expressed in the thesis are entirely those of the author based on the results and his interpretation of published material cited in scientific journals and books.

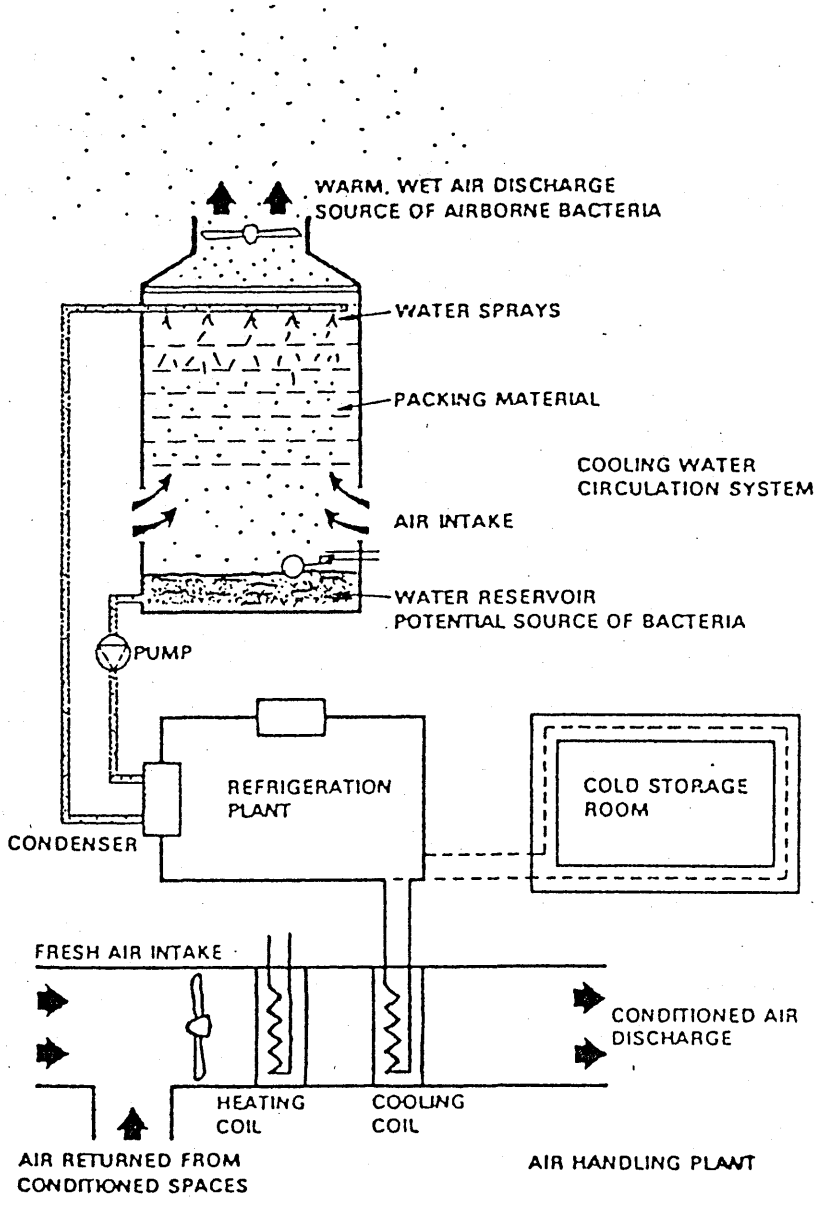

FIGURE 2.15

Air conditioning system incorporating a cooling system. The cooling tank is usually mounted on a roof (cooling tower).

positioning and design of cooling towers (181).

As a cause of community acquired pneumonia, the incidence of the disease varies between 1% to 15% (182). A milder infection caused by the same organism was responsible for an influenza-like illness in a public health office in Pontiac after which the condition was termed Pontiac Fever. Recently another outbreak of similar type caused by a legionella organism of a different subtype (L. micdadei) was described in Scotland (Goldberg 1989).

The essential feature of the illness appears to be inhalation of airborne material which is contaminated with legionella. Cigarette smoking (177) and debilitation (184) are predisposing factors.

Legionnaires' disease and the public health may eventually be linked in the U.K. in a similar manner as the 1952 London fog and the measures mentioned in chapter one. As a result of a hospital outbreak of the disease (185) a report of importance to "the future development of public health function" has advised on improvements required in the supply of health services in England (186).

CHAPTER 3

HUMIDIFIER FEVER IN TWO FACTORIES

3.1 INTRODUCTION

The investigation of two factories was performed by the author in close co-operation with the factory medical officers in one factory and with an Employment Medical Adviser (from the Glasgow EMAS office, St. Vincent Street, Glasgow) in another. Both the factory medical service and EMA requested assistance to undertake some form of investigation into symptoms affecting employees within the buildings which appeared work-related.

The factories were both situated in modern industrial estates in East Kilbride. The major function of the first factory investigated was assembly and production of microprocessor semiconductor circuits. The second factory was a printers which produced quality magazines, books and forms.

3.2. Workplace descriptions

The microprocessor factory employed more than 1000 personnel who performed various tasks including electronic assembly, manufacture of electronics, office work and ancillary work, which were separated by corridors and partitions. The

ventilation of the building was controlled by 6 air handlers (American Air Filters) installed in 1976 which artificially moistened the air by spraying water through baffle plates into the airflow from a recirculating water storage bath. The water spray was controlled by a humidistat to maintain a humidity of at least 45% to prevent the development of static electricity. The precise water consumption of the unit was not known. The maximum air flow capacity was 14.4 m^3/S with an estimated average airflow in winter of $5m^3/S$ and water consumption of 95 1/day varying according to the degree of air recirculation and the temperature of the external air intake. Summer water consumption was not known but was thought to be a small fraction of the winter figure. A water sample from the humidifier is shown in plate 2.1.

The printing factory was of barn design with natural ventilation, employing 30 people. The fresh air intake was heated to 17°C and a relative humidity of 45% (to avoid paper shrinkage and creasing) was maintained by two ceiling mounted spinning disc humidifiers (WEKO, type LDFT, Biel AG), controlled by a wall mounted humidistat (plates 3.1 and 3.2). The maximum water consumption of each humidifier was 7 l/hour but the actual water consumption was unknown. Both humidifiers were found to

PLATE 3.1

The printing factory and the ceiling-mounted spinning-disc humidifier in the upper background. Note the presence of fine dust (anti-offset powder) on the horizontal surfaces of the printing press.

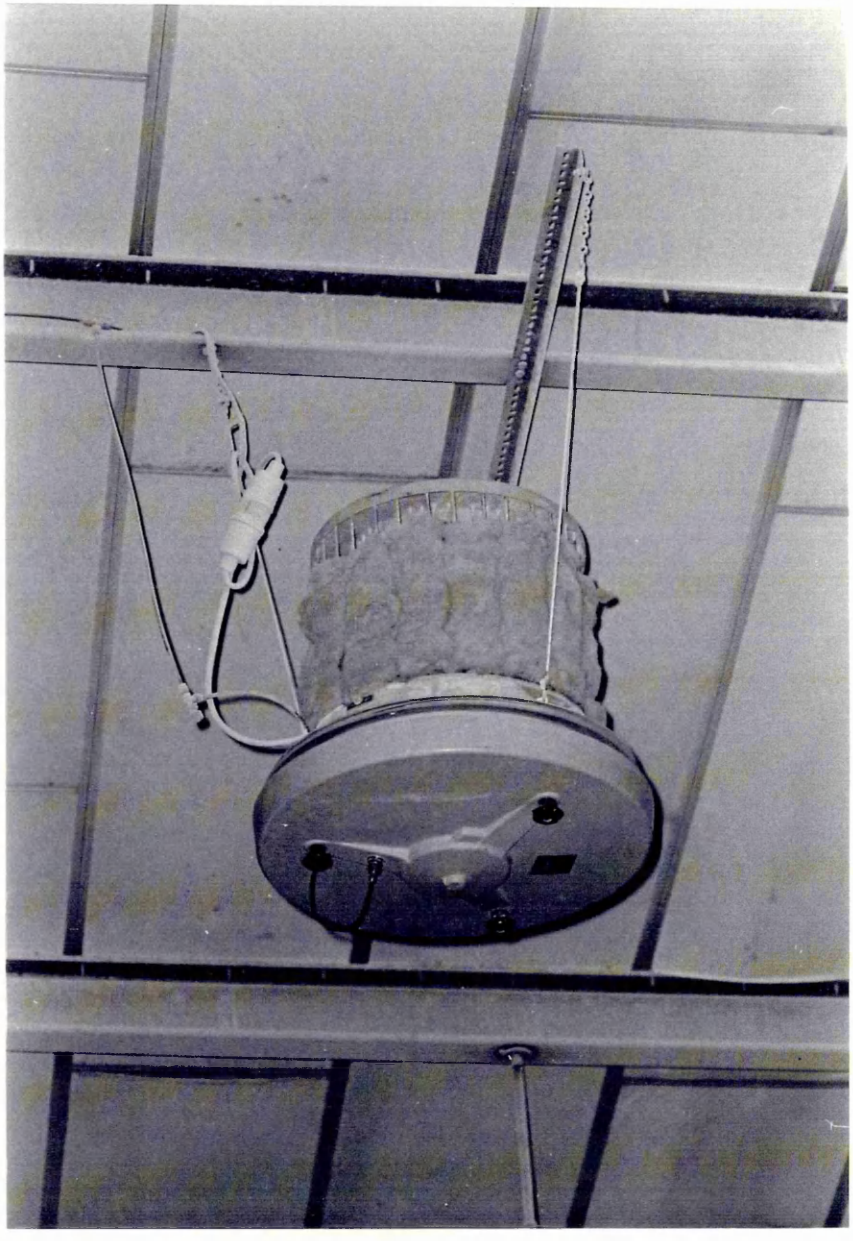

PLATE 3.2

Close-up of the spinning-disc humidifier responsible for humidifier fever in the printing factory. The fibrous material (baffles) was compressed allowing the escape of large droplets.

be heavily contaminated by "anti offset powder" (a vegetable starch used to allow paper sheets to run cleanly through the printing press and not to adhere to the previous and subsequent prints), and to contain viscid fluid.

3.3 The workers

The area where individuals became symptomatic in the microprocessor factory was the electronic testing area which was initially investigated in September 1983 when 50-60 personnel worked on a rota basis. In July 1986 the same ventilation system from the testing area was extended to include an assembly area where a further 200 individuals worked. All 22 workers who worked in the main section of the printing factory outwith a naturally ventilated office area, were assessed either during July 1985 or January 1987.

The subjects who were available for study were selected according to restrictions which were designed to satisfy the managerial staff in both factories and also the need to gather a satisfactory number of subjects for scientific study. In the microprocessor factory the decision was taken that any symptomatic volunteers and any others who were asymptomatic who wished to be assessed, should be studied in the 1983 group and

similarly in 1986. This was a non-union workforce and the study relied heavily on the enthusiasm of the factory medical service to balance the managerial staff in what we acknowledged to be a sensitive subject within the building. No visitor was allowed to be unaccompanied within the factory premises and photography was not permitted. On the other hand, the printing factory was more simple to investigate and most of the workers consented to interview and a venous blood sample. The printing factory was staffed by the SOGAT union and the approaches to the other staff members were directed with consent first of the union, then management, then worker.

Such restrictions on studies within factories are common although perhaps unavoidable and seldom reported. The author's experience was that at least as much time was required for establishing the need for a study within each factory as was required to examine the exposed and affected populations. The degree of compromise within each factory was unavoidable although the results obtained remained interesting.

3.4 The questionnaire

The design of the questionnaire was influenced by the interventionist nature of each investigation. The source of the

disorder was quickly identified after the auther or EMA were contacted and the humidifiers switched off. The questionnaire standardised the approach to each patient through the Yes/No answering scheme on each of eleven symptoms, the timing and duration of the symptoms, smoking history, gender, duration of exposure (judged by duration of employment although nocturnal workers probably received higher exposure), and the relationship of the symptoms to work or if symptoms resolved when off work for any reason.

These components of the questionnaire were based on the symptom pattern previously recognised to suggest humidifier fever (127) or extrinsic allergic alveolitis (2). Wheeze was included in the list primarily as an acknowledgement of being a common respiratory symptom and not because occupational asthma was suspected, however during these studies work-related asthma was described by others in relation to the output of a contaminated humidifier (187).

The questionnaires were completed by a doctor during each interview, the interview ending when a venous blood sample was taken. 85/88 questionnaires were completed correctly and available for study when gathered. Exposure details (years of

TABLE 3.1 THE QUESTIONNAIRE UTILISED IN THE STUDY OF TWO FACTORIES AFFECTED BY HUMIDIFIER FEVER

Name: Emp. No.: Dept. No.: Shift:

D.O.B.: Date Interviewed: Years Employed:

Have you suffered from any of the following symptoms in the last 4 months?

1.	Chest tightness	Yes/No
2.	Headaches	Yes/No
3.	Shivering or Chills	Yes/No
4.	Breathlessness	Yes/No
5.	Cough	Yes/No
6.	Wheeze	Yes/No
7.	Poor appetite	Yes/No
8.	Weight loss	Yes/No
9.	Fever	Yes/No
10.	'Flu-like' symptoms	Yes/No
11.	General tiredness	Yes/No
12.	Other symptoms - specify:-	Yes/No

Were symptoms worse on return to work after holiday or weekend off? Yes/No

Did these symptoms persist for whole of working week? Yes/No

Smoking history:

Do you smoke?	Yes/No
Have you ever smoked?	Yes/No
How many daily?	------

Blood taken? Yes/No

service) were unavailable for 3 subjects in the microprocessor factory who were employed by external contractors. A sample of the questionnaire is show in table 3.1.

3.5 Consideration of the results

The results obtained by this technique are shown in tables 3.2 and 3.3. In total 88 subjects were assessed, 74 were symptomatic and 14 asymptomatic. 66 (26 from 1983 and 40 from 1986) originated in the microprocessor factory and 22 (16 from 1985 and 18 from 1987) from the printing factory. 12 in the printing factory were included in the 1985 and 1987 studies. Only one subject was studied in both 1983 and 1986 microprocessor outbreaks which we were told by the factory management probably reflected the high turnover of staff within the factory although this could not be verified from staff records. The factory medical service offered a similar explanation.

The commonest symptoms overall within both groups were recognised as "flu-like". Fever appeared more commonly in the printers. Many more subjects within the microprocessor group complained of symptoms more in keeping with extrinsic allergic alveolitis or asthma than classical humidifier fever although more than one of these conditions could occur (in principle) in

TABLE 3.2 QUESTIONNAIRE RESULTS AND SEROLOGY FROM THE MICROPROCESSOR FACTORY

MICROPROCESSORS 1. AUGUST 1983 - MAY 1984

NAME	GENDER	AGE	PPT'N	TOTAL IgG Aug / May	SBI	1	2	3	4	5	6	7	8	9	10	11	M.S.	Night	Persist	Smoker	Exp.
NC	F	45	+	16.6/10.4	40.7/ 8.2			x						x	x	x	x				4
LB	F	50	-	8.4/ 6.0	0/0	x			x	x	Sore throat, burning eyes							x		x	5
MM	F	50	+	11.1/ 9.1	30.0/17.5		x				Nausea				x	x		x			4
EM	F	39	-	8.4/ 6.0	0/0	x			x							x	x	x		x	5
RMcD	F	36	-	15.4/13.2	20.3/15.9			x	x	x					x	x	x	R	x	x	4
JB	F	28	+	20.5/13.9	9.5/ 2.2	x			x		x				x	x	x	x	x		5
ED	F	45	+	15.4/13.5	52/45	x	x	x	x	x		x		x	x	x	x		x		8
MF	F	39	-	13.1/11.8	18.4/21.9	x	x	x	x		x				x	x	x	x	x		4
DA	M	28	+	37.3/25.4	100/100	Crackles		x	x	x		x	x	x	x	x	x		x		1
JM	M	38	+	9.8/5	21.5/14.6			x	x	x	x			x	x	x	x		x	ex	2
MMcK	F	27	+	13.9/12.5	1.2/0	x	x	x	x	x	x	x		x	x	x	x				5
IS	F	40	-	14.3/12.5	0/0				x		Sore throat, metallic taste					x				ex	6
LD	F	31	-	13.2/10.4	0/19				x	x					x			x		x	2
DW	M	36	+/-	16.2/12.5	0/0	Sore throat				x		x		x	x	x	x		x		4
HD	M	42	+	13.2/ 9.7	42.5/ 7.7		x	x						x	x	x	x				9
WG	M	43	+	13.9/ 9.1	66.7/42.6	x	x	x	x			x		x	x	x	x			x	5
MMcC	F	38	+	41.3/30	18.6/13.2	x	x			x		Dysphonia				x	x	R	x		1
MD	F	44	+	15.7/14.5	15.4/10.4	x			x		x					x	x	x	x	x	4
DR	F	36	-	9.4/ 7.2	0/0	x	x			x	x			x	x	x	x	x	x	x	3
MC	F	37	-	14.6/ND	6.9/0				No symptoms									x			8
MCr	M	41	-	12.5/ND	0/0				No symptoms											ex	10
EG	F	36	-	9.7/ND	0/0				No symptoms											x	4
AL	F	39	-	10/ND	0/0				No symptoms									x			5
MH	M	35	-	12.5/ND	0/0				No symptoms									R		x	4

MICROPROCESSORS 1. AUGUST 1983 - MAY 1984

NAME	GENDER	AGE	PPT'N	TOTAL IgG Aug / May	SBI	1	2	3	4	5	6	7	8	9	10	11	M.S.	Night	Persist	Smoker	Exp.
JM	M	36	-	16.2/ND	2.0/0				No symptoms									R		x	6
JS	F	33	-	9.1/ND	0/0				No symptoms												9

MICROPROCESSORS 2. 1986

NAME	GENDER	AGE	PPT'N	TOTAL IgG	SBI	1	2	3	4	5	6	7	8	9	10	11	M.S.	Night	Persist	Smoker	Exp.
MMcN	F	34	+	16.9	13	x	x	x	x	x				x	x	x	x		x		7
SH	F	48	-	13.7		x			x	x	x						x		x	x	10
AF	F	24	-	11.9				No symptoms													0.2
JC	F	33	-	14.3				Sore throat/rash				x	x		x	x	x	x		x	2
MM	F	50	-	8.55				Rash	x							x	x	x	x		9
DM	F	42	-	16.9	21		x	x		x					x		x	x	x		7
SK	F	28	-	9.6		x	x		x		x	Asthma/sneezing					x		x		2
JG	M	40	-	8.5			x	Sore throat		x	x			x	x			R	x		3
DC	F	37	+	16.3	16	x	x	x	x	x	x	x	x	x	x	x	x	x	x		7
TC	M	26	-	15			x			x		x				x	x	R	x		2
RB	M	31	-	7			x	Headache		x					x		x		x		4
GH	M	26	-	15				x					x	x	x	x				x	2
CS	F	21	-	8.55			x	x	Sore eyes			x		x		x	x		x		4
SF	M	25	-	15			x								x	x	x				3
GB	M	23	-	15			x	x							x	x	x				2
JMcL	F	50	+	15	35	x	x		x	x	x					x			x		12
JGF	M	25	-	12.5			x			x					x	x	x		x		2
MC	F	40	+	16.3	11				Nasal blockage							x	x		x		10
ES	F	22	-	16.3	15	x		x							x	x	x		x		10
MG	F	34	-	13.7		x		x	Back pain		x				x				x		3

MICROPROCESSORS 2. 1986

NAME	GENDER	AGE	PPT'N	TOTAL IgG	SBI	1	2	3	4	5	6	7	8	9	10	11	M.S.	Night	Persist	Smoker	Exp.
GS	F	25	-	15		x	x	x	x	x	x						x		x		2
LO'C	F	24	-	12.5		x		x	x		x					x	x	x			2
PT	M	39	-	11.9											x	x	x		x		4
MP	M	29	+	14.3	23		x		Leg muscle pain			x				x	x		x		6
WM	M	31	-	8.55		x	x	x	x			x	x			x			x	x	7
LD	F	39	-	13.1		x						x			x	x	x		x		NA
KG	M	38		15.6					x	x	x						x		x	x	7
LB	F	30	-	11.3				x												x	4
ML	F	55	+	26.5	14					x	x	x					x				9
MC	F	33	-	12.5			x			x					x	x					3
JW	F	29	-	9.1			x		Hay fever/swollen glands						x	x			x		NA
TH	M	23	-	13.7			x			x	Lack of concentration				x	x	x		x		3
MA	F	35	-	11.9			x				Lack of concentration				x	x	x				4
SR	F	47	-	9.6					x						x	x				x	NA
DK	F	23	+	18.3	26		x	x								x	x				6
LC	F	38	-	10.7		x	x	x		x	x				x	x	x	x	x		10
GR	F	57	+	16.9	37	x	x	x	x	x	dieting /GP influenza			x	x	x			x	x	9
BW	F	49	+	20.4	25	x	x		x										x		12
IM	F	40	+	15	17	x	x	x	x	x	x	x	DK	x	x	x			x	x	11
DA	M	31	+	23	100		x	x	x			x		x	x		x		x		4

TABLE 3.3 QUESTIONNAIRE RESULTS AND SEROLOGY FROM THE PRINTERS

PRINTING 1985-1987

NAME	GENDER	AGE	PPT'N	TOTAL IgG 85 / 87	SBI 85 / 87	1	2	3	4	5	6	7	8	9	10	11	M.S.	Night	Persist	Smoker	Exp.
IB	M	36	++	ND/20.4	ND/118		x	x		x				x	x		x				11
GB	M	22	-	ND/9.63	ND/0			No symptoms													1.5
WB	M	24	++	12.5/15	93/0		x	x	x					x	x					x	9.5
WB Snr	M	47	+++	16.3/17.6	95/93		x	x	x	x				x	x	x	x	x	x		17
PB	M	43	++	20.7/19	110/88		x	x						x	x		x				6
EC	F	22	+	11.9/13.7	114/95		x							x	x		x				4
MC	F	58	++	ND/16.3	115/92	x	x	x						x	x		x			Ex	14
FG	F	54	+	15.6/ND	16/ND			No symptoms													11
JH	M	26	+	15.6/13.7	99/62		x	x						x	x		x			x	3
WJ	M	60	+	21.1/16.9	105/100		x		x					x	x		x			x	10.5
DMcI	M	49	++	15.6/11.3	104/79		x	x						x	x		x				16
IMcI	M	37	+	ND/14.30	96/0			x						x	x		x			x	11
JMcC	M	52	-	ND/11.30	0/ND			No symptoms													1.5
JL	M	57	-	ND/10.20	0/0			No symptoms												x	17
JN	M	37	-	ND/11.90	ND/0			No symptoms													1.5
AMar	M	39	+	ND/14.30	ND/108	x		x						x	x		x		x		4
AMas	M	43		16.30/11.3	89/90			No Symptoms													3.5
IMcK	M	40	+++	21.10/14.3	104/108		x	x		x					x	x	x	x	x		8
GN	M	51	+	17.60/12.5	66/39		x	x							x	x	x	R	x	x	14
RS	M	50	+	10.20/8.02	75/64			x						x	x	x	x			x	2
MT	F	39	+++	22.6/13.7	114/113	x	x	x						x	x		x				10
DO'N	M	60	+	ND/17.60	ND/107			x						x	x		x				8

KEY TO TABLES 3.2 and 3.3

PPT'N	:	serum precipitins
IgG	:	immunoglobulin G
SBI	:	specific binding index
Exp	:	exposure in years
+	:	present
-	:	absent
x	:	positive finding
F	:	female
M	:	male
night	:	night shift worker
R	:	rotating night and day shift
ex	:	ex-smoker
N.A.	:	not available
N.D.	:	not done
crackles	:	bilateral basal ausculatory crackles
persistence	:	symptoms persisted through the working week
Monday symptoms	:	symptoms appeared on the first day after a break from work.

1.	chest tightness	7.	poor appetite
2.	headache	8.	weight loss
3.	shivering or chills	9.	fever
4.	breathlessness	10.	flu' like symptoms
5.	cough	11.	general tiredness
6.	wheeze		

any one subject. Thus cough, breathlessness, appetite disturbance, chest tightness and wheeze were more common in this group who also appeared more willing to describe other symptoms which were not specifically mentioned on the symptom list.

14 of the microprocessors were smokers and 5 ex smokers. Both are shown as "ever smokers" in table 3.4. Cigarette smoking might be expected to produce certain of these symptoms in the absence of humidifier disease and probably represented the other major source of inhaled material in either factory. From table 3.4 the deduction can be made that although many of the smokers had symptoms which could be considered to have been smoking related, several other smokers also had symptoms which are more in keeping with humidifier disease, particularly humidifier fever. Cigarette smoking offers one explanation for the higher incidence of cough, wheeze and breathlessness in the microprocessors thereby representing a background incidence of non-humidifier related symptoms. These symptoms and poor appetite, weight loss and general tiredness appeared more common in the microprocessors and despite similarities of the antigen (see later) suggests that this population differed in the degree of exposure to antigen, susceptibility to the development of the disease process, or simply to reporting of symptoms in comparison

TABLE 3.4 THE SYMPTOMS AND SMOKING PATTERN IN EACH FACTORY
Current smokers are shown in parenthesis
Ever-smokers are ex and current smokers in total

	MICROPROCESSORS		PRINTERS		TOTAL	
		Ever-smokers		Ever-smokers		Ever-smokers
No. of symptomatic subjects	58	19(14)	16	9(8)	74	28(22)
1. Chest tightness	25	10(6)	1	1(0)	26	11(6)
2. Headaches	33	4(3)	12	5(2)	45	9(5)
3. Shivering or chills	27	8(5)	14	7(5)	41	15(10)
4. Breathlessness	28	12(9)	4	2(2)	32	14(11)
5. Cough	28	12(8)	3	0(0)	31	12(8)
6. Wheeze	19	9(7)	0	0(0)	19	9(7)
7. Poor appetite	16	5(4)	0	0(0)	16	5(4)
8. Weight loss	6	3(3)	0	0(0)	6	3(3)
9. Fever	17	6(4)	14	7(6)	31	13(10)
10. "Flu" like symptoms	38	10(8)	16	8(7)	54	18(15)
11. General tiredness	45	13(9)	4	1(1)	49	14(10)
12. Others (specified in 3.1)	18	3(1)	0	0(0)	18	3(1)

with the printers. A higher proportion of the microprocessors were female (77%) but the printers were generally older with longer exposures as estimated from years of service (see appendix A1.2 for statistical analysis). More of the printers were or had been smokers (40.9%, 8/9 current smokers) than the microprocessors (34.5%, 14/19 current smokers), but this was not statistically significant and is unlikely to be the cause of the different symptom spectra in each factory. Cigarette smoking is known to influence the development of occupational asthma (188), extrinsic allergic alveolitis (189) and humidifier fever (137) and is likely to be highly relevant within this group of workers. The serological effects of cigarette smoking will be discussed in the next chapter. However, from a symptomatic viewpoint symptoms of similar type commonly appear in smokers and non smokers alike in both factories, and humidifier fever symptoms are not uncommon in the smokers. Therefore in these populations smoking does not appear to have influenced the development of humidifier disease to the same extent as has been reported previously in other disorders of presumed similar aetiology (190,191).

The Monday-pattern of symptoms was noted by 58/74 (79.3%) of the symptomatic subjects but as has been mentioned previously

(2.6.1 section on climate) the occurrence of symptoms was strongly influenced by the intermittent nature of the humidification system and particularly so during the summer. This type of humidifier system also explains the apparent persistence of symptoms throughout the working week which was observed in 43/74 (58.1%). Persistent symptoms only occurred in the night shift workers in the printing factory suggesting higher antigen exposure in response to cooler nocturnal air intake into the humidifiers. The majority of symptomatic subjects in the microprocessor factory were day shift workers where 22/29 of these workers and 12/18 night workers had persistent symptoms through the working week.

The relationship of symptoms and serology will be discussed in Chapter 4.

The questionnaire and blood samples were repeated again nine months later (after 1983) in all 19 symptomatic subjects in the microprocessor group and sixteen months later in 10/22 of the printers.

In the microprocessors, symptoms had settled in thirteen subjects and persisted in six, who were all night shift workers.

Later we established that the suspect humidifier was not dismantled as initially suggested but had been retained within the factory and modified by increasing the rate of water circulation by fitting an exhaust to remove greater quantities of the water stored in the bath. The humidifier was retained for use only when maximal humidification was required and this would most likely occur at night (see Chapter 2, section on climate).

10 subjects from the printing factory consented to a repeat interview, pulmonary function assessment and serology 14 months after the 1987 outbreak of humidifier fever. The spinning disc humidifier had been replaced by mains water atomizers and symptoms in the factory had almost disappeared. The results of this assessment are shown in table 3.5.

The 10 subjects from the printers all noted a marked improvement in symptoms and in most, they had entirely recovered. 2 patients with shortness of breath attributed (probably correctly) the symptoms to cigarette smoking in one and previous pulmonary tuberculosis in the other. These two subjects had lung function test results which were in keeping with these underlying abnormalities. Unfortunately, the facility for portable lung function studies was not available during the acute

TABLE 3.5 FOLLOW-UP ASSESSMENT IN THE PRINTERS

NAME	SYMPTOMS	EECO	FVC	FEV1	FEF 25-75	FEF 50	FEF 75-85	PF	PH
RS	dyspnoea	11	4.27	3.00	2.03	2.72	0.38	483	smoker
JW	none	3	4.61	2.70	3.84	5.41	0.87	411	
AMasin	none	3	3.09	2.38	3.29	3.47	0.91	339	
DO'N	none	3	3.48	2.72	2.64	3.27	0.59	413	
EMcC(neeC)	none	2	2.99	2.88	4.15	4.56	1.99	371	
MC	dyspnoea	2	2.18	0.95	0.48	0.36	0.19	83	previous PTB
MT	headache	2	1.96	1.68	2.23	2.72	0.56	267	
FG	none	3	2.77	2.08	2.16	2.61	0.51	301	
PB	none	3	3.6	2.8	3.41	3.62	1.12	502	
WB Snr.	none	2	3.83	3.07	3.83	4.15	1.24	528	

Key:

EECO — End-expired carbon monoxide, measured by Eco check (PK Morgan Ltd., England)

FVC — Forced vital capacity

FEV1 — Forced expiratory volume in one second

FEF 25-75 — Forced expiratory flow at 25-75% of expirate

FEF 50 — Forced expiratory flow at 50% of expirate

FEF 75-85 — Forced expiratory flow at 75-85% of expirate

PF — Peak flow rate (L/min)

PH — Past history

PTB — Pulmonary tuberculosis

• Measured by portable Vitalograph Compact Spirometer (Vitalograph Inc., Kansas, USA)

attacks of illness but the abnormalities of pulmonary function during humidifier fever and associated diseases have been demonstrated by others previously. In the study of Ashton et al (136) a slight reduction in FEV1, vital capacity and flow rate after 75% of the vital capacity was expired but no changes in peak flow rate, FEF 50, or transfer factor were noted (although on the subsequent day a 13% fall in transfer factor was recorded which was thought to have been caused by a higher ambient temperature on that day). Others (129,132) found physiological abnormalities consistent with extrinsic allergic alveolitis and demonstrated a reduction in vital capacity and reduced transfer factor. The response to challenge testing with humidifier antigen was less consistent in another group where a fall in vital capacity was recorded but no change in transfer factor (133).

One explanation of these varying findings may be in varying dosages of inhaled antigen. Hendrick (192) has suggested that a response is more likely when the dose is increased and that the systemic response is a more sensitive measure of disease activity than is pulmonary physiology.

The end expired carbon monoxide was utilised to confirm the

smoking history in these subjects and was consistent with the history in all, as has been observed in a similar group (193).

Several individual points were noted in each workforce. A number of the workers (including the subject who developed extrinsic allergic alveolitis) were initially thought to have functional illness. One menopausal subject was disappointed that her symptoms of flushing, pyrexia and sweating returned after hysterectomy when she went back to work. The subject (DO'N) responsible for servicing the humidifier (hosing with a water jet) in the printers would presumably have regular exposures to concentrated antigen but had no more frequent episodes than the other day shift workers.

The most striking feature of the second outbreak of humidifier fever in the microprocessors was the lack of recognition of the recurrence of symptoms despite previous experience of humidifier disease. Symptoms were present for many months before the more classical outbreak occurred when the humidifier was finally removed. The symptoms have since entirely settled.

CHAPTER 4

IMMUNOLOGY OF HUMIDIFIER-RELATED DISEASES

4.1 INTRODUCTION

A further feature of similarity between humidifier-related extrinsic allergic alveolitis and humidifier fever is the presence of an immunoglobulin disturbance within serum, a feature common to other forms of EAA (194) into which humidifier fever is, perhaps, best categorized (195). Serum IgG, either detected by precipitins on gel diffusion or by enzyme-linked immunosorbant assay (using humidifier water or baffle plate sludge as antigen), is often elevated in workers who have been exposed to a contaminated humidifier. Symptomatic workers from previous outbreaks of humidifier disease have been reported to have elevated IgG where there was a good correlation between the presence of IgG and disease (128,129,131,132,148). As in other forms of EAA exposed asymptomatic subjects also may have specific IgG (161) which in one study of a volunteer sample detected antibody in 63% (137).

4.2 Antibody measurement in the assessment of humidifier fever.

The traditional method of detecting serum precipitins is by

double gel-diffusion as described by Ouchterlony (196) however, the techniques of enzyme immune assay (EIA) or radio-immunoassay have the advantages of detecting antibody of lower concentration than on gel-diffusion and also a more precise quantification of the amount of antibody present (197). An EIA was developed as part of the laboratory investigation of symptoms which developed in the microprocessor factory and printing factory discussed in Chapter 3. Serum was obtained from 26 workers who volunteered symptoms during an outbreak of humidifier fever in the microprocessor factory in September 1983 and again after the humidifier was altered and most symptoms resolved in June 1984. Another 40 sera were obtained from those workers who reported symptoms during the 1986 outbreak of the same illness. 22 sera were collected from the printing factory which included 16 from the original outbreak of humidifier fever in 1985. 12 of these individuals and 6 others donated sera during the recurrence of the illness in 1987 and 10 of these donated further sera 18 months later after the humidification system was replaced. For comparison, control sera were obtained from 60 healthy non-exposed donors (volunteer laboratory personnel), 160 factory workers who described symptoms suggestive of legionellosis (kindly supplied by Dr. R. Fallon, Ruchill Hospital) and 40 subjects who developed Pontiac fever (183) in a local outbreak of

the illness.

4.3 The Antigen

The antigen for the assay was prepared from water extracted from the humidifier in each factory and concentrated x 20 (Minicon Concentrator, Amicon, Danvers MA, USA) and stored at -20°C. The sludge was extracted 1 in 4 v/v saline for 1 hour at room temperature and the extract harvested, dialysed, freeze dried for storage and reconstituted at 1mg/ml for use. The EIA technique is described in the appendix (A3). Serum precipitins were detected by Ouchterlony gel-diffusion. IgG subclass antibody activities were estimated by a similar technique (kindly performed by Dr. D.M. Kemeny). Serum IgG was measured by nephelometry (Beckman ICS Analyser 11). Total serum IgE was measured by radioimmunoassay (RIACT, Pharmacia U.K. Ltd.) according to the manufacturer's instructions.

4.4 Serological results of two factory outbreaks of humidifier fever. The antigen from each source was demonstrated to react with lines of identity with serum from an index case (Plate 4.1) from each of the factories and since this implied that the major antigens were similar in both factories, one standard antigen preparation from the sludge extract was used throughout.

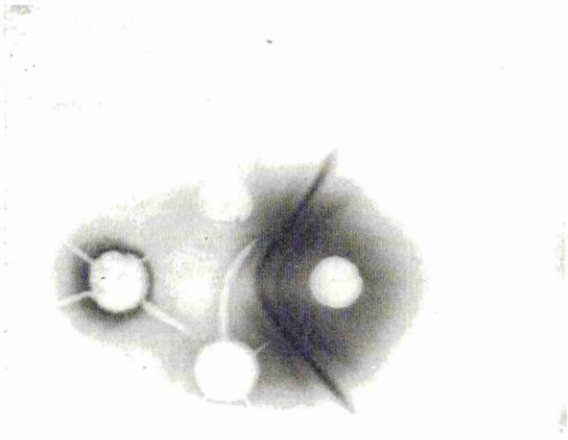

PLATE 4.1

Gel-diffusion plate showing lines of identity between the antigens from each factory (wells positioned at 12 and 6 o'clock) and a serum sample from a microprocessor factory worker (subject with extrinsic allergic alveolitis described in 2.7) in well at 3 o'clock. A normal, non-exposed individuals serum is in well at 9 o'clock.

The optimal proportion for each variable involved in the EIA was assessed by checkerboard analysis. For the detection of IgG antibody activity, the optimum concentration of antigen for initially coating the wells was 20 mg/ml, and the serum dilution optimal at 1 in 100. The profile of the assay performance for one strong-positive and one weak positive precipitating serum, one negative normal control and one negative myeloma (high non-specific serum IgG level) is shown in figure 4.1. Antibody positive and negative sera are discriminated at 1 in 100 dilution and give a quantitative estimate of antibody activity. The assay is unaffected by the myeloma serum at the working dilutions.

The specificity of the EIA was demonstrated by inhibiting the development of colour by preincubating the diluted serum, 200ul, with 5 ul (5ug) of antigen overnight before proceeding with the assay.

4.5 EIA and precipitins were compared by calculating the specific binding index from the optical density of 31 test sera and 31 control negative (non-exposed) sera in one assay. After calculating the mean plus two standard deviations of the optical density, which was equivalent to the antibody activity of normal

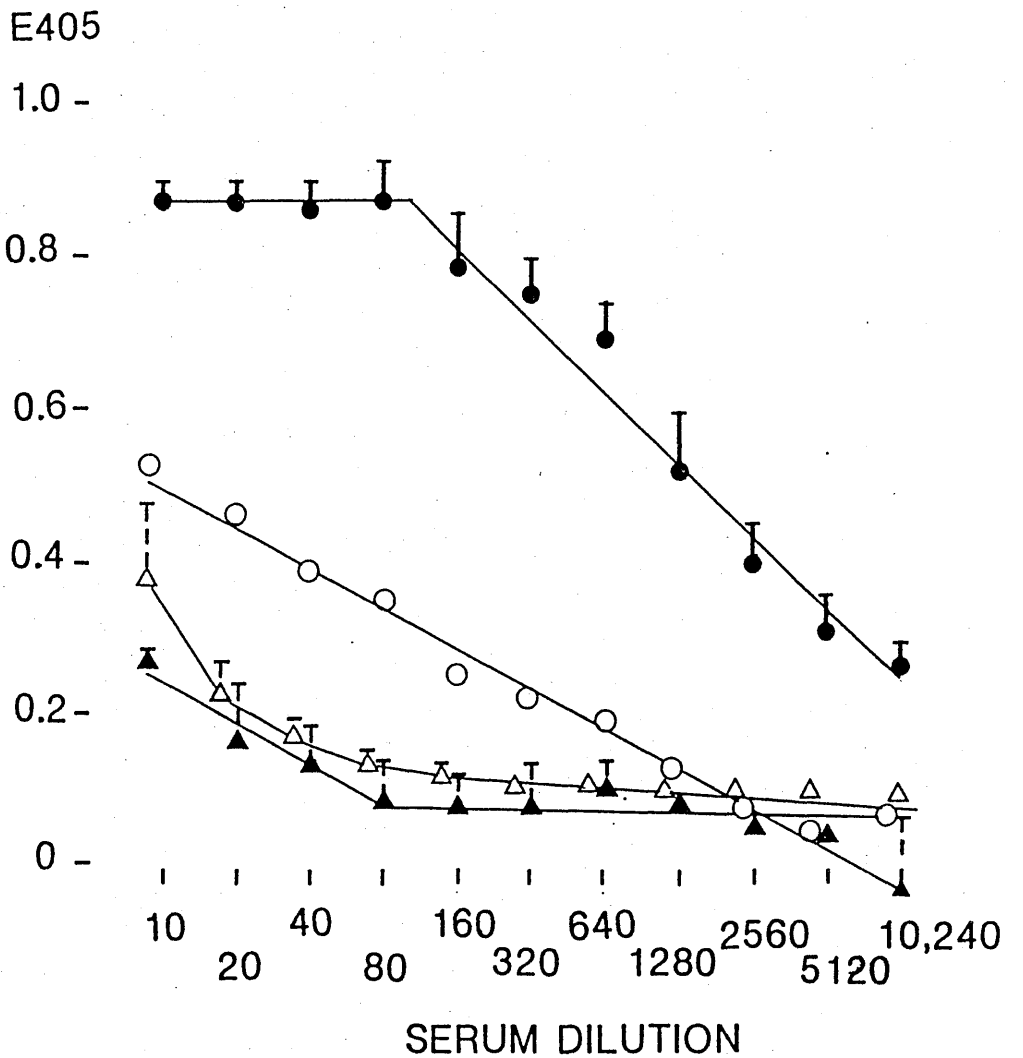

FIGURE 4.1

IgG assay performance profile. A range of results of several samples is plotted for a strong precipitin positive serum (●), a weak precipitin positive serum (O), a negative normal control (▲) , and one negative myeloma (Δ). The optical density is represented on the axis labelled E405.

serum, the SBI (see appendix for definition) ranged from 0% at the top of the normal range to 100%, which was the designated value of a strong positive precipitin serum. The figures corresponding to the optical density and SBI are shown on the ordinates of figure 4.2. Twelve subjects were precipitin positive and 19 precipitin negative. All of the positive precipitin subjects had greater than normal SBI and 5 of the 19 precipitin negative sera had positive SBI. For all 88 test sera there were 44 precipitin positive and 44 precipitin negative with corresponding SBI means values of 58.1 and 2.4 respectively ($p<0.001$).

4.6 IgG antibody and humidifier disease. The statistical analysis comparing asymptomatic exposed subjects with symptomatic exposed subjects is presented in the appendix (A2) where IgG antibody is significantly different between the groups. The control (non-exposed healthy) group were used to generate the normal range and had zero antibody activity by definition. Antibody activity was seen only in the factory workers in which there was a suspicion of humidifier disease. No antibody activity was found in the miscellaneous group of symptomatic factory workers who had negative legionella serology and no cause for the symptoms found. Similarly the Pontiac fever group (183)

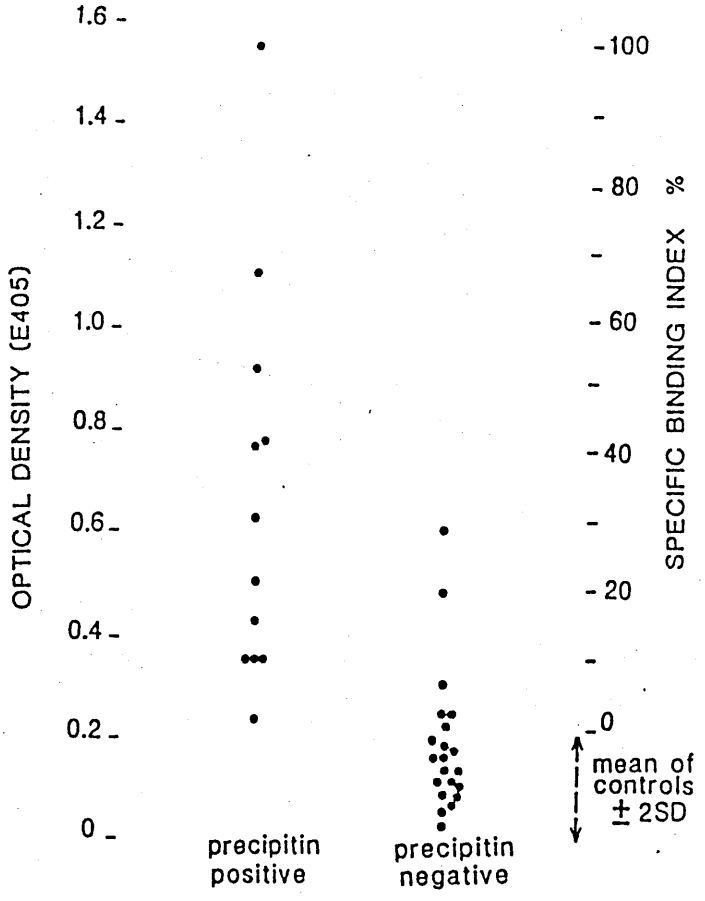

FIGURE 4.2

Determination of specific binding index. The mean ± 2SD of the optical density was determined which was equivalent to the antibody activity of normal serum. The specific binding index range from 0% to 100%, which was the designated value of a strong positive precipitin serum.

were also devoid of antibody activity, as were the myeloma sera.

4.7 Factors influencing antibody activity. The symptoms profile of each individual subject is listed in Chapter 3 together with details of age, gender, work history and smoking history. Females had significantly less IgG antibody than males (Appendix A1.3). Duration of service was significantly associated with IgG antibody (Appendix A1.5). Smoking was associated with lower antibody titres but the difference between smokers and non-smokers was not significant. The presence of symptoms was significantly associated with greater antibody activity ($p= 0.041$).

4.8 IgG subclasses are shown in figure 4.3. The response is predominately IgG1 subclass with very little antibody activity in the IgG2 subclass. The absolute values within each subclass for each individual were closely correlated (Table 4.1) demonstrating that individuals with the highest total IgG antibody level tended to be the highest subclass responders.

4.9 Hypergammaglobulinaemia was significantly more marked in the antibody positive symptomatic subjects than asymptomatic and antibody negative symptomatic subjects (Table 4.2). A closer

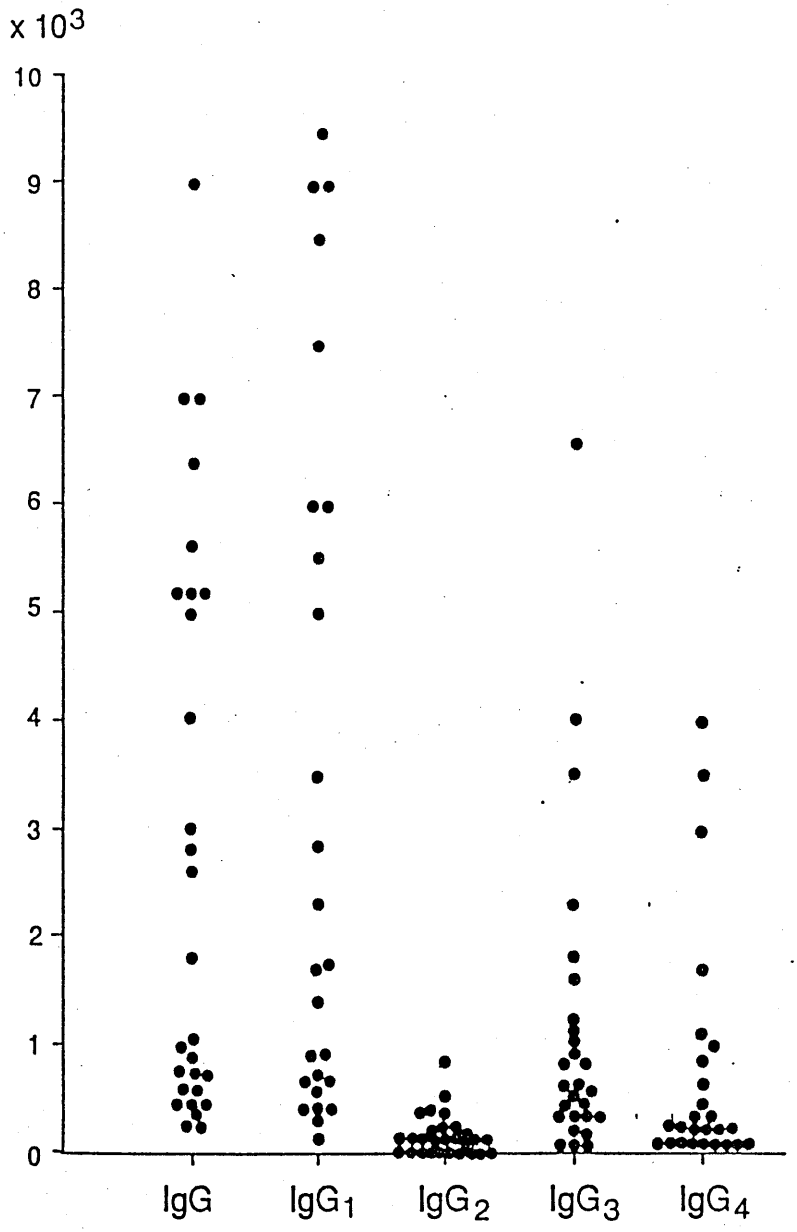

FIGURE 4.3

IgG subclasses. The 26 serum samples from the initial outbreak of humidifier fever in the microprocessor factory. Note the elevation of IgG_1 subclass.

Table 4.1

The correlation coefficient between the titre of antibody, of total IgG and IgG subclasses

	Total IgG	IgG1	IgG2	IgG3
IgG1	0.791	-	-	-
IgG2	0.704	0.630	-	-
IgG3	0.541	0.438	0.729	-
IgG4	0.553	0.501	0.614	0.709

Table 4.2

The median and range of the total serum IgG level (mg/ml) in the symptomatic and asymptomatic factory workers, as well as these levels in the antibody positive and negative categories. The Spearman Rank coefficient between groups 1 and 2 was 393.5 $p = 0.029$, group 1, a and b was 18.5 $p = 0.016$, group 2, a and b was 734 $p<0.001$.

	Serum total IgG level	
1. Asymptomatic n = 14	11.9	9.1 - 16.3
a. Antibody positive n = 4	15.6	10.2 - 16.3
b. Antibody negative n = 10	10.1	9.1 - 12.5
2. Symptomatic n = 74	15.0	7.0 - 37.3
a. Antibody positive n = 40	16.3	10.2 - 37.3
b. Antibody negative n = 34	12.5	7.0 - 16.2

association was observed between the serum antibody response to humidifier antigens and total IgG level than humidifier disease and total IgG. This association was quantitatively related by regression analysis of the antibody titres and the total IgG level (figure 4.4). No such correlation was observed with the high total IgG in the control myeloma sera.

4.10 Longitudinal serological analysis was performed by obtaining serum from the two study populations after intervals of 9 months in the microprocessor factory and 18 months in the printers, during which the humidifier systems within the factory were either modified or used differently (see before). Table 4.3 lists the results for the microprocessor factory where all serological parameters reduced when exposure to humidifier antigens was reduced. The most significant difference was the reduction by 24.7% of the mean total IgG level. Similar results were seen in the printers (figures 4.5 and 4.6). The humidifiers were cleaned and serviced more regularly after August 1985 and totally removed after January 1987 when a marked reduction in total IgG level and almost absence of specific antibody was noted by June 1988.

4.11 Consideration of the results

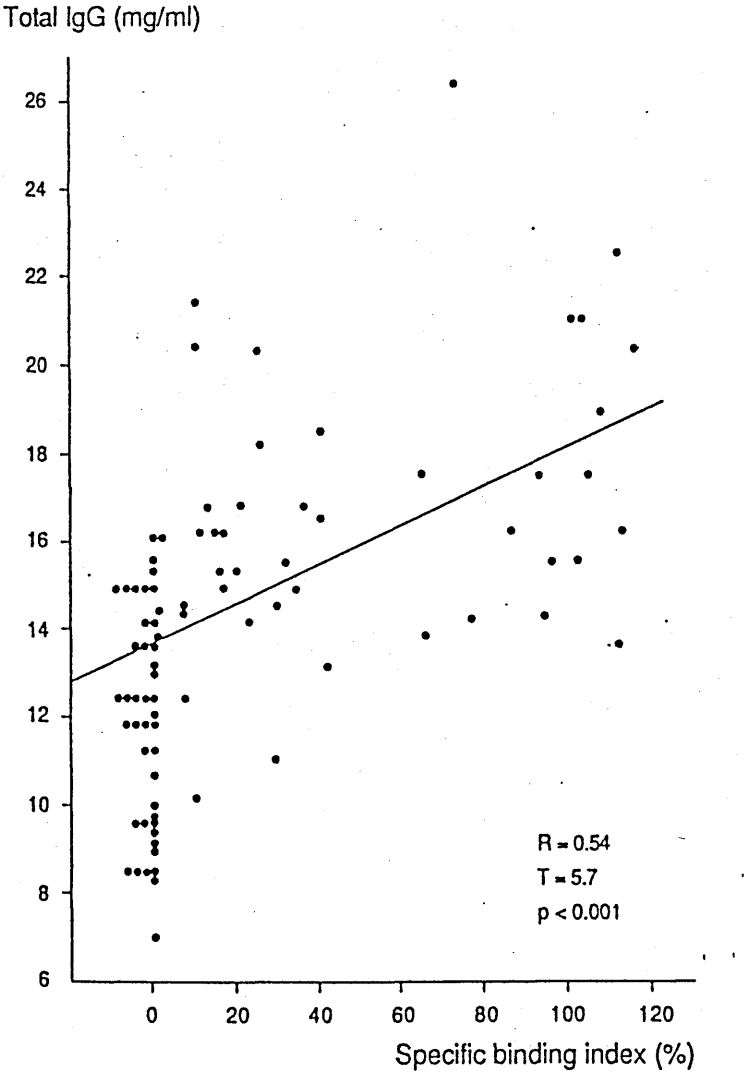

FIGURE 4.4

The relationship of total IgG and specific binding index. The correlation coefficient is shown alongside with the significance.

Table 4.3

Serological changes (means ± s.d.) in 26 workers after alteration of contaminated humidifier

	Pre	Post	T	P
IgG antibody	14.99 (25.14)	10.9 (22.4)	2.60	0.015
IgG1	4618 (3391)	3510 (3196)	1.44	0.170
IgG2	257 (297)	121 (98)	2.13	0.050
IgG3	1193 (1169)	993 (849)	0.96	0.680
IgG4	1036 (1303)	942 (919)	0.68	0.510
IgM antibody	11.2 (22.4)	8.55 (21.9)	1.50	0.140
IgA antibody	4.66 (19.7)	3.96 (19.6)	0.01	0.320
Total IgG	15.7	11.4	4.80	0.001

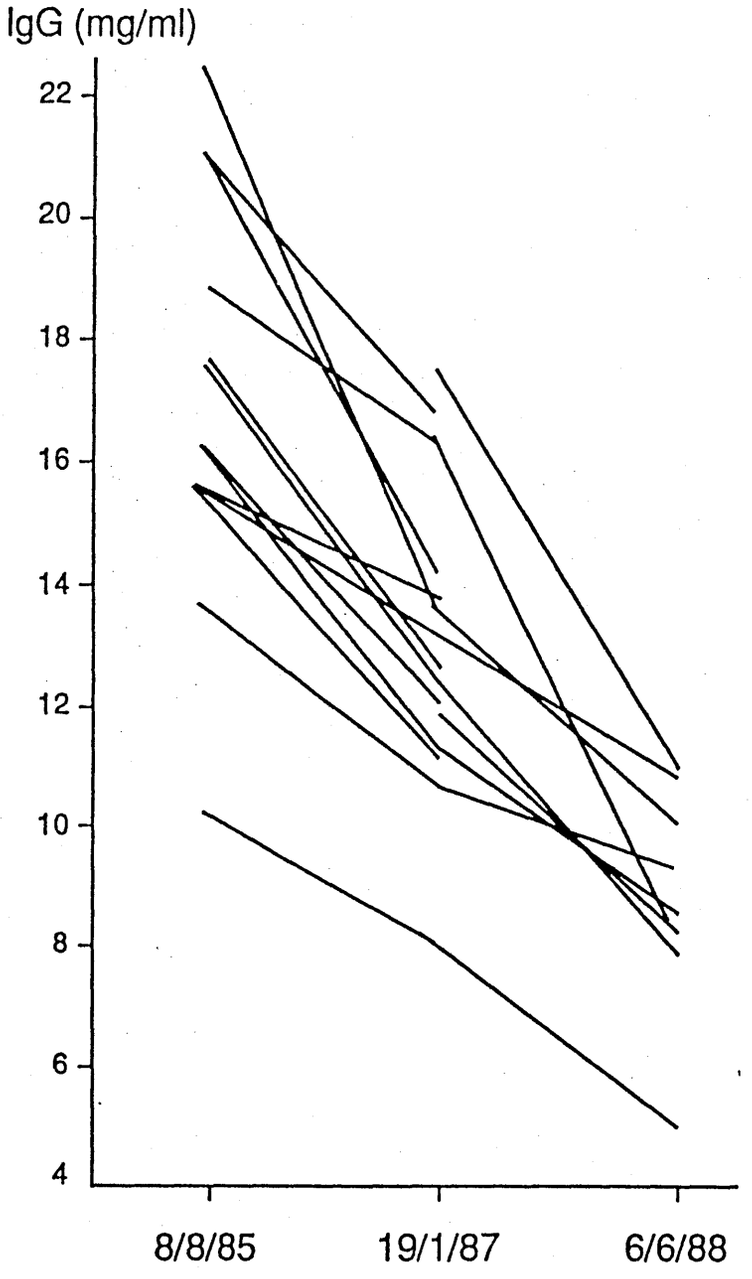

FIGURE 4.5

Total IgG before and after removal of the contaminated humidifiers in the printing factory.

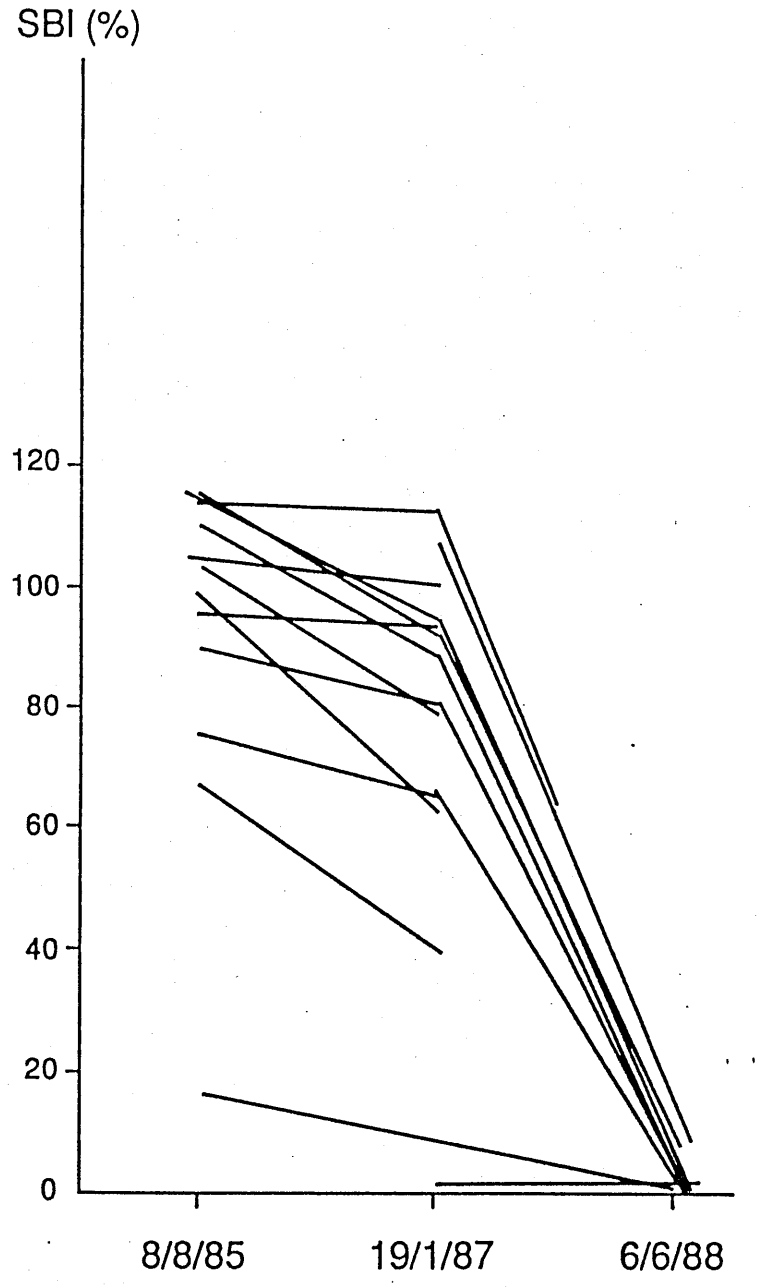

FIGURE 4.6

Specific IgG (SBI) before and after removal of the contaminated humidifiers in the printing factory.

The progressive fall of antibody titres provides an objective measure of an antigen removal in those who were symptomatic with elevated antibody. Symptomatic antibody negative subjects formed a major group (45.9%) for which there is no serological marker. The distribution of symptoms and the presence of precipitins is shown in figure 4.7 where there is a trend towards higher numbers of symptoms and the presence of precipitins but the relationship is not strong. Cigarette smoking and the effect on the antibody response is considered further in Chapter 6, but the negative relationship observed previously in similar conditions (162,197) was not as strong in this group. There is no obvious explanation for the lower antibody level in females.

The finding of the predominant IgG, sub-class component of total IgG, which also reduced most after antigen removal, can be compared with similar findings after removal of gluten from the coeliac diet (197a) but the explanation in the humidifier group is speculative. Immunoglobulin G subclasses have also been studied in farmer's lung (197b) where IgG_1 was elevated in symptomatic and control farmers, whereas IgG_3 was elevated only in symptomatic subjects. No such relationship was found between IgG_3 and the presence of symptoms in the humidifier group

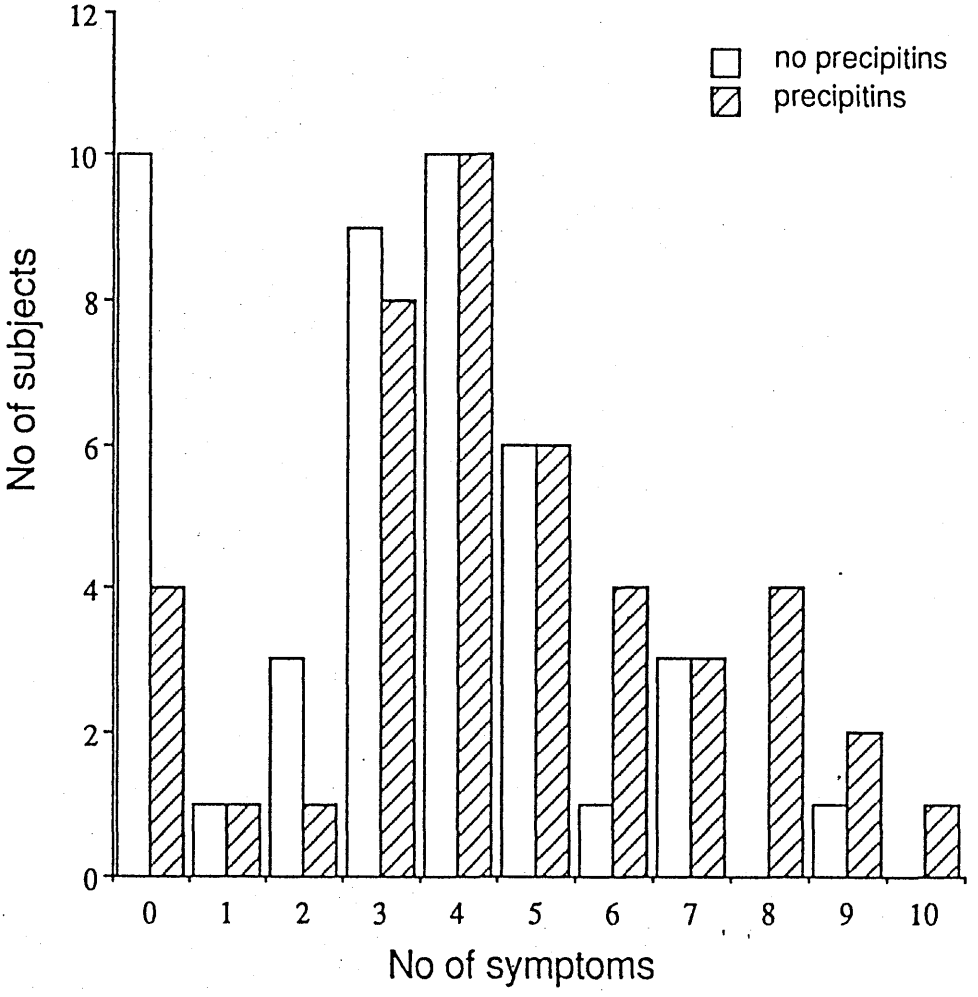

FIGURE 4.7

The sum of symptoms and presence of precipitins for the 88 subjects assessed in each outbreak of humidifier fever. The absence of precipitins is often accompanied by the presence of symptoms.

described above.

CHAPTER 5

PREVENTION AND RESOLUTION OF HUMIDIFIER-RELATED DISEASE

5.1 INTRODUCTION

One objective of this chapter is to demonstrate the difficulties of managing a disorder which depends on the interaction of several professional groups. Most of these individuals were not based within the buildings or companies directly affected by humidifier fever and, as could be predicted for professionals, with specific training, difficulties arose when new information appeared which was foreign to a specific group. The author's contact with each of these non-medical specialties was of major benefit when investigating each episode of humidifier fever and subsequent resolving procedures. As a consequence of this increasing experience the author became aware of a general lack of understanding among non-medical professionals (although not exclusively non-medical) of potentially important complications which could arise from air handling machinery and affect unsuspecting individuals.

5.2 Management and staff beliefs

Two factory outbreaks of humidifier-related disease

were described in Chapter 3.

In the microprocessor factory, neither the factory medical officers nor occupational nurse was aware of problems which could be air-conditioning related and appeared uncertain about the nature of the disorder which affected the staff. The staff were concerned most that the problem was not Legionnaires' disease - a similar question which was promptly raised by the plant engineer, management engineer, general management, and the air-conditioning company responsible for installation and advice concerning maintenance of the air-conditioning system. Each of these groups had previously concluded that either the illness was hysterical or a form of "sick-building syndrome".

Part of the difficulty within this factory was caused by poor communications between management, staff, and the medical officers. Each group had a tendency to filter information, confusion resulted and perhaps distrust. The affected subjects were reassured by the author with simple explanation and were content that the problem would be transient. The management possibly considered the economic implications of new air conditioning machinery more important than the author's medical advice to dismantle the contaminated humidifier after the initial

outbreak of illness since the air handler was retained and alterations made to increase water turnover within the system. This may be a further reason why the medical staff did not recognize the recurrence of sporadic symptoms within the factory as humidifier fever until the second major outbreak of symptoms because the medical staff had been wrongly informed that the contaminated air-handler would not operate again. After recurrent illness was finally recognized a plan was prepared to replace this cold water system with individual steam humidifiers positioned throughout the factory building. These humidifiers are now operating and symptoms within the staff have disappeared.

In the printing factory, management and staff worked more closely. The staff were aware of the development of symptoms in colleagues who were usually healthy. This factory population did not at any time consider hysteria as a cause of the symptoms. Concern again, by management and various representatives, was expressed about Legionnaires' disease. The workforce were acquainted with an illness known as "humidifier fever", because of previous episodes of the illness in printing factories elsewhere which were known to the union representatives. The humidifiers in the factory had been maintained according to the manufacturers instructions on a

regular basis without mishap until the factory general maintenance man was unable to perform his usual duties because of illness. During this phase, humidifier fever developed. An attempt was made to increase the frequency of cleaning in order to reduce contamination of the system but this was ineffective and eventually these humidifiers were removed. Mains-water atomizers were installed at a cost of £3,000 (in comparison with estimated installation costs of £12-15,000 for steam humidifiers which would also have higher running costs). The atomizers were purchased after consulting the author and subsequently the regional water authority for reassurance that the mains water did not present a further hazard if atomized. The machinery was purchased from a firm specialising in the supply of humidification machinery for printing (J.S. Industrial Services Ltd., Kent, U.K.) who also installed the equipment. The author was informed that the previous humidifiers were purchased from the manufacturer on the basis of very little background information in Cologne at a German Trade Fair and installed by a local plumber. The management freely admitted complete ignorance of medical consequences of contamination of the equipment, as well as a no more than a passing interest in air conditioning.

5.3 Air conditioning engineers are generally responsible for installation and servicing of air handling plant and humidifiers. The firm contracted to the microprocessors factory (Thermal Transfer Ltd, Unit 8, New Broompark, Edinburgh EH5 1RS) were keen to learn of developments in the factory and again were concerned that the problem was not Legionnellosis. After discussion, an information document was written by the author entitled "Humidifiers and disease, a guide for engineers". The objective of this was to increase awareness of the condition and thereby perform a health education and disease prevention role. The text is shown below. This was then circulated to businesses which utilised humidified air conditioning.

5.4 HUMIDIFIERS AND DISEASE, A GUIDE FOR ENGINEERS

Humidifier fever

In 1959 a flu-like illness was described in a pattern makers workshop which was caused by a contaminated humidifier, Since then a similar disorder has been described in printers, textile workers, microprocessor assemblers, stationery suppliers and a hospital operating theatre.

The illness, humidifier fever, is not caused by an infectious agent but arises as a response to inhalation of contaminated water droplets from a biologically rich humidifier system which usually incorporates cold water storage. The problem has probably become more common in the past decade as a result of the increase in air conditioning and sealed ventilation in buildings constructed since the 1960's and the energy crisis of the 1970's. Any type of contaminated cold water humidifier can be incriminated, for instance spinning disc, evaporative or spray.

The illness is usually identified in a group of workers concurrently who may complain of any of the following; headache, chills, sweating, muscle and joint aches, shortness of breath, cough and lethargy. Symptoms generally develop late in the working day or in the evening at home. Often the disorder presents on the first working day after a break and is one of the causes of 'Monday Fever', gradually receding as the working week progresses only to return again after the next break. This pattern of humidifier fever relies on the continuous use of a humidifier. If the humidifier operates intermittently this pattern may not develop and the symptoms may be less clearly work-related. For this

reason, the disorder has been more often identified as a winter illness when humidification is more continuous than in summer when the external air intake temperature is more often than not, able to carry enough moisture to maintain the desired humidity in the factory.

Blood from workers who have inhaled the contaminated droplets often contains serum antibodies which will react in the laboratory to extract from the humidifier system, e.g. water, sludge or baffle (eliminator) plate sludge. This reaction, however, only confirms exposure to humidifier contaminants and does not in itself indicate disease. There is evidence to suggest that humidifier fever can develop in subjects who do not have these antibodies, perhaps in response to a particularly high output from a contaminated humidifier which has been dormant for some time, however, symptomatic subjects generally have antibodies detected.

Humidifier asthma

This illness has a similar cause as humidifier fever, however work-related wheezing is the predominant symptom.

Humidifier lung or
extrinsic allergic alveolitis

This form of humidifier disease is rare in the U.K. and represents a form of inflammation within lung tissue (alveoli) which occurs as a result of the inhalation of organic material in certain individuals who seem susceptible to the disorder. Humidifier lung is the U.S. equivalent where the illness is caused by a fungus.

Each of the disorders described above is probably the result of an immune reaction within the lung with constitutional symptoms occurring as a consequence.

Treatment and prevention are closely linked. If the cause is removed the symptoms will settle spontaneously. There are no known long term complications which may either be because there are none or because of the relatively recent description of the disorder.

Awareness of the need for regular maintenance, the avoidance of recirculation of water within a system and the avoidance of humidification unless to satisfy the demands of an industrial process are the mainstays of prevention of humidifier disease. The addition of biological cleansers to the water

storage unit is not recommended because of the unknown effects which these agents have (if any) after inhalation, and should perhaps only be used when a building can be closed for a prolonged period.

Steam humidifiers may be installed as an alternative and so far have not been associated with medical problems but this is an expensive method of supplying moisture. Mains water sprays are an attractive compromise however, since mains water is not sterile there is a theoretical risk of spreading bacterial or viral infection.

Suspect humidifiers can be visually inspected and will probably show obvious evidence of contamination. A water or sludge sample can be compared with other samples from known outbreaks of humidifier disease and blood samples from the relevant workers. This type of disease monitoring is primarily an area of research which may ultimately clarify the cause of humidifier disease.

Kenneth Anderson
Department of Respiratory Medicine
Glasgow Royal Infirmary
August 1988

A list of references for this paper may be obtained from the author.

5.5 Validation of the effect of this document in industry may be gauged by the correspondence which developed with several companies who wished further information or assistance. The company name, position of the employee responsible for enquiring, town and product are shown in table 5.1.

Most of the companies in table 5.1 also requested analysis of humidifier water which was performed by Enzyme Inhibition Assay (EIA), (the technique is described in Appendix A3). The companies based in England were content with advice by telephone and, because of distance, were advised to contact the local environmental health officer for information concerning local legionella investigation. IBM utilised humidification only in areas where microcomputers were tested in microclimates in a sealed testing room while personnel were excluded. Water samples were sent by post from factories 2 and 5 and personally collected by the author from companies 1, 3, 4, 6, and 10. The type of humidifiers in use by the companies who allowed water analysis and results of EIA are shown in table 5.2.

Table 5.1 Companies who requested advice concerning humidification systems

COMPANY	POS. OF ENQUIRING EMPLOYEE	TOWN	PRODUCT
1. British Aerospace PLC	Safety Manager	Prestwick	Aircraft
2. Barrie Knitwear	Manager	Hawick	Knitwear
3. Castle Cement Ltd	Quality Control Supv.	Coatbridge	*Slag cement
4. British Petroleum (Grangemouth) Ltd	Occupational Hygienist	Grangemouth	Petrochemicals
5. Robertson of Dumfries Ltd (Head Office:Austin Reed)	Manager	Dumfries	Knitwear
6. Wm. Collins Ltd	Occupational Hygienist	Glasgow	Book binding/printing
7. Thermal Transfer (UK) Ltd	Manager	Manchester	Air conditioning installation
8. Phillips (UK) Ltd	Occupational Hygienist	Durham	Electronic equipment
9. IBM	Occupational Hygienist	Greenock	Electronic equipment
10. Media Services Ltd	Environmental Health Officer	Cumbernauld	Computerised Data storage

*Humidity at 3 levels of 45%, 55% and 65% was required in the laboratory testing area to comply with British Standard 4550, and not precisely for the the industrial process of slag cement manufacture.

Table 5.2 Humidifier water sample analysis

Factory	Humidifiers (number)	Water appearance	EIA
1	Evaporative (3)	clear	negative
2	Spinning disc (1)	clear	negative
3	Spinning disc (3)	cloudy grey + slime	negative
4	Steam (9)	three brown discoloured	one slight positive
5	Spinning disc (4)	clear	negative
6	Drip bank (3)	*yellow	negative
7	Steam (5)	clear	negative

*thought by engineer to represent an antimicrobial additive (see later).

All these samples were submitted for legionella analysis kindly performed by Dr R Fallon, Ruchill Hospital. No legionella species currently recognised was identified using a fluorescent antibody technique.

5.6 British Petroleum requested a more extensive investigation of various water storage units and facilities supplying water. A visit was arranged in February 1989 when the author and B.P. occupational hygienist visited the appropriate areas of the oil refinery accompanied by the site engineer for the area. The visit also provided an important opportunity to perform the EIA on water samples from sources other than humidifiers.

Steam humidifiers were utilised in computer control centres in different areas of the refinery either in duplicate or in triplicate with use of each unit planned on alternate weeks. Three of these units were corroded, one badly, and the water stained brown. A small reaction on EIA was detected from one of the contaminated units. The comment from the site engineer was that these corroded units had not been switched on for some time because of corrosion in the equipment. Since then these units have been replaced.

Contaminated steam humidifiers have never been incriminated as a cause of humidifier-related disease and the presumption is that since the water is boiled and steam released, then no organic material is released into the air (121). While

this assumption is probably reasonable for well maintained units, the detection of organic contamination within the steam humidifier above shows that steam humidifiers are a potential source of pathogenic material.

Water samples were obtained from 22 other sites in the refinery, which comprised 17 shower units, 1 roof mounted cooling tank, 1 main supply tank for humidifiers and 3 concrete cooling towers containing a large volume of contaminated recirculating water with a (2%) daily loss by evaporation of 3 million gallons. The cooling tower samples were taken from within the tower and outside from the surrounding moat. The water was discoloured brown by organic material which was adherent to the wooden framework within the tower. None of these samples were found to contain humidifier antigen by EIA.

5.7 Prevention of humidifier-related disease depends on the consideration of a number of factors.

5.7.1 The need for humidification must be confirmed. The system adopted should not recirculate, store water, or release droplets (121). Steam humidification is presently the optimum since steam production is assumed to inhibit organic

contamination but the expense of running these systems is higher than cold water systems (127). Water atomizers are available which do not incorporate water storage thereby reducing some of the risk of water droplets.

5.7.2 The addition of chemical biocides has been discouraged recently (123,121) partly because the effects of inhalation of these substances is unknown. Many substances are available for this purpose, most of which have effects as sensitizers (personal communication : B Backhouse, ICI Belgium). Some difficulty can be encountered when a particular chemical has had the original manufacturer's name altered for marketing by the wholesaler to the consumer who may then be unable to identify the substance supplied. "Vantocil IB", a polycationic compound is usually used as a swimming pool sanitizer and also occasionally as a humidifier disinfectant. In high concentration "Vantocil" causes skin irritation and sensitisation. "Proxyl AS", another sensitizer, and "Vantock", a quaternary ammonium commonly used as a fabric conditioner, are other products of this nature manufactured by ICI.

Panacide, a complex phenolic, which can be tolerated as oral medication for parasitic worm infestations in humans, is

produced by "BDH", Poole, Dorset (personal communication : Peter Short) in three variations; "D", "OA" for emulsions and "M" which is water miscible.

These chemical manufacturers appreciated that caution was important when adding the compounds to water which was of unknown rate of consumption and which might be added to water which had already been treated with another substance with which inter-reaction was possible.

A compound "Panacide B" was added to the microprocessor factory humidifier but use of the substance was terminated by the development of what management described as "people-orientated problems". Exposure to biocides should be minimalised by increasing air flow through the building and addition to the water only when the building is unpopulated.

5.7.3 Filtering intake air can remove dust, particles and organisms by using filters of varying characteristics according to the need for a clean environment and might remove the need for the use of biocides. High efficiency particulate air filters (HEPA filters) have a minimum particle removal efficiency of 99.97% for 0.3 micron particles and in theory would reduce

contamination of humidifiers (198). Jacobs et al (199) recently reported a case of recurrent extrinsic allergic alveolitis caused by contamination of a home air conditioning unit which was successfully prevented by the installation of an electrostatic dust filter. The area of antigen or microbial removal from recirculated or intake air would be worthy of further assessment but there are no reports in the medical literature of studies of this type.

5.7.4 Hygiene of a high standard within the building is desirable. The printing factory described in chapter 3 was heavily contaminated by vegetable starch which possibly provided a nutrient for organic proliferation. As part of preventative maintenance, the working environment should be clean. A regular system of direct maintenance on the humidifier should be organised perhaps more often during warmer months and redundant periods (e.g. holidays) when organic growth is encouraged and water consumption reduced. Air conditioning ducting is often neglected, which is because of design, difficult to clean. No current guidelines describe any mechanism for cleaning apart from dismantling.

5.7.5 Ultraviolet air disinfection (UV) has been reviewed

recently (200) but the effect on intake and output air from an air handler has been doubted by some experts (127). Despite this criticism UV light has been known for some time to effectively reduce airborne infection (201). The control of several infectious organisms including Legionella (201a) is improved by UV in the indoor environment, particularly in the medical setting, but since not all organisms are uniformly sensitive then UV cannot be considered a complete substitute for other methods of prevention of contamination.

CHAPTER 6

HUMIDIFIER FEVER AND RELATED DISORDERS

6.1 INTRODUCTION

In this final chapter the major findings of the thesis are discussed and compared with what has been reported previously in building-related disease, and conditions of presumed similar aetiology. The immunological observations can be compared with other forms of lung disease of various causes. Factors which influence the development of illness caused by humidifiers and other similar disorders, particularly cigarette smoking are mentioned with findings of studies within these groups of cigarette smoking workers. The chapter ends with suggestions for future studies in humidifier-related disease and improvements within the air conditioning industry.

6.2 The symptom pattern of humidifier fever is an important indicator of the presence of the disorder. None of the symptoms defined in our groups or those reported by others is specific and could easily be mistaken for respiratory illness of infections or other cause. Other disorders with a "Monday" pattern of illness-symptoms most marked on the first day back at work after

a break - include the feverish complaint which affects cotton workers some of whom have byssinosis (202) and workers exposed to metal fumes (203). Grain workers (204) can also experience similar symptoms which also occur, particularly in smokers, after exposure to polymer fumes (205). Lowell (206) highlighted the consistency of symptoms which occurred in subjects who developed disorders caused by the inhalation of antigenic dusts and suggested that each produced symptoms through a final common pathway which as yet still escapes description. Similar feverish symptoms are also a result of inhalation of endotoxin (a lipopolysaccharide found in the walls of Gram negative organisms and blue-green algae) which is thought by some to be the common link between humidifier fever (207) and fever in cotton workers (208). Endotoxin was detected in the humidifier water in the microprocessor factory described in chapter three (kindly performed by Dr. B. Pietrowitz, Glasgow and also by Prof. R. Rylander, Gothenburg, Sweden) at levels estimated between 62 ng/ml and 145 ng/ml but no airborne measurements were taken within the factories.

The timing of symptoms is clearly important in humidifier fever. Most reports describe symptoms which decrease in severity as the week progresses, although consistently

occurring 4-12 hours after exposure. Some authors (128,129) have previously commented that this pattern depends on exposure to humidifiers which operate continuously followed by removal of the subject from the source of antigen. The author's experience of the microprocessor factory and the printers was that symptoms were unrecognised and intermittent for a prolonged period before the disorder was noted, probably because of intermittent humidification in both factories.

The climate measurements presented in chapter two clearly show the importance of low nocturnal temperature and the relationship to the outbreaks of humidifier fever, not particularly on return to the workplace immediately after the holiday except when the temperature was low enough to require humidification.

The reason for the apparent "tolerance" of antigen inhalation after repeated exposure is not clear. Immunological tolerance is the description of the phenomenon which limits the lymphocytic response to inherent antigens (self) which occurs as a result of balanced immunological control mechanisms (209) and perhaps inhaled antigen may produce a similar sequence of events within lung when lymphokines and other cellular factors are

released as part of the lung defence process. Other work of interest in this area includes the observation that after serial antigen challenges sensitized animals may recover with a subsequent refractory period to further challenges for a period of months (210).

6.3 The antigens in humidifier water found in the printers and microprocessor factory were highly related, possibly reflecting the similar geographical sites. This similarity of antigen between outbreaks of humidifier fever from different sites has been noted by some and disputed by others. Edwards (211) found cross reaction with antigen from Cardiff, Sweden and later from the USA (195), whereas, Longbottom (212) suggested that the antigenic nature varied, thought in reflection of the organic contaminants present in the humidifier. The humidifier water in our outbreaks cross-reacted with others - but not all - from various sites in the U.K. (measured in the laboratory where Dr. Longbottom performed her analysis mentioned above). The explanation of cross reaction in some cases and not in others may be an indicator of growth conditions encouraging different organisms or a change in the water samples during transport or processing. There is no published material which shows that antigen/water from one outbreak has been used as an antigen

challenge in a subject affected by humidifier fever at a different site where there was or was not an antigenic link. No one organism has been consistently found in humidifiers which were associated with illness in exposed workers. No organism was cultured from the printing factory humidifier described in chapter three. In the microprocessor humidifier, during the first episode of illness, or environmental pseudomonad was found which grew at 24°C and not 37°C. Pre-absorption of a precipitin positive serum with this organism did not remove antibody activity against the antigen extracted from the sludge, suggesting that the organism was of doubtful significance. A water sample from the second microprocessor outbreak was found to contain Aureobasidium pullulans, (performed by the "Clyde River Purification Board", East Kilbride in a laboratory in close proximity to the microprocessor factory; cultures in Glasgow were negative) an organism incriminated in a previous case report of allergic alveolitis in the USA (154) and a suspected cause of sequoiosis (213) and sauna-takers disease (214). Once again, no antigenic activity was found to be this organism after pre-absorption of precipitin positive serum.

Unlike the antigens responsible for farmer's lung (215) and pigeon breeders disease (216), there is no published work

which has studied antigen specific for humidifier fever or associated allergic alveolitis.

6.4 Immunological studies in humidifier fever were discussed in Chapter 4. As has been noted previously (166) the presence of precipitins alone is not diagnostic of humidifier fever or allergic alveolitis of any cause (217). Our own study (and others) shows that antibody measurement in humidifier fever adds to the clinical recognition of the disorder and also clarification of the symptoms which are most specific to those subjects who also have immunological disturbance. Those subjects who were most symptomatic had evidence of more immunological upset, a feature which has been recognised in other forms of extrinsic allergic alveolitis (218,219). Following the removal of the source of the antigen in the printing factory, a marked reduction was noted of the circulating specific and total IgG. This fall in immunoglobulin was less marked in the microprocessor factory where this was presumably caused by the retention of the contaminated humidifier. Antibody measurement, therefore, offers an objective measurement confirming the removal of the source of antigen. Total IgG measurement also provides an early, inexpensive, although less specific, guide to the presence of an immunological disturbance when a more elaborate

enzyme-linked assay is not available. Pyrexial illness in a worker exposed to humidified air may be assumed to be not uncommon, but humidifier fever is unlikely to be the cause of most of these events. As a first step in investigation, a serum IgG might offer a more reasonable approach than the time and expense of sampling various parts of the humidifier and then organising either a gel-diffusion test for precipitins or more elaborate search for specific antibody.

Immunological alterations in serology are also found in other disorders which are not usually classified as forms of extrinsic allergic alveolitis. The author recently studied a group of workers with prawn-related occupational asthma who had serum precipitins but no clinical evidence of alveolitis (220,in press). The fundamental question of the relevance of circulating antibody and the pathogenesis of extrinsic allergic alveolitis remains unanswered (221), however, IgG antibody may be useful as a clinical adjunct.

6.5 Cigarette smoking is known to be a factor which variously complicates occupational and environmental lung diseases of many causes (222,188,223), and is accepted as one (if not "the") major public health issues of the industrialised world

(224). Smoke from cigarettes forms a major component of indoor respirable particulate (225,17) which is the acknowledged cause of lung diseases in passive smokers (226).

Immunological mechanisms, especially in lung, are also influenced by smoking (227) where the suppressive effect of smoking was observed in individuals susceptible to extrinsic allergic alveolitis (190). Similarly, circulating IgG specific for pigeon antigen was present in 55% of non-smoking and 4% of smoking pigeon fanciers (189). This effect of smoking has also been noted in outbreaks of humidifier fever. In the investigation reported by Cockcroft et al (162) significantly fewer smokers developed antibodies than non and ex-smokers. A relationship was not found for the development of symptoms which were "probably" humidifier-related according to their own criteria. Another group (135) noted that all seven workers with humidifier fever were non-smokers, but no further analysis of smoking and serology was presented. Smoking habit was found to significantly influence total IgG and the presence of precipitins and symptoms during the first outbreak of humidifier fever in the microprocessor factory (138) reported in Chapter 3. In that group all the workers with symptoms strongly suggesting humidifier fever were non-smokers and none of the smokers

investigated had precipitins to the humidifier sludge. The mean serum IgG was 12.1 (±2.8) for the eight smokers and 16.8 (±6.8) for fourteen non smokers. This analysis was repeated for the 88 subjects from each of the four episodes of humidifier fever reported in chapter three and although non-smokers appeared to have more symptoms, the differences for the presence of symptoms or antibody was less marked. One possible explanation of this phenomenon is misclassification of smoking history, which may be particularly relevant since during the second microprocessor factory episode, the factory management were introducing a no-smoking policy within the building. Non-smoking was expected during work hours in the printers because of the nature of the work but smoking was allowed in rest areas away from printing and machinery. The effect of these manoeuvres is to considerably reduce the time available for a smoker to smoke and although the worker would continue to consider himself a smoker, biologically he might behave as a non-smoker. A non-smoking policy might also influence the accuracy of a smoking history (227a) which is often questioned (228,229), usually with the assumption of some individuals not stating correctly that they are smokers and not, as also seemed possible in our factory groups of over-estimating cigarette consumption.

One method of confirming cigarette smoking is by measuring metabolites of the smoke either in breath, serum, urine or saliva (230). The author reported a study of this type in a group of volunteer pigeon fanciers (193), who as a population also tend to restrict cigarette smoking - at least while in contact with the pigeons (P. Lynch - personal communication). In this study, 86 volunteers had a smoking history taken and also donated serum for measurement of circulating specific IgG by enzyme-linked immunosorbant assay with pigeon gamma-globulin as antigen. End-expired carbon monoxide was then determined with a hand-held meter (ECO-check, PK Morgan Ltd., England). The results are shown in figure 6.1. Of the 86 subjects studied, 67 were non-smokers. Antibody titres were significantly higher in non-smokers than smokers using Kruskal-Wallis one-way analysis of variance ($H=7.3$, $P=0.007$) and in subjects with end tidal carbon monoxide concentrations of less than 10 parts per million (ppm) than in those with concentrations of 10 ppm or more ($H=8.2$, $P=0.004$). The data suggests that 10 ppm reasonably divides the smokers from the non-smokers. On this basis 4 smokers (out of nineteen) would have been misclassified as non-smokers and one non-smoker (at 11 ppm) as a smoker. In most cases (66/67) the non-smoking history appeared accurate. This study is believed to be the first study which reports a measured smoking marker

where smoking is thought to interfere with the immune response.

As an extension of this work in the area of smoking histories and markers of cigarette exposure, and while investigating the factories affected by humidifier fever the author noted that few studies of smoking and smoking markers had been performed in specific groups susceptible to environmental lung disease where smoking is implicated in the aetiology (231). The studies of accuracy of habit reporting were not conducted in such groups although Robertson and colleagues (232) have assessed a group of office workers in this way. For these reasons a study using serum thiocyanate, a marker with a more prolonged half life than others (233) which has another advantage of an assay which has readily available materials. The method is shown in the appendix (A2). Serum was gathered from several groups, 280 pigeon fanciers who volunteered for assessment at a national pigeon show; 136 consecutive asbestos-exposed workers who were referred for assessment to the Glasgow Royal Infirmary, Dept. of Respiratory Medicine and interviewed personally by the author; 60 prawn processors, assessed in a factory survey; 80 factory workers exposed to the output of contaminated humidifiers. Likely non-smokers were defined as those with thiocyanate (SCN) less than 70 umol/l, likely smokers > 100

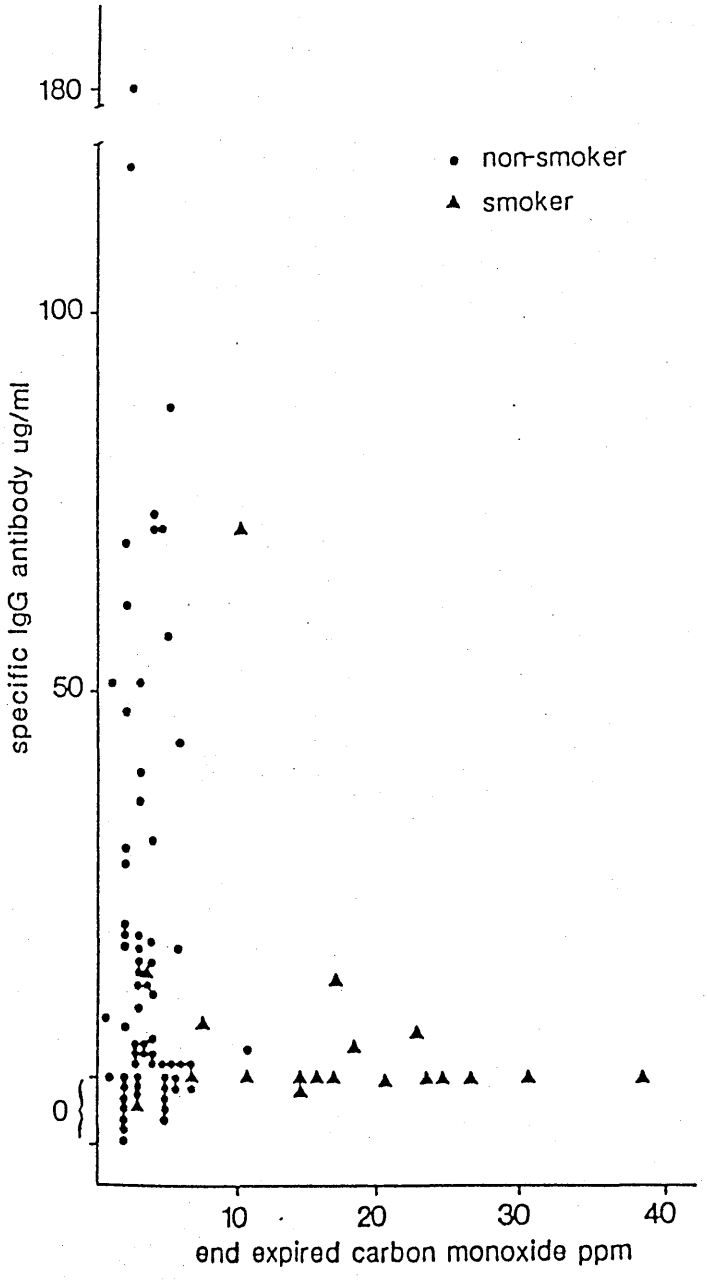

FIGURE 6.1

End expired carbon monoxide and pigeon antibody. 86 measurements from 67 non-smokers by history (•) and 19 smokers by history (▲).

umol/l with subjects 70-100 umol/l,accepted as difficult to classify. The distributions of smoking history and SCN are shown in figure 6.2. The totals for the groups are in table 6.1. 399 histories (82.7%) appeared accurately given within the ranges of <70 and >100 while 69 were doubtful within these ranges. 39 (11.8%) of the 330 subjects <70 umol/l were smokers by history. The given history in these subjects was more accurate than expected and therefore supports observations in similar subjects made previously in the absence of a biochemical smoking marker. The group of smokers with SCN levels in the non-smoking range is presumably a reflection of the pattern of inhalation. Whether or not these smokers would behave as "non-smokers" in response to the inhalation of potentially pathogenic substances is not known. This group possibly reduces the apparent effect of smoking and perhaps should be considered in the analysis of studies incriminating an effect from cigarettes. As a subgroup, the humidifier subjects appear to have given the least accurate smoking histories - both overestimating and underestimating the amount smoked. Air recirculation within the microprocessor factory is a possible, although improbable, explanation of a trend towards higher thiocyanate levels in the non-smokers in the mid-range but this is even less likely in the non-smokers of the higher range, where the smoking history must

Table 6.1 Serum thiocyanate and given smoking history for 548 subjects in four selected groups

Selected Group	Numbers of Subjects					
	<70 umol/l		70-100 umol/l		>100 umol/l	
	Smokers	Non-Smokers	Smokers	Non-Smokers	Smokers	Non-Smokers
Prawn processors	3	23	6	0	20	0
Pigeon fanciers	11	196	9	18	39	7
Asbestos workers	14	56	12	6	42	6
Humidifier exposed workers	11	16	6	23	7	17
TOTALS	39	291	33	47	108	30

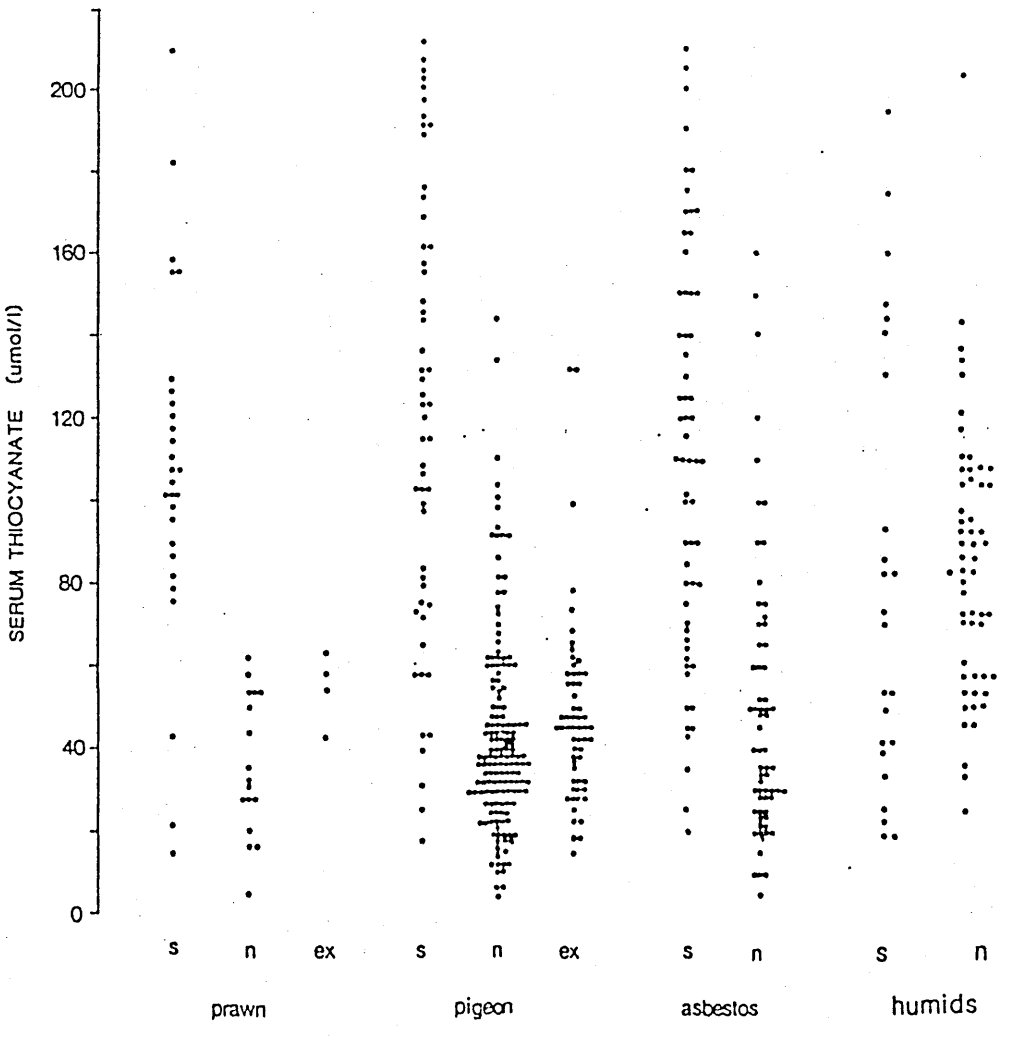

FIGURE 6.2

Serum thiocyanate and smoking history for 548 subjects in four selected groups. (prawn = prawn processors; pigeon = pigeon fanciers; asbestos = asbestos workers; humids = humidifier exposed workers from factories described in Chapter 3). The least consistent histories and thiocyanates were obtained in the humidifier group. The presence of smokers in the non-smoking range is discussed further in the text.

be considered unreliable. When the humidifier group are removed from the group analysis the accuracy of the history increases to 90% within the low and high ranges further suggesting that the humidifier histories as less accurate than the other groups.

The complexities of antigen exposure and cigarette smoking in the microprocessor and printing factories may have, through the mechanisms discussed above, produced an antibody and symptomatic response in cigarette smokers. The absence of a relationship between smoking and the development of antibody to contaminated humidifiers has also been noted by others (137) who suggested that airborne antigen exposure was the crucial factor influencing the precipitin response in the smokers. Similar findings were reported by Cormier et al (233a) after antigen challenge in animals where short duration cigarette exposure did not prevent the development of precipitins after exposure to Micropolyspora faeni. Our work suggests that the pattern of cigarette smoking and the given smoking history is also important.

The precise mechanism involved which is influenced by cigarette smoking is not known. As stated in chapter one, the pulmonary alveolar macrophage is exquisitely sensitive to inhaled

material and cigarette smoke. Antigen presentation to T-lymphocytes is impaired in macrophages from smokers (234) and, since air flow obstruction is often present in smokers, the assumption that less antigen is inhaled also seems reasonable.

6.6 The control of humidifier-related disease was discussed in chapter five. One further observation of note was the relative lack of training in staff who were in executive control of the humidifier machinery. Only two company representatives (Factories 3 and 8, table 5.1) were aware of the disease known as humidifier fever. Some of these employees have professional training but most (and probably the vast majority in the country who are responsible for humidification equipment) will have no knowledge of the subject. These companies would benefit by the collation of a humidifier register by a central agency which would then be responsible for dispensing information. Such input is available through the Health and Safety Commission who, in collaboration with the Printing Industry Advisory Committee, have produced a leaflet about humidifier fever, however a national "code of conduct" for air conditioning machinery might improve on this without a need for government legislation.

6.7 Further studies in humidifier-related diseases

6.7.1 The source of contamination of the machinery is unclear. A series of humidifiers might be studied in isolation, away from a factory workforce and the water content monitored by microbiological and immunological methods.

6.7.2 Blotting techniques have not been applied to characterise the antigen from any of the reported U.K. outbreaks of disease.

6.7.3 The effect of steam humidification on indoor microbiology and health of workers has not been studied.

6.7.4 Longitudinal studies of the health of workers who have been exposed to contaminated humidifiers have not been performed. There are also no long term studies of the effect of air conditioning on respiratory health.

6.7.5 Broncho-alveolar lavage (Chapter 1) has been performed in a few cases of humidifier-related extrinsic allergic alveolitis (145) but no studies have been reported in humidifier fever. Such studies would be most interesting after antigen

challenge in antibody positive asymptomatic and antibody negative asymptomatic subjects, and might produce some explanation of the basis of susceptibility. Lavage studies might also examine whether or not humidifier fever and humidifier extrinsic allergic alveolitis are similar or unrelated diseases.

6.8 Humidifier-related disease from current evidence is of three types:-

1. Humidifier fever is a pyrexial illness in most subjects, although other symptoms are not uncommon. The Monday pattern of symptoms only occurs with rigid working practices and continuous humidification - both of which are relatively uncommon. Intermittent symptoms should be expected which will probably follow outdoor temperature variation, which may be a particular feature of symptoms in summer. The disease is probably less common in smokers, although not exclusive to non-smokers. Serum precipitins are often found to extracts from the humidifier but antibody need not be present for symptoms to be caused by a humidifier in a similar fashion to other causes of organic dust toxicity.

2. Humidifier-related extrinsic allergic alveolitis is rare. Symptoms are more persistent than in humidifier fever and

may be accompanied by more marked shortness of breath, weight loss and prostrating illness. Crackles may be heard on chest auscultation. The chest radiograph may be abnormal with bilateral pulmonary infiltration. Pulmonary function tests may indicate a restrictive ventilatory defect with impairment of gas transfer.

3. Humidifier-related asthma is also likely to be rare. Serial peak flow recording and antigen challenge may show typical features of the disease.

6.9 The complexities of humidifier fever:

The difficulties of investigating each factory were discussed in chapter five. Fingret (235) recently reported the problems encountered during an outbreak of illness which was of major importance to individuals working and near one building. Such reports are rare. Our own experience was that professional groups were mainly helpful, conveying a sense of common interest in an avoidable health hazard which affects the working environment - not a specific occupational hazard but one which is found in many factories, offices, homes and transport. The microprocessor factory management were keen to institute a health promotion scheme, following the general trend elsewhere (236),

but, paradoxically, seemed reticent to follow advice which would resolve a more acute and debilitating pathology in the building.

Despite these difficulties a large number of symptomatic subjects were assessed, and an unexpected number of subjects with seronegative disease were found. Others (237) have discussed a possible relationship between pulmonary mycotoxicosis and the development of extrinsic allergic alveolitis which may also be a relevant association where the source of antigen is a contaminated humidifier.

This thesis would not have been possible without developing productive associations with architects, air conditioning engineers, factory management, government health specialists (EMAS), biochemists, scientists, occupational hygienists, environmental health officers, immunologists, meteorologists, bacteriologists, union representatives, medical and nursing personnel and other individuals including technical representatives of companies who freely provided information and assistance. This co-operation reflects the interest in the subject in the U.K. and Europe.

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APPENDIX

A1. Statistical Analysis

The data were analysed throughout with MINITAB (Pennsylvania State University) and SPSS (SPSS Inc. Chicago Il. USA).

The homogeneity of distribution of the groups was studied between asymptomatic and symptomatic factories, with regard to gender, age, duration of service, smoking habit, shifts worked and antibody. The method used was a oneway analysis of variance which was unlikely to produce false rejections of the null hypothesis (i.e. Ho = uniformity of distribution), despite a possible criticism of validity in view of the categorical nature of some of the variables and non-normal distribution of others.

A1.1 Distribution of patient characteristics in symptomatic and asymptomatic groups

	Asymptomatic			Symptomatic			p value	
	Number	Mean	SD	Number	Mean	SD		
Gender	14	0.57	0.51	74	0.41	0.49	0.298	n.s.
Age	14	40.00	10.58	73	38.30	10.22	0.573	n.s.
Service (Exposure)	14	5.36	4.65	71	5.85	3.80	0.674	n.s.
Shift	14	0.42	0.75	74	0.45	0.72	0.885	n.s.
Cigarette smoking	14	0.64	0.74	74	0.40	0.63	0.218	n.s.
Specific IgG (SBI%)	14	8.50	23.60	74	32.00	41.10	0.041	sig
Total IgE	14	30.00	31.10	74	98.50	279.50	0.366	n.s.
Total IgG	14	12.20	2.50	74	15.05	4.66	0.031	sig

A1.2 Distribution of patient characteristics in the two factories

	Microprocessors			Printers			p value	
	No. of samples	Mean	SD	No. of samples	Mean	SD		
Gender	66	0.32	0.47	22	0.82	0.39	<0.000	sig
Age	66	37.10	9.10	21	43.00	12.30	0.017	sig
Service (exposure)	63	5.00	2.95	22	7.95	5.43	0.002	sig
Shift	66	0.60	0.78	22	0.00	0.00	<0.000	sig
Cigarette smoking	66	0.45	0.68	22	0.40	0.59	0.780	n.s.
Specific IgG (SBI%)	66	13.33	22.97	22	73.18	45.60	<0.000	sig
Total IgE	66	94.80	285.80	22	65.90	145.50	0.650	n.s.
Total IgG	66	14.22	4.69	22	15.76	3.71	0.160	n.s.

A1.3 Distribution of patient characteristics between genders

	Female			Male			p value	
	No. of samples	Mean	SD	No. of samples	Mean	SD		
Age	49	39.30	9.91	38	37.50	10.70	0.415	n.s.
Service (exposure)	46	5.87	3.37	39	5.64	4.53	0.791	n.s.
Shift	49	0.65	0.75	39	0.20	0.61	0.003	sig
Cigarette smoking	49	0.47	0.71	39	0.41	0.59	0.678	n.s.
Specific IgG (SBI%)	49	18.20	29.70	39	40.90	46.90	0.007	sig
Total IgE	49	100.50	325.80	39	71.40	131.40	0.601	n.s.
Total IgG	49	14.05	3.81	39	15.31	5.19	0.194	n.s.

A1.4 The age, duration of service, smoking habit, and antibody measurements were next subjected to analysis by Pearson's-product-moment-correlation. Similar cautions already mentioned with regard to analysis of variance apply here also.

A1.5 Correlation of Variables

Variable	No. of Sample	Mean	SD
Age	84	38.59	10.30
Service	84	5.78	3.94
Cigarettes	84	0.45	0.66
Specific IgG	84	28.70	39.60
Total IgE	84	88.79	263.39
Total IgG	84	14.75	4.52

Correlations	Age	Service	Cigarettes	Sp.IgG	T.IgE	T.IgG
Age	1.00	0.5700	0.110	0.310	0.06	0.120
		p<0.0001	p=0.315	p=0.004	p=0.58	p=0.27
Service		1.0000	0.080	0.340	-0.01	0.170
			p=0.427	p=0.001	p=0.91	p=0.12
Cigarettes			1.000	-0.12	0.050	-0.18
				p=0.260	p=0.59	p=0.10
Specific IgG				1.000	-0.10	0.570
					p=0.32	p<0.001
Total IgE					1.000	-0.12
						p=0.27
Total IgG						1.000

A1.6 Discriminant analyses were performed using normal or abnormal antibody level as classes and the categorical variables as discriminating variables. This method is a reversal of the normal procedure where continuous variables are used as discriminating variables for categorical classes.

A1.7

Groups defined :	1.	Specific Binding Index	(0,>0)
	2.	Total IgE	(<80,>79)
	3.	Total IgG	(<15,>15)

85 Subjects analysed (3 missing data on service)

A1.8 Canonical Discriminant Functions

	Specific Binding Index	T.IgE	T.IgG
Function	1*	1*	1*
Eigenvalue	0.6817	0.1851	0.4418
% of variance	100.0000	100.0000	100.0000
Cumulative %	100.0000	100.0000	100.0000
Canonical correlation	0.6370	0.3952	0.5535
After function	0.0000	0.0000	0.0000
Wilks' Lambda	0.5946	0.8438	0.6936
Chi-squared	39.2470	12.8230	27.6230
D.Freedom	15.0000	15.0000	15.0000
Significance	0.0006	0.616	0.0241

A1.9 Standardized Canonical Discriminant Function Coefficients

	Specific Binding Index	Total IgE	Total IgG
Service	0.64644	0.37284	0.54575
Shifts	0.31026	- 0.19675	0.06756
Cigarettes	- 0.41464	0.10104	- 0.27502
Chest Tightness	0.16321	0.92970	- 0.29449
Headache	- 0.06027	0.33382	0.06716
Chills	0.12357	- 0.51337	0.46775
Breathlessness	0.11351	- 0.08318	0.49273
Cough	- 0.06192	- 0.43685	0.53773
Wheeze	- 0.21094	0.52566	- 0.18355
Appetite Loss	0.09358	- 0.73556	- 0.09887
Weight Loss	- 0.24629	0.77363	0.12174
Fever	0.42417	0.42576	- 0.01054
Flu	0.21177	- 0.36042	- 0.01298
Tiredness	0.07925	0.28430	- 0.09475
Other	- 0.35365	0.12387	- 0.19017

A1.10 Structure matrix: Pooled-within-groups correlation between discriminating variables and canonical discriminant functions (variables ordered by size of correlation within function).

A1.10.1 Specific Binding Index Group

Service	0.55825
Fever	0.52468
Chills	0.45705
Flu	0.35711
Other	- 0.35191
Headache	0.30288
Cigarettes	- 0.26738
Chest Tightness	0.19136
Wheeze	- 0.13787
Shift	- 0.12087
Breathlessness	0.11113
Appetite Loss	0.09758
Weight Loss	- 0.09342
Cough	0.08487
Tiredness	0.06426

A1.10.2 Total IgE Group

Weight Loss	0.31771
Appetite Loss	- 0.31116
Wheeze	0.27793
Service	0.24806
Cough	- 0.24240
Cigarettes	0.19650
Chills	- 0.18890
Flu	- 0.17370
Breathlessness	0.16971
Chest Tightness	0.13812
Other	0.13740
Shift	- 0.07423
Tiredness	0.07423
Fever	- 0.06773
Headache	- 0.06598

A1.10.3 Total IgG Group

Service	0.54917
Chills	0.50425
Other	- 0.40791
Cough	0.39659
Fever	0.35854
Breathlessness	0.35378
Headache	0.34682
Flu	0.27595
Shift	- 0.19532
Cigarettes	- 0.14970
Chest Tightness	0.12659
Appetite Loss	0.10355
Weight Loss	0.07977
Tiredness	0.05428
Wheeze	0.00918

Comment: Significant discriminant functions were obtained for the IgG measurements, but not for the IgE measurements.

A2 SERUM THIOCYANATE (AUTOMATED)

Principle

Thiocyanate reacts with ferric nitrate to give ferric thiocyanate which gives a golden brown colour measurable at 500 nm. Centrifugal analysis is preferred to enhance accuracy and precision.

Reagents

Precipitating reagent - 10% trichloracetic acid (TCA)

Colour reagent - Ferric Nitrate (120g Ferric nitrate to 250 ml with 2 M nitric acid).

Calibrants

25-500 umol/l aqueous, routinely calibration is performed using the 500 umol/l standard only.

QC

Low QC is undiluted bovine serum albumin

High QC is 1 in 2 dilution of BSA with 500 umol/l standard

Target ranges

LO	HI
n50	50+100

Method

To 200 ul of calibrant, QC or sample in a microfuge tube, add

200 ul of 10% TCA, cap and vortex thoroughly. Centrifuge in a microfuge for 6 minutes. Analyse 200 ul of supernatant using the Centrifichem System as follows:-

1. Switch on power selecting temperature 25°C, wavelength 500 nm. Allow 10 minutes for machine to warm up.

2. Perform 2 CLEAR cycles to ensure rotor is clean.

3. Store water blank - i.e. according to the included example sheet set up the Centrifichem analyser and run a sample ring containing water in every reagent well. After obtaining a print-out of results change AUTO to HOLD. ABS to CONC and conc factor to 500 as directed on example sheet.

4. Perform 1 CLEAR cycle before removing sample ring and drying analyser.

5. Load up sample ring with the following:

   Sample Well

Position 0	200 ul water
Position 1	200 ul water
Position 2 & 3	200 ul, 500 umol/l standard
Position 4	200 ul "low QC"
Position 5-28	200 ul test sample supernatant
Position 29	200 ul "high QC"

Fill any empty spaces with water.

A3. Enzyme Immunoassay (EIA)

The enzyme immunoassay (EIA) was an indirect sandwich technique (238) where the antigen dissolved in sodium carbonate buffer (0.IM, pH 9.6) was coated overnight at 100 nl per well in irradiated polystyrene microelisa plates (M129B, Dynatech, Virginia, USA). The plates were then washed thrice in wash buffer (PBS pH 7.2 containing 0.05% Tween-20). Test, standard and internal-control sera were diluted in wash buffer and incubated in duplicate in the wells at 100 nl/well for 2 hours at room temperature. The plates were then washed as above before adding 100 nl of an alkaline phosphatase conjugated monoclonal antihuman IgG, IgM or IgA (ICN Immunobiologicals, Lisle, IL, USA) diluted I:1000 with wash buffer. After a further 2 hours incubation the plate was again washed before adding 50 ul/well of 1 mg/ml alkaline phosphatase substrate (p-nitrophenyl phosphate, Sigma) in 10% diethanolamine pH 9.8. After approximately 15 min or when sufficient colour had developed in positive control sera (i.e. optical density, E405 nm>1.00) the reaction was halted by adding 50 9nl of 3M sodium hydroxide. The optical density; E405 nm of each well was recorded on a spectrophotometer

(Multiskan, Flow Lab. Irvine, Scotland). The results were expressed as a percent specific binding index (SBI) which was calculated as follows:

$$SBI = \frac{OD_x - OD_{LC}}{OD_{HC} - OD_{LC}} \times 100$$

where OD_{HC} = OD of positive control

OD_{LC} = mean OD of negative controls + 2SD

OD_x = OD of unknown sample.

The positive control serum was chosen from a strongly precipitin positive serum and was designated an arbitrary value of 100%. The negative control value was the mean value plus 2 standard deviations of the readings for sera from healthy unexposed subjects (usually 8 per plate). The between plate variability (coefficient of variation) for 10 tests was 15.8%, and the within-plate variability was 9.6% for a medium titre serum.

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