Microbial and immunological investigations and remedial action after an outbreak of humidifier fever

J H EDWARDS

From the Medical Research Council Pneumoconiosis Unit, Llandough Hospital, Penarth, S Glam, UK

ABSTRACT Humidifier fever (Monday sickness) occurring in office staff in a factory processing rayon presented as pyrexia with a polyuria and leucocytosis on the first day back to work after a break during the winter half of the year. Chest radiographs showed no abnormalities but pulmonary function tests indicated mild airways obstruction in the affected group as a whole. Respirable dust samples taken on a Monday when 11 cases occurred were not pyrogenic, indicating that a mechanism other than direct pyrogen activity produced the pyrexia. Efforts were then directed to determining an immunological basis for the episodes. In particular, *Thermoactinomyces vulgaris*, previously held responsible for humidifier fever, was studied. During the episode of 11 cases, the number of viable airborne spores of this organism was far higher than on Mondays when no cases occurred. In a second episode of nine cases, however, the airborne viable count was of the same order as non-episode Mondays.

Extracts of *T vulgaris* produced lines of precipitation in gel diffusion studies with roughly half the office staff sera tested, but no correlation was observed between precipitin line formation and disease. A similar proportion of normal sera reacted against this extract. Extracts of dust lying on the topside surface of the suspended ceiling above the office, however, produced precipitin lines with sera from 16/18 affected individuals and 2/18 non-affected individuals ($p < 0.001$) as did extracts of humidifier material.

Extensive microbial analysis failed to detect any one fungus or bacterium that produced antigens capable of reacting with positive serum, but extracts of amoebae correlated absolutely with humidifier material and ceiling dust extract in gel diffusion studies. A reaction of identity observed between the amoebae and ceiling dust extracts showed the presence of identical antigens. In similar studies the high degree of cross reactivity with antigens and sera from Spanish and Swedish outbreaks was obtained, which suggested a common antigen source in humidifier fever.

That these antigens were produced by microbial development on rayon fibre could be shown by incubating rayon dust from the factory atmosphere with sterile water and testing with sera from affected individuals. Bales of rayon entering the factory did not have this potential to develop antigens, indicating microbial contamination after handling and processing. The initial source of contamination was considered to be the humidifier disseminating microbial spores and cysts throughout the factory and on to the suspended ceiling above the office. These were capable of secondary development on settled rayon fly under wet conditions, and evidence for this was obtained. Remedial action included cleaning the humidifier, modifying the baffle plates, running water to waste, and installing a prefilter. Dust was eliminated from the office area, and new accommodation, including the building of an office block detached from the main factory, was arranged for the office workers. So far no further cases have been reported.

In environments where people are present for any length of time temperature and humidity control is

necessary to maintain comfortable working conditions. Similarly, certain work processes require fairly strict control of these variables, and this is achieved in several ways. In one of the more common methods¹ air is drawn in from the factory en-

Received November 1978
Accepted 29 March 1979

vironment and mixed with a variable proportion of external air. This is warmed and passed through a humidifier chamber where fine droplets of water are sprayed into the moving air current. Larger droplets are removed by baffle plates that cause turbulence and particle deposition. The temperature-controlled, humidified air is then distributed.

Normally these systems present few health problems except when infective or allergenic materials find their way into the humidification system. This can occur when organic material present either in the air taken into the humidifier, or in the water used for humidification, provides nutrient for air- or water-borne microbes. Biomass build-up occurs in the humidifier chamber itself or on the baffle plates due to air/water-borne particle deposition and may be propagated if the water that has not evaporated in the humidifier is recirculated. One of the first indications of an allergic response due to humidification was provided by Pestalozzi in 1959² and since then numerous reports have appeared.^{3,4} Although, as might be expected from the open nature of such systems, microbial analysis has shown the presence of many species of organisms in humidifier water,^{1,5} by analogy with other hypersensitivity lung diseases one might expect one organism to be the predominant sensitiser. In 1970 Banaszak *et al*³ reported that *Thermoactinomyces vulgaris* was the organism responsible for antigenic stimulus in humidifier fever since the organism had been isolated from an air conditioning system, was associated with other forms of hypersensitivity lung disease, and produced lines of precipitation on gel diffusion testing with sera from cases of humidifier fever. Inhalation challenge with extracts of *T. vulgaris* produced pyrexia in a case of humidifier fever. Our experience, however, is that extracts of *T. vulgaris* can react non-specifically on gel diffusion and the organism can activate the alternative pathway of complement.⁶ This latter property may have caused the induced pyrexia reported by Banaszak *et al*.³

An opportunity to study the role of *T. vulgaris* and other organisms in humidifier fever arose when an outbreak of the disease occurred in a local factory processing rayon into non-woven fabrics. Our investigations were undertaken to correlate airborne *T. vulgaris* organisms with disease incidence and also to determine the presence of other organisms that may have been active either non-specifically by pyrogen activity or specifically by immunological mechanisms. Since rayon was being processed continuously at the factory it had to be determined whether incoming bales were a source of contamination or whether this arose during handling and processing. Also, with material and sera

available from two other outbreaks of humidifier fever in Spain and Sweden, it was possible to compare sources from geographically unrelated areas for similar characteristics. Finally, during the course of this study, remedial action was taken, and this is presented.

Materials and methods

DISEASE OCCURRENCE AND DISTRIBUTION

The disease manifested itself as a pyrexial episode occurring on Mondays during the winter months or on the first day back to work after a holiday. The individuals affected were, in total, 20 of 50 office workers, but the number responding at any one time varied, and episodes were not seen every Monday. The other features of the disease were polyuria and leucocytosis. There were no abnormal radiograph findings, presumably reflecting a milder form of the disease compared with other studies.^{3,4} Lung function studies [VA^1 = alveolar volume (allowing for mixing); TL^1 = transfer factor (for carbon monoxide); $Kco = TL^1/VA^1$ (transfer per unit volume); PFR = peak flow rate; FEV_1 = forced expiratory volume (at 1 sec); FVC = forced vital capacity], performed on a Monday when 11 cases were recorded, showed evidence of a mild airways obstruction in the group of individuals most often affected. Both men and women were affected with an even spatial distribution within the office. There was no correlation between non-smoking and increased serological activity as has been seen in farmer's lung,⁷ neither was the presence of rheumatoid factor detected (cf Banaszak *et al*³). A more detailed account of these findings is to be presented elsewhere.

DESCRIPTION OF WORK AREA

The factory processed rayon, and the main humidifier, although located above the main office, extracted and delivered air only out of and into the factory, which was separated from the offices by a fire-brick wall. A scale diagram of the main office area is shown in fig 1. Some gaseous interchange took place through holes in the wall and doors but the main pressure equilibration vents between the factory and the offices had been closed a few years before the investigation. An integral part of the scheme was a suspended ceiling over the office area producing a void space 117 × 21.3 m in area and averaging 5.7 m to the roof. During the period when the pressure equilibration vents were in operation and due to inadvertent gaseous interchange—that is, doors open—rayon dust (fly) from the factory, as well as materials associated with building—for example, calcium sulphate and other debris—had

Fig 1 Diagram of main office area showing suspended ceiling and void space. Walls within the office area are not included. Air conditioning of office was provided by three extraction fans. A larger extraction fan was housed in roof distant to offices. Fresh air was obtained only by opening windows and doors. A subsidiary office, 30 m from main office, also had an extraction fan operating thereby drawing dust in from void space.

settled on the top surface of the suspended ceiling.

The air conditioning for the offices merely extracted air from the office area into the void above. Equilibration took place through window/door leaks but mainly through the unsealed joints between the 1.0×0.7 m foil-coated boards that formed the suspended ceiling. This could be observed directly using the flame direction of a lighted match and by the presence of dust streaks down walls under the joints.

AIR SAMPLING IN OFFICE AREA

Airborne microbes

For 14 consecutive days air was sampled by an Andersen sampler⁸ at two points, one in the main office and the other in a minor office where two of the most frequently affected individuals worked. Air was drawn over nutrient agar plates at 28.3 l/min for five minutes, and samples were incubated at 50°C for two days to detect thermophiles. This was repeated with Sabouraud agar plates incubated at 37°C to detect fungi. After the two-week period air was sampled on three successive Mondays.

Respirable dust

Respirable fractions of dust were collected daily

over the two-week period on $0.8 \mu\text{m}$ Millipore filters and assayed gravimetrically. A comparison was made with the external environment.

Detection of pyrogenic material in office dust and microbial extracts

Dust from the Millipore membrane retaining the respirable fraction particles, collected on the Monday when 11 cases of humidifier fever were recorded, was used. It was resuspended in saline such that 1 ml contained the amount of dust calculated to be inhaled by a sedentary worker over a work period of eight hours⁹ scaled down in the ratio 70 to 2.5 (the weight of an average man (70 kg) compared with the average weight of the rabbits (2.5 kg) used in the pyrogen study).

One ml of dust suspension was inoculated into the marginal ear veins of two rabbits and temperatures were recorded by indwelling rectal thermometers over a seven-hour period. The experiment was repeated with the dust suspension at $\times 2$, $\times 5$, and $\times 10$ this concentration. The ceiling dust itself was tested for pyrogenicity at $400 \mu\text{g/kg}$, likewise extracts of the organisms associated with the ceiling dust and office atmosphere—that is, *T. vulgaris*, *Cladosporium herbarum*, and a bacillus.

INVESTIGATIONS INTO CEILING DUST AND HUMIDIFIER MATERIAL

Microbial analysis

Fungi were grown on Sabouraud's agar and corn meal agar at room temperature and 37°C . Bacteria were cultured on nutrient agar at room temperature, 37°C , and 50°C . Protozoa were detected by growth on *Klebsiella aerogenes* streaked on non-nutrient agar plates at room temperature.

Preparation of extracts

Dust extracts—The suspended ceiling tiles had a thin aluminium covering on their uppermost surface, and it was possible to reach several of these. The dust was mopped off these tiles with a wet sponge and extruded into a plastic bucket containing sterile deionised water. After several tiles had been cleaned the dust suspension was shaken and soluble material obtained by filtration. Concentration was by air dialysis. Typically, 200 g dust yielded 50 mg soluble extract. To unprocessed suspension, phenol was added to 1% to prevent microbial development.

Humidifier material—The sludge from the humidifier chamber or baffle plates was extracted with water at 10% w/v, filtered, and concentrated. Humidifier recirculating water was filtered and concentrated.

disease. The same extract produced the same percentage-positive reaction in normal sera tested, as was expected from our previous experience with this organism.⁶ Extracts of *C herbarum* did not react on gel diffusion with sera tested.

There was no trend in the amount of respirable dust collected over the two-week period. Low levels of dust were observed from 0.082 mg/m³ to 0.204 mg/m³ in the general office (average 0.174) and 0.084 mg/m³ to 0.247 mg/m³ (average 0.15) in the smaller office. Outside air averaged 0.063 mg/m³. No correlation was seen between episodes of disease and airborne dust concentration.

PYROGEN RESPONSES TO DUST AND ORGANISMS IN OFFICE ENVIRONMENT

Pyrogen was not detected in the office dust sample even with $\times 5$ and $\times 10$ relative amounts of dust (fig 3a). Ceiling dust (fig 3a) and calcium sulphate were also non-pyrogenic at up to 400 $\mu\text{g/kg}$. Extracts of *T vulgaris* and *C herbarum* produced pyrexia (fig 3b). Interestingly, the response to *T vulgaris* was biphasic at 400 $\mu\text{g/kg}$ reducing to a late monophasic response at 40 $\mu\text{g/kg}$. After these results *T vulgaris* was considered unlikely to be the causative agent in our episodes.

INVESTIGATIONS INTO CEILING DUST

The investigation was extended into ceiling dust which, although non-pyrogenic, presumably con-

Fig 3a Pyrogen responses in rabbits to materials associated with a humidifier fever environment; ceiling dust 400 $\mu\text{g/kg}$, respirable airborne dust $\times 1$, $\times 2$, $\times 5$, $\times 10$. Note absence of response to respirable airborne dust from office at up to $\times 10$ that inhaled by individuals, also the non-pyrogenic nature of ceiling dust at 400 $\mu\text{g/kg}$.

Fig 3b Pyrogen responses in rabbits to extracts of organisms associated with humidifier fever environment. Note biphasic pyrogen response to *T vulgaris* extract at 400 $\mu\text{g/kg}$, reducing to a late monophasic reaction when dose was 40 $\mu\text{g/kg}$. *C herbarum* extract was also pyrogenic whereas an extract of *Bacillus* often found was not.

tained active material. An extract of this dust at 50 mg/ml produced lines of precipitation on gel diffusion with 16/18 affected cases and 2/18 non-affected sera. Statistically, this is significant ($p < 0.001$). With many sera more than one line was observed.

The isolation of organisms produced many fungi and bacteria, some of which were identified—for instance, *C herbarum*, *Paecilomyces variotii*, *Mucor pusillus*, *Aspergillus fumigatus*, *A niger*, *T vulgaris*, *Bacillus subtilis*; others were not identified but yeasts, bacilli and some Enterobacteriaceae were detected. Extracts of all organisms were made regardless of identification. None of the organisms yielded extracts reacting as fully with positive sera as did an extract of the dust itself.

INVESTIGATIONS INTO HUMIDIFIER MATERIAL

The inability to isolate organisms responsible for the antigens in the ceiling dust then led to investigations into the humidifier itself. The baffle plates and mixing chamber were thickly coated with wet rayon fly which formed a dark sludge with a light buff coloured drier surface. A metallic sheen was seen, which tended to float on water and adhere to glass surfaces. Extracts of this sludge and also concentrated recirculating water reacted even more strongly in gel diffusion than extracts of ceiling dust with positive sera. Microbial analysis showed different fungi and bacteria from the ceiling dust—for instance, *Fusarium*, *Flavobacterium*, *Sphaerotilus*.

However, again no one extract was responsible for the antigens seen in the sludge extract. Microscopic analysis showed protozoa of two main groups, ciliates and amoebae. Purified ciliates did not produce an antigenic extract whereas amoebal extracts produced as described yielded up to five precipitation lines on gel diffusion with positive sera.

A known amoeba, *Naegleria gruberi*, grown on *K aerogenes* or other Gram-negative organisms yielded antigens that produced lines of identity on gel diffusion with ceiling dust extract,¹² and there was an absolute correlation between precipitation reactions to ceiling dust, sludge extract, and *N gruberi* extract, although the order of reactivity was sludge > ceiling dust > amoebae. Certainly, amoeba extracts did not account for all the antigenic components in sludge.

The discovery of amoebae in the humidifier (provisionally identified as *Enchinamoeba exundans*) prompted an equivalent search in the ceiling dust and this also proved positive for amoeba belonging to the Vahlkampfiidae, provisionally *Vahlkampfia* sp.

POTENTIAL SOURCES OF ANTIGEN DEVELOPMENT IN FACTORY DUST AND INCOMING RAYON BALES

Overnight extracts of the factory rayon fly were negative antigenically as were 14-day extracts of autoclaved factory dust with sterile water. The addition of sterile water to the factory rayon fly led to antigen development (fig 4) but not the addition of factory or laboratory water to sterile fly. No antigens developed when incoming, that is, fresh rayon, was incubated with sterile water.

These experiments indicate that airborne factory dust can develop antigens and that "contamination" occurs only after the incoming rayon is processed. The spores and cysts may be attached to the rayon fly either during processing through machines (a dry process) or be picked up from the atmosphere when the fly becomes airborne. The source of these microbes could be the humidifier, since although the bulk (that is, >99%) material has been removed, the remaining sludge (about 100 g) is still potentially antigenic.

Further inquiries showed that on three distinct occasions the valve controlling the steam supply to a calorifier located over the office area with the humidification system stopped working. Steam filled the void space, and the entire suspended ceiling had pockets of water that sometimes caused the tiles to sag and leak into the office. This factor it seems led to secondary microbial development, since there was an appreciably increased presence of soluble sugars in the ceiling dust detected, using the

Fig 4 Antigen development in airborne factory dust, obtained from dust filtration units, incubated with factory or laboratory water for 14 days at room temperature (extracts A, B, C, E, G, H, I). Extracts D and J—water used concentrated $\times 200$. Extract K—overnight extraction at 4°C of non-sterile dust with factory water. Extracts tested by immunodiffusion, development with positive serum.

A Dust plus factory water, B Dust plus sterile factory water, C Sterile dust plus factory water, D Factory water concentrated, E Sterile dust plus sterile factory water, F Baffle plate sludge extract, G Dust plus laboratory water, H Dust plus sterile laboratory water, I Sterile dust plus laboratory water, J Laboratory water concentrated, and K Dust, overnight extraction at 4°C . Note development of antigens only in incubations of non-sterile dust and factory or laboratory water—that is, A, B, G, and H compared with C and I. Factory and laboratory water supplies did not contain viable microbes capable of antigen production, D and J. That development of micro-organisms was necessary is shown by extracts E and K.

phenol/sulphuric acid method of Dubois *et al*,¹³ compared with factory rayon fly. To test whether the incoming rayon already had the necessary spores/cysts for antigen development, 36 samples from the centre of incoming bales were incubated with sterile water and processed as for factory rayon fly samples. These results showed no antigens to be produced indicating that the factory rayon fly became contaminated from the humidifier air as it became airborne.

ANTIGENIC COMPARISONS WITH MATERIALS FROM OTHER OUTBREAKS

Lines of identity formed between Spanish, Swedish, and our humidifier sludge extracts reacting with either local positive sera or with Spanish or Swedish sera. This suggests common antigen sources and types of antibody response in these three outbreaks.

REMEDIAL ACTION

During the study period some attempts were made to provide a solution to the problem. After the initial 11-episode outbreak (fig 2) the fans extracting air from the office were switched off and a large fan was installed in the roof, removing air from the void. The office heating system was modified to take in external air and force this into the office. These actions sought to increase the air pressure in the office relative to the void space. Manometer readings, however, showed little difference, if any, between void and office, possibly due to the relatively large void volume ($12.6 \times 10^3 \text{ m}^3$) compared with the office ($1.5 \times 10^3 \text{ m}^3$). Also at this time the humidifier was cleaned. The next nine episodes (fig 2) called for more drastic remedial action, and the office workers were rehoused in temporary buildings while the problem was considered. The suspended ceiling was removed in its entirety ($2.5 \times 10^3 \text{ m}^2$) and the area thoroughly wet cleaned. The carpet (470 m^2) in the office area was removed as an extract of this showed antigen to be present. The fire-brick wall separating the factory and offices was sealed off. Finally, the humidifier (plus two others in the factory) was cleaned; 20 kg sludge was removed. The baffle plates were replaced by non-metallic plates that are easily removed and cleaned (now once a month). Most importantly, the intake air had a prefilter incorporated that removes about 99.9% of all the rayon fly. The water from the humidifier is run to waste and not recirculated. A recent examination of the humidifier showed less than 100 g of sludge in the whole system indicating fairly successful cleaning and preventative procedures. A new ceiling was placed on the office area, and half the workforce containing equal numbers of previously affected and non-affected individuals returned to the office area; so far (24 months) no episodes have been reported.

Discussion

The findings of our investigations suggest that in this outbreak at least the thermophilic actinomycete, *T. vulgaris*, did not play a causative role as had been suggested by Banaszak *et al.*³ Possibly the precipitation lines seen in this American outbreak were due to non-specific reactions and the pyrexia observed on inhalation challenge was due to endogenous pyrogen from *T. vulgaris*. It is also unlikely that thermophiles can develop in a normal humidifier since the running temperature is too low ($< 25^\circ\text{C}$). Again, farmers handling mouldy hay can be exposed to vast numbers of *T. vulgaris* spores¹⁴; yet our experience is that of more than a thousand sera tested over the past decade, only one farmer had

precipitins to *T. vulgaris* over and above the blur line that occurs with about half normal sera. The source of antigen described here is partly or wholly protozoal but the laboratory development of the total antigenic complex has not as yet been achieved, possibly due to our inability to recreate the precise conditions prevailing in the humidifier. From the experiments performed the following sequence of events probably led to the episodes observed.

The airborne rayon fly became deposited in the humidifier. Air/water-borne fungi and bacteria developed on the rayon (modified cellulose). Concurrently, protozoa developed on the fungi/bacteria setting up a sewage fungus complex.¹⁵ The products and spores or cysts of these organisms were blown into the factory and adhered to factory rayon fly. This passed initially through the equilibration vents between the factory and the void and settled on the suspended ceiling. Steam saturation provided the necessary moisture for setting up a secondary complex, which when dried produced the very fine antigenic dust.

Sensitisation to antigenic components in the dust occurred in some office workers and led to pyrexial illness on Mondays. The mechanism of the in-vivo pyrogen granule release is not known, but certainly antigen-antibody complexes can cause pyrogen granule release from polymorphonuclear leucocytes¹⁶ as can lymphokines derived from lymphocyte-antigen interactions.¹⁷ When tested about 44% of the factory workforce also had precipitins against humidifier sludge in the absence of disease. Thus it seems that either the office workers are sensitised differently from the factory workers or the presentation of the antigenic components are different in the office compared with the factory. Indeed, both factors may be operating but the latter is favoured since it is unlikely all the sensitised factory workers had a different type of sensitisation compared with the office workers, and the episodes were experienced only by people in the office.

The determination of undefined protozoa as antigen sources¹² led to our obtaining a named free-living water-borne amoeba to prepare extracts. *N. gruberi* provided a suitable antigen source but other amoebae have since been shown to be antigen sources. Warhurst¹⁸ showed antibodies to Acanthamoeba in the sera of humidifier fever cases, and this is important in view of the limited pathogenic role of strains of Acanthamoeba.¹⁹

Amoebae and protozoa probably provide the common thread running through outbreaks of humidifier fever. Several workers have reported a multitude of organisms in humidifiers,^{1,5} and the existence of variations of the sewage-fungus complex seems likely. Our cross-reactions with the Spanish

and Swedish sera indicate a distribution of similar antigens in humidifier fever, yet this differs from the report by Longbottom,²⁰ who found only minor antigens to be common in outbreaks at three different locations in Britain. Also other organisms can mediate a pyrexial response—for example, Gram-negative organisms in sewage sludge disease.²¹

As in other organic dust diseases removal from exposure eradicates the disease, and this was so in our cases when housed in the temporary offices. A permanent solution to the problem for the office workers has been to construct a separate, detached office block to high environmental standards. Further remedial action has eliminated dust in the old office area, and the humidifier now contains less than 1% sludge compared with before cleaning. Yet within the factory the airborne dust still retains its potential to develop antigen when incubated with water for 10–14 days, and it seems logical that the microbes responsible were derived from the factory atmosphere since incoming rayon bales incubated with water did not develop antigen. The stringent cleaning routines operating, however, and the awareness of the factory management of the problem makes the build-up of antigenic material to an unacceptable level extremely unlikely.

Clinical investigations were undertaken by Dr A Axford; airborne dust collections by Mr J W Skidmore; pulmonary function testing by Mrs C Bevan and Mrs I Ashton; chest radiographs by Mr W Audsley—all of the MRC Pneumoconiosis Unit, Penarth; fungal analyses by Dr J Mullins, Asthma Research, Sully; and initial protozoal investigations by Dr A Griffiths, Microbiology, Cardiff. Vital help was provided by Drs A Jones and M Jones, Employment Medical Advisory Service, Cardiff. The kind participation of the employees at the factory under study, particularly Mr J Tonge, Mr C Verbeeck, Mrs M Dew, Mr P Harbord, Mr R Stone is greatly appreciated as is the help and guidance of Dr J C Gilson, MRC Pneumoconiosis Unit. Finally, the artistic and photographic expertise of Mr D Llewellyn and Mr R Boothby, Llandough, is self-evident.

References

- ¹ Pickering CAC, Moore WKS, Lacey J, Holford-Strevens VC, Pepys J. Investigation of a respiratory disease associated with an air-conditioning system. *Clin Allergy* 1976;**6**:109–18.
- ² Pestalozzi C. Febrile Gruppenerkrankungen in einer Modellschreinerei durch Inhalation von mit Schimmelpilzen kontaminiertem Befeuchterwasser (Befeuchterfieber). *Schweiz Med Wochenschr* 1959;**89**:710–3.
- ³ Banaszak EF, Thiede WH, Fink JN. Hypersensitivity pneumonitis due to contamination of an air conditioner. *N Eng J Med* 1970;**283**:271–6.
- ⁴ Sweet LC, Anderson JA, Callies QC, Coates EO, jun. Hypersensitivity pneumonitis related to a home furnace humidifier. *J Allergy Clin Immunol* 1971;**48**:171–8.
- ⁵ Keller H, Spengler H, Latscha U. Befeuchterfieber. *Schweiz Med Wochenschr* 1972;**102**:865–72.
- ⁶ Edwards JH, Baker JT, Davies BH. Precipitin test negative farmer's lung—activation of the alternative pathway of complement by mouldy hay dusts. *Clin Allergy* 1974;**4**:379–88.
- ⁷ Morgan DC, Smyth JT, Lister RW, Pethybridge RJ. Chest symptoms and farmer's lung: a community survey. *Brit J Ind Med* 1973;**30**:259–65.
- ⁸ Andersen AA. New sampler for the collection, sizing and enumeration of viable airborne particles. *J Bacteriol* 1958;**76**:471–84.
- ⁹ Cotes JE. *Lung function. Assessment and application in medicine*. 2nd ed. Oxford: Blackwell, 1968:388.
- ¹⁰ Edwards JH. The double dialysis method of producing farmer's lung antigens. *J Lab Clin Med* 1972;**79**:683–8.
- ¹¹ Working Party of the British Society for Mycopathology. In: Evans EGV, ed. *Serology of Fungal Infection and Farmer's Lung Disease*. Leeds: University Printing Service, 1976:9.
- ¹² Edwards JH, Griffiths AJ, Mullins J. Protozoa as sources of antigen in 'humidifier fever'. *Nature* 1976;**264**:438–9.
- ¹³ Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. *Anal Chem* 1956;**28**:350–9.
- ¹⁴ Lacey J, Lacey ME. Spore concentrations in the air of farm buildings. *Trans Brit Mycol Soc* 1964;**47**:547–52.
- ¹⁵ Curtis EJC. Sewage fungus, its nature and effects. *Water Res* 1969;**3**:289–93.
- ¹⁶ Cranston WI. Humidifier fever. Mechanisms of pyrogenic responses. *Thorax* 1977;**32**:653–63.
- ¹⁷ Chao P, Francis L, Atkins E. The release of an endogenous pyrogen from guinea pig leukocytes in vitro. *J Exp Med* 1977;**145**:1288–98.
- ¹⁸ Warhurst D. Humidifier fever. Amoebae and allergic lung disease. *Thorax* 1977;**32**:653–63.
- ¹⁹ Stamm WP. Humidifier fever. Pathogenicity of free living amoebae. *Thorax* 1977;**32**:653–63.
- ²⁰ Longbottom JL. Humidifier fever. Serological testing in humidifier fever. *Thorax* 1977;**32**:653–63.
- ²¹ Rylander CR. Humidified fever. Responses to airborne sludge. *Thorax* 1977;**32**:653–63.