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Humidifier fever[1]

MRC Symposium (1977). *Thorax*, **32,** 653–663. **Humidifier fever.** In enclosed environments, it may be necessary to regulate temperature, ventilation, and humidity to maintain comfortable working conditions. Several systems can be used although in terms of installation and running costs a simple radiator system is far more economical than air conditioning with complete temperature and humidity control. Humidity control requires the introduction of water into a moving current of air, and in such a system baffle plates are often used to eliminate large droplets; also any unused water is usually recirculated. Organic dust drawn into the system and settling on the baffle plates and in the mixing chamber may be utilised by micro-organisms introduced from the atmosphere and from the water supply, and a biomass builds up. Microbial material is then voided into the working atmosphere by the ventilation system.

Under appropriate exposure conditions susceptible individuals may succumb to an episode of humidifier fever, an influenza-like illness with pyrexia and malaise as the main symptoms, but cough, chest tightness, dyspnoea and weight loss may also be seen. The episodes usually occur after absence from work for a few days and have been termed 'Monday sickness'. Individuals are often able to return to work the next day and appear refractory to further exposure. The disease is of the winter months probably due to the larger amount (up to 90%) of fresh air drawn into the humidifier during the summer.

In the blood of exposed subjects precipitins are usually present to extracts of baffle plate material and recirculating water although they are not necessarily indicative of disease. Skin tests may be positive and inhalation challenge has reproduced the disease in susceptible individuals. Many organisms may be isolated from baffle plates and recirculating water but only amoeba extracts have produced consistently positive reactions with sera from affected individuals.

Remedial actions such as changing from water to steam humidification or running recirculation water to waste have proved effective in some factories. Other measures may be considered, for example, adding microbicidal agents or prefiltering intake air.

The pyrexial episode may be due to immune complex-complement or alternative pathway-complement activation, inducing the release of leucocyte pyrogen; alternatively, sensitised lymphocytes can release lymphokines capable of inducing leucocyte pyrogen release.

It is now realised that the respiratory route of introduction of airborne materials into the pulmonary tract may not only be responsible for diseases characterised by bronchopulmonary distress but may also give rise to solely constitutional symptoms. The groups of diseases termed extrinsic allergic alveolitis, hypersensitivity pneumonitis, or organic dust pneumoconiosis can provide such cases. Exposure tends to be occupational and, in general, the patient is aware of the presence of dust. However, recently, with the advent of modern ventilation techniques, an organic 'dust' disease associated with contaminated humidifiers (Banaszak *et al.*, 1970) or other aerosol-producing contaminated water systems (Friend *et al.*, 1977) has extended the concept of environments producing extrinsic allergic alveolitis to include those with no obvious dust burden.

The increased use of air conditioning and humidification in factories, hospitals, and homes strongly indicated that a reappraisal of the conditions of use, construction, and biological control for ventilation systems was necessary. Thus, on 14 and 15 December 1976 a meeting of engineering, scientific and medical experts in the field of humidifier fever was held at the Medical Research Council Pneumoconiosis Unit to assess our current knowledge on this latest of recognised organic dust diseases.

The meeting was divided into four parts. The first section on engineering aspects was designed to cover factors governing the need for ventilation design, limitations of the use of such systems, and prevention of microbial build-up. The second section dealt with

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the medical aspects of the disease including actual outbreaks of humidifier fever and related conditions occurring in sewage workers. The third section contained papers on the biological aspects of the problem such as serological responses to materials from humidifiers and analyses of the contribution of individual microbes to the antigenicity of humidifier extracts. Also in the third section papers were presented on the development of microbial build-up and the possible mechanisms of the disease. The final section was discussion.

Engineering aspects

G. W. Brundrett (The Electricity Council Research Centre) introduced the engineering aspects with a paper on the need for ventilation in rooms where people are present for most of the working day. Minimum needs are physiological and designed to maintain an ambient carbon dioxide level below 0·5% by volume (American Conference of Governmental Industrial Hygienists, 1971). For sedentary work this is 4·5m³/h (about 1/6 of an air change per person per hour in a sitting room). Pleasant, comfortable conditions require more fresh air to control body odour, relative humidity, and cigarette smoke. Ventilation requirements to eradicate body odour problems are affected quantitatively by racial differences (Shehadeh and Kligman, 1963) and qualitatively by sex differences (Russell, 1976). They are also dependent on the bathing habits of the room occupants, the airflow required for weekly bathers being approximately 50% more than that required for those who bathe daily or on alternate days (Yaglou *et al.*, 1936). Body size is also important for adults (Lehmberg *et al.*, 1935) although children are more odorous. The volume of the room also affects the ventilatory requirements, the necessary airflow being inversely proportional to room volume.

Relative humidities in excess of 70% may be uncomfortable, and those below 40% may be unpleasant due to static electricity. Ideally, air supplies should be adequate to maintain a relative humidity between 50% and 60%. There is some contribution to the humidity and temperature by the worker; breathing and other moisture loss from the body amounts to about 40g/h. Also outside air will have a variable moisture content and this will require varying dehumidification in passing through the ventilatory unit; the controls should, therefore, be linked to outside temperature conditions (Brundrett, 1976).

Smoking adds considerably to the environmental control problems. Since 80% of the mainstream smoke is retained in the smoker's lung it is the sidestream smoke which contributes most to the contamination of the room air. Carbon monoxide is the major contaminant (50–90mg per cigarette), and for acceptable conditions the ventilation system must maintain CO content to less than 9 ppm. Fresh air rates are normally doubled in those rooms where smoking is permitted (Brundrett, 1975).

S. Ratcliffe (Department of Health and Social Security) reported that comfortable conditions defy precise specification in engineering terms but data derived empirically and given in guides such as that published by the American Society of Heating, Refrigeration and Airconditioning Engineers reduce the range of uncertainty. In general, comfort satisfaction related much more to temperature than to humidity, and in the UK an indoor temperature of 65°F (18°C) is accepted as being the minimum for comfort in day rooms, while the relative humidity may lie within the range 50–80%.

According to the function of a room, adequate conditioning may be obtained by systems ranging from the installation of radiators or warm air ventilation to complete air conditioning with full control of heating and humidification. It is economically important to assess realistically which system is required because for the three systems the capital cost and the running costs are very roughly in the ratio 1:2:4 and 1:7:15 respectively, depending largely on site factors. Complete conditioning with very small tolerances would add considerably more to the capital and running costs. The variability of demand on the air conditioners differs considerably between internal rooms and those rooms on the perimeter of a building. This is due mainly to solar and other heat gains and losses through the glazing. Where possible, in hospitals, rooms requiring full air conditioning are located internally. In new installations it is the policy of the Department of Health and Social Security to use steam humidifiers, but many older installations have wet humidifiers, all of which require a collection pond of some sort in the air stream. Although this could provide a breeding ground for bacteria these installations have given perfectly adequate service, and at present it is not planned to change them all to steam humidifiers.

O. M. Lidwell (Central Public Health Laboratory, Colindale, London) described the normal flora of the air and pointed out that, in terms of starter inocula for humidifiers, the atmosphere is a ready source of bacteria, algae, protozoa, etc, as well as a carrier of numerous pollen grains. The ability of organisms to develop in the conditions found within humidification systems is, however, another matter. Whereas Pseudomonads, for example, can develop in little more than pure water, the growth requirements for protozoa are more demanding. The internal humidifier environment will also influence the type of

organism which may develop, for example, whether metal structures are coated or not. The flow rate of water through the equipment influences the buildup of organisms. For example, when the outlet of a hospital humidifier was blocked the number of organisms rose from 10/ml to 1000/ml, one strain of organism being particularly dominant. Additives will also influence the flora; the addition of disinfectants may even increase the number of particular species of bacteria such as Proteus.

The major factor governing the site of deposition of airborne material in the respiratory tract is particle size. Particles under 5μ are particularly likely to reach and be retained in the alveoli whereas larger particles tend to be deposited in the bronchioles, bronchi, and trachea. Nasal retention becomes increasingly effective above 5μ. With mouth breathing a substantially higher fraction of particles less than 5μ are deposited in the alveoli, and at 2μ and smaller, retention is almost exclusively alveolar with a maximum retention, above 80%, for 2-μ particles.

Humidifiers, by the nature of their design, are likely to produce fine droplets as from sprays or spinning discs, and this leads to the production of even finer particles. In establishments such as hospitals, it is obviously of greater importance to the exposed population than in others to avoid the dispersal of contaminating organisms which may be pathogenic.

A reduction in the number of organisms released into the environment may be brought about by several means:

1 Better input filters—resulting in a reduction of the microbial inoculum.

2 Steam injection—eliminating the need for a recirculating water system with its potential for the build-up of organisms and avoiding some of the problems of spinning discs.

3 Cleaning—frequent cleaning reduces the number of organisms but is often a difficult process.

4 Heating the water tanks, thereby killing most of the multiplying organisms; 60–70°C would be adequate for most species.

5 Adding chemicals to the system—a seemingly simple operation but with many problems, for example, dose maintenance and possible inactivation by organic material, resistant strains developing; in addition, the bacteriocide would be inhaled.

6 Ultraviolet light—this could be used on the intake air, in the humidifier chamber, as a treatment for recirculating water, or on the outlet air. Its effectiveness in the first and last of these positions is likely to be limited.

7 Avoiding free water and wet parts.

Medical aspects

J. Pepys (Brompton Hospital, London) introduced the section on medical aspects with a general outline of the types of response one might observe to inhaled material. The traditional criteria of allergic pulmonary reactions, that is, exposure to a specific allergen affecting a small fraction of the exposed population who show increasing sensitivity with time, must now be modified in view of increased understanding of the factors involved. The three main areas in which knowledge has expanded are (*a*) the immune reactivity of the subject, (*b*) the nature of the inhaled material, and (*c*) the circumstances of exposure.

The immune reactivity of the subject is fundamental to the nature of the immune response elicited by allergen. The atopic patient is capable of responding by an immediate reaction mediated by IgE. This reaction is highly sensitive, responding to molecular concentrations of allergen, not inhibited by corticosteroids, and capable of releasing large quantities of histamine. The atopic state may also be mediated by short-term sensitising antibody thought to be IgG2 and IgG4.

Immune complex reactions with complement activation have recently undergone further classification, and one or more of three main responses may occur—a systemic response with pyrogen release, a syndrome of asthma, and an alveolar response. The continued presence of immune complexes may be related to the solubilisation of the complexes, and the antibody affinity, particularly low affinity antibody, may allow immune complexes to promote increased tissue damage. Such low affinity antibody could be difficult to detect with conventional precipitin tests and may account for the variable positivity of these tests. Other mechanisms capable of activating complement have now been identified as important in allergic lung disease, particularly activation of the alternative pathway of complement which may simulate the Arthus response. The acute phase reactant C-reactive protein is also capable of fixing complement. The demonstration of precipitating antibodies implies only an exposure to the antigenic source and is not necessarily causal in any illness. However, precipitins remain a useful test for establishing the source of the causal antigens except that the presence of teichoic acids contaminating antigenic materials may produce false positive reactions.

The differences in antigenic reactivity may also be important. Perhaps those antigens which are highly soluble produce predominantly changes in ventilation–perfusion ratios without marked radiographic changes. The spores of *Micropolyspora faeni* may have diffusible antigens on the surface membranes

capable of preferentially producing soluble aggregates or effective complement consumption. The retention of the spore in the lung may also produce a foreign body granulomatous response.

The following two papers contained accounts of humidifier fever outbreaks.

C. A. C. Pickering (Wythenshawe Hospital, Manchester) described a systemic and pulmonary illness that occurred in workers employed in a printing and stationery factory, constructed in 1950, performing type composition and lithographic work (Pickering *et al.*, 1976). Three hundred and fifteen workers were employed with a 7–37 years' service record, most having had 20 years of service. Initial symptoms developed in 1965 and the investigation of nine male workers was performed in 1970. All described an influenza-like illness of fever, malaise, cough, chest tightness, and dyspnoea with associated loss of weight and night sweats. The illness occurred during the months of November to March. It was worst early in the working week, symptoms beginning in the early afternoon or evening of the first working day. The air conditioning system was suspected because, for economic reasons, there was a 2% recirculation during summer but a 50% recirculation during the winter months. Four units of the same type were used, two supplying the main floor. The system consisted of coarse fibre filters, a water spray chamber contained within a series of baffle plates, and a heat source. The baffle plates were found to be contaminated with a black jelly, and extracts of this material produced strong precipitins in all affected workers and in 75% of asymptomatic exposed workers. Precipitins were also found to *Trichoderma viride*, *Phoma* sp, Fusarium, and other fungi but no specific causative organism was identified in the affected workers.

Sampling of the main production area atmosphere revealed no specific organism. Complete relief of symptoms was obtained by employing steam in place of the water spray. Panacide treatment was not found to be effective in reducing water contamination.

J. A. R. Friend and J. Gaddie (City Hospital, Aberdeen) described an acute systemic and pulmonary illness that affected part of the workforce of a modern factory involved in the folding, cutting, and gumming of stationery. The factory, constructed in 1971, employed 560 persons and was 200×60 m in size, incorporating a main production area, a maintenance workshop, and a small printing department. The illness developed three years after the opening of the factory. Twenty-four workers were affected, 15 working mainly in the maintenance workshop and nine in the main production area. Eighteen workers were examined; all were men, age range 20–66 years, working an 0800–1630 hours shift. All complained of an illness occurring towards the end of a Monday shift and persisting for 24 hours, consisting of chest tightness, arthralgia, cough, dyspnoea, and shivering; physical examination revealed pulmonary crepitations in six subjects. A minority had a leucocytosis with eosinophilia and half had evidence of atopy on skin testing with common allergens. Thirteen workers had evidence of a restrictive pattern on lung function but all had normal chest radiographs. Adjacent to the maintenance workshop was a pump room containing three vacuum pumps and two compressors, supplying vacuum and compressed air to machines on the factory floor. The atmosphere of the pump room was hazy, and inspection of cooling water in the pump room revealed contamination, with a brownish scum and a stale smell. The associated external cooling tower had slats which were covered with a brown jelly-like material which on culture revealed five antigenic types of Gram-negative chromogenic rods, iron-fixing bacteria, and many fungi. Using freeze-dried extracts of the contaminated water as antigen, 23 of the 24 affected workers showed strong precipitins; 5 of 30 unaffected workers also showed positive precipitins. There were no consistent precipitin reactions to individual organisms isolated from the water.

Skin testing with the contaminated water extract revealed immediate positive responses in 9 of 15 workers tested. Those affected workers with strong precipitins worked close to the pump room, most in the adjacent maintenance workshop. Bronchial challenge tests performed on two affected workers, beginning with 10^{-6} mg/ml of freeze-dried extract by nebulisation, showed no symptoms at low concentration but four hours after inhaling 1 mg/ml there was a progressive fall in forced vital capacity and transfer factor for carbon monoxide, associated with headache and fever. No response occurred in two normal volunteers unassociated with the factory. The dispersion of the antigenic material from the contaminated water was thought to arise as a result of aerosol production in the effluent from the vacuum pumps in the pump room, which were of the 'liquid ring' type in which a rotating turbine creates a vacuum with the help of the recirculating water. The air–water effluent from these pumps passes to separating tanks where the water falls to the bottom for recirculation, but large volumes of air were discharged into the pump room atmosphere and dispersed through doorways, and possibly the ventilation system, to other areas of the factory. After the air effluent had been vented outside the factory no further episodes of the illness occurred.

C. R. Rylander (Gothenburg University, Sweden) described his findings in sewage sludge disease. This

is a condition in which workers in sewage disposal and treatment plants develop episodes of chills and malaise. Their work involves heat treatment of sludge to produce a powder. Dust concentrations, measured by Millipore filter, are between 0·5 and 4 mg/m^3 but often higher. Between 10^4 and 10^7 bacteria, mostly Gram-negative, are found per cubic metre. Symptoms also occur in ordinary sewage treatment plants.

Studies were made on control groups from a nearby oil refinery and on unexposed, occasionally exposed, and frequently exposed workers from the treatment plant. The heavily exposed group showed a high incidence of fever and purulent conjunctivitis, and significant increases in white cell count, IgG, IgA, C-reactive protein, and fibrinogen degradation products. There was also a reduced level of IgG antibody to the lipid A antigen of *Escherichia coli*. Pulmonary function was not studied in detail.

It was suggested that the disease may be caused by endotoxin activating the alternative pathway of complement. In discussion it was suggested that the disease was related to return to work after holidays and weekends, although detailed studies had not yet been undertaken.

In a further presentation, Rylander reported on some experimental findings on the pulmonary defences to airborne bacteria.

Previous work, dating back to the 1880s, had shown that different bacteria were treated differently when inhaled, and this related to their pathogenicity. More recent work had confirmed these findings but shown that a high proportion of inhaled bacteria are rapidly cleared from lungs (up to 95% within six hours). Moreover, bacteria in airways are rapidly rendered non-viable. This bactericidal effect is probably more important in defence than clearance, and many factors may play a part, for example, macrophages and their enzymes, cellular and humoral immune mechanisms, and lysosymes. In addition polymorphs are clearly important.

Cotton dust inhalation increases the numbers of polymorphs (and later on macrophages) in animal lungs, and lungs so treated clear bacteria more rapidly. Controlled experiments had shown that this effect was probably due to the polymorphs. In inhalation experiments using cotton dust or bacteria the increase of polymorphs in bronchial lavage specimens has been used as an index of response. Differences have been observed between different organisms, for example, there is little response to inhaled *Bacillus subtilis* but a significant response to *E. coli*, Enterobacter, and bacterial lipopolysaccharide. Further studies are in progress to determine the mechanisms of leucocyte mobilisation which may be related to endotoxin in bacterial walls.

Biological aspects

J. L. Longbottom (Cardiothoracic Institute, London) presented experimental results on serological testing in humidifier fever.

In three outbreaks of humidifier fever, sera from workers in the factories were tested for precipitating antibodies to extracts from baffle jelly (Nottingham), freeze-dried water from a cooling tower (Aberdeen), and sludge from a ventilation system (Leeds). Positive reactions were obtained as follows:

	Nottingham	*Aberdeen*	*Leeds*
Symptomatic workers	100%	95%	100%
Asymptomatic, exposed workers	75%	23%	30·7%
Asymptomatic, non-exposed workers	29%		
Blood bank controls	None	None	None

From the sources of the extracts a variety of fungi, bacteria, free-living nematodes, and amoeboid cysts were isolated. So far, extracts of pure cultures of these organisms have not given corresponding precipitin reactions.

Although there was some cross-reactivity, the sera of the patients from each outbreak showed a specificity for their own respective antigen preparations. Crossed immunoelectrophoresis showed that the cross-reactivity was associated with weaker antigens whereas the main antigens in the three extracts were specific.

Patients from these factories who had apparently not been exposed to the antigens for over a year still had remarkably high levels of precipitating antibodies. These were mainly IgG but also possibly IgM antibodies.

J. H. Edwards (MRC Pneumoconiosis Unit, Cardiff) presented findings on a local outbreak of humidifier fever. The main symptom was a pyrexial episode that occurred some time after work on Mondays or on the first day back to work after a holiday. There was associated myalgia and a transient leucocytosis. Individuals were usually well enough to return to work the next day. The attack differed in some respects from previously reported outbreaks in that the people affected, that is, about 20 of 50 office workers, were not directly supplied by air from the humidifier, which was necessary for an industrial process using regenerated cellulose (rayon) in an adjacent part of the factory. However, finely particulate rayon material from the factory environment had settled on a suspended ceiling above the office, and extracts of this dust, as well as various extracts from

the humidifier, reacted with the sera of affected individuals in the office at a statistically significant level.

The relationship between disease and precipitins did not hold for the factory workforce, where approximately 44% sera tested had precipitins in the apparent absence of disease. Investigations on the source of antigenic material were made and included the isolation of many bacteria and fungi from both ceiling dust and humidifier material. Apart from a few weak reactions, extracts of such organisms were antigenically negative. However, microscopic examination of the wet material from the humidifier revealed large numbers of protozoa to be present. These were isolated and two main groups emerged, ciliates and amoebae.

Purified ciliates failed to produce antigenic material that reacted with positive sera whereas extracts of the amoebae isolated did react. A known amoeba, *Naegleria gruberi*, was extracted, and this produced precipitin lines with positive sera that joined with lines from ceiling dust extract, indicating the presence of antigenically identical material. Amoebae were also isolated from the dry ceiling dust, indicating a presence of amoebal cysts in the dust (Edwards *et al.*, 1976).

Although *N. gruberi* produced antigens capable of reacting with positive sera, the morphology and antigenic reactivity of amoebae isolated from the humidifier and ceiling dust suggested that several different strains might be present such that one strain might not necessarily be responsible for all the antigens produced.

A possible explanation for the development of antigenic material in the ceiling dust was that on three recorded occasions a steam valve located in the space above the suspended office ceiling became stuck, and water vapour saturated the atmosphere, creating conditions for the development of bacteria, fungi, and protozoa, using the regenerated cellulose particles as initial substrate.

D. C. Warhurst (Amoebiasis Diagnostic and Research Unit, St. Pancras Hospital, London) reported that, in humidifier samples received from the outbreaks at Leeds and Aberdeen, ciliates, flagellates, and amoebae were found. *Acanthamoeba polyphaga*, *A. castellanii*, and *A. astronyxis* were among the organisms isolated.

Sera from 25 affected and unaffected workers involved in the outbreaks and from 28 healthy persons were tested by the indirect fluorescent antibody (IFA) technique against amoebae from culture using *A. polyphaga* isolated from a corneal ulcer (Elliott strain), *A. castellanii* isolated from a nasopharyngeal swab (Ryan strain), and *N. gruberi* from fresh water (CCAP 1518e). Only two out of 28 healthy persons had IFA titres of 1/100 or more against these strains. Twenty-five workers were then tested. Of 10 with no precipitins against humidifier extracts, eight had a titre of 1/50 or less against Elliott and Ryan strains. Of 15 with precipitins to humidifier extracts, 13 showed IFA titres of 1/100 or greater against both acanthamoebae. The other two positives had 1/50 titres to at least one strain. Using 1/50 IFA titre as cut-off point, two out of 10 with no precipitins were positive by IFA and 13 out of 15 precipitin positives were positive by IFA against Acanthamoeba. None of the sera gave a reaction to *N. gruberi* trophozoites, but five of the precipitin positive sera reacted with *N. gruberi* cysts, and it could be seen that the cyst wall was involved. When there was a high titre to Acanthamoeba there was a reaction at 1/100 with Naegleria cysts.

Although *A. polyphaga* and *A. castellanii* have 22 out of 26 antigenic components in common, some sera were species specific. In sera from symptomatic patients at Leeds and Nottingham there was a link with high titre to Acanthamoeba trophozoites and Naegleria cysts which did not hold true for two of four sera from Aberdeen.

On the basis of the work done there does appear to be a relationship between the presence in sera of precipitins to humidifier extracts and the presence of antibodies, detectable by the IFA technique to Acanthamoeba trophozoites and in some cases to Naegleria cysts.

W. P. Stamm (Amoebiasis Diagnostic and Research Unit, London) reported that the first cases of primary amoebic meningoencephalitis in man were recognised in 1965 since which time about 85 cases have been reported worldwide.

The acute disease (most frequent in adolescents) is caused by *N. fowleri* when patients die within 10 days. The disease caused by Acanthamoeba is more chronic and less common.

All *N. fowleri* infections have been associated with warm water in temperate climates. The amoebae of the genus Acanthamoeba have no specific associations and are considered to be opportunistic with a wider age range of affected individuals and conditions of infection. In four cases of eye infection with corneal ulcer in the UK and in three cases in the USA, amoebae of the genera *A. polyphaga* or *A. castellanii* have been isolated. It is not yet known whether amoebae of the genus Acanthamoeba are the cause of the corneal ulcer since treatment with drugs to which the amoebae are sensitive do not necessarily clear the ulcer. In a group of recruits in Czechoslovakia, a high percentage showed Acanthamoeba in nasopharyngeal swabs, and many of these had recently had upper respiratory tract infections.

It is suggested that nasopharyngeal swabs for

Acanthamoeba should be carried out on humidifier fever patients.

D. E. Hughes (Microbiology Department, University of Wales, Cardiff) then reported on microbial growth under static conditions that could prevail in water tanks supplying water for humidification. Such static situations are quite usual (Gray and Williams, 1971) and limitations are imposed upon microbial development by availability of nutrients and other necessary factors, of which the most important is often water. Microbes can live and grow in a wider range of water activities than other organisms, yeasts and fungi being more tolerant to lack of water than bacteria. Cryptobiotic forms, such as spores and cysts, not only contain little water but can survive in its absence. Water growth relationships vary with temperatures, pH, and chemical composition of the media; also microbial development can alter the water relationship of an environment, for example, oxidation of organic foodstuffs by organotrophs (heterotrophs) yields water $(CH_2O)n+\frac{1}{2}O_2 \rightarrow H_2O+CO_2$, and more complicated processes have been envisaged for the release of water from inorganic materials such as goethite ($Fe_2O_3.H_2O$) (Vishniak, 1971).

As a group microbes have the widest range of organic utilisation, extending from CO_2 to large polymers; for example, a typical Pseudomonad may utilise several thousand different compounds as a sole carbon source. It is not surprising, therefore, that microbes are universally distributed and have been found developing in paints, films, plaster, fabric, and paper (Hughes, 1973). These foci may be small but this can change rapidly when favourable conditions for reproduction are presented. Oxygen and carbon dioxide requirements vary; for example, obligate anaerobes or aerobes and CO_2 normally regarded as an end product may have a nutritional role or a role in sporulation (Smith and Anderson, 1973). Thus the presence of specific allergens (especially fungal) may depend on CO_2 build-up under static conditions.

In situations where growth is limited by gaseous requirements agitation plays a role in determining not only growth rate but also population distribution. For some organisms motile cells may move into more favourable conditions, and the formation of crustose growths on surfaces or aerial spores may lead to the release of allergens into an air stream. Microbes also show the widest tolerance to temperature, some surviving below 0°C and above 100°C. Also survival may be seen in non-polar solvents such as oils.

Thus, taking water, nutrient, gaseous, and temperature requirements into consideration, rarely is the full growth potential of an organism reached or, if reached, maintained for any length of time. Usually a succession of organisms results. The problem might therefore be to prevent the primary coloniser which incidentally might not be the allergen. Growth under exponential conditions, although not achieved for any length of time, can result in a surprising build-up of organisms. As an example, large oil emulsion hydraulic systems have been found to produce up to 1 ton wet cells in four to five days so that little oil remained.

Where a steady-state population is reached protozoa may be found which, although unable to metabolise substrates, grow by grazing on other microbes.

The accumulation of microbial biomass is often dependent on the formation of attachment organelles or compounds such as polysaccharides which lead to the formation of flocs or slimes. In these there will be a range of viable as well as dead organisms plus empty cell walls, sheaths, and spores. These may well be allergenic and become airborne if an air current passes over or through the slime/floc.

Remedial measures would require a fairly complete survey of the microbial populations, the amounts of the biomass's source of nutrient, and the physical factors that might be expected to regulate growth rates. It is possible the solution may be as simple as changing the water supply or as complicated as continuous addition with monitoring of a specially developed biocide. Perhaps it could be stressed that the most important aspect of such diseases of industrial systems is that the microbial ecologist should be involved in the design of plant from its initial conception.

Finally, the nature of the pyrogenic response was presented.

W. I. Cranston (St. Thomas' Hospital, London) reported that fever has long been recognised as a sign of disease but it was not well understood until the discovery of endotoxins. However, these compounds could not offer a complete explanation of febrile mechanisms and a pyrogen which was not an endotoxin was extracted from mammalian white blood cells (Beeson, 1948). Repeated doses of endotoxin give rise to tolerance whereas repeated doses of the pyrogen do not (Beeson, 1947). There are certain differences between endotoxin and leucocyte pyrogen. Endotoxin is heat stable, the pyrogen is not. The pyrogen is a protein of molecular weight 25 000, endotoxin is a complex lipopolysaccharide. Direct infusion of endotoxin in saline gives a temperature rise after one hour whereas if the endotoxin is first incubated with whole blood the temperature rise takes only 10 minutes. The leucocyte pyrogen can also be produced by polymorphs, monocytes, Kupffer cells, and other tissues. In the presence of phagocytosis (the usual conditions for pyrogen production), it is not possible to demonstrate the presence of any pyrogenic material in the cell in the initial hour. During that time the addition of inhibitor of protein synthesis

will inhibit its final production. After one or two hours, leucocyte pyrogen begins to appear, and the addition of these inhibitors will no longer inhibit its release, although iodoacetic acid will still do so to some extent. There appears to be an initial stage of protein synthesis followed by release (Atkins and Bodel, 1971). These pyrogens can be found in the circulation of animals during infection, and evidence suggests that their site of action is on the brain in the pre-optic area of the hypothalamus.

In hypersensitivity states there is evidence that circulating antibodies and antibody/antigen complexes can trigger the white cells to produce leucocyte pyrogen, but the mechanism is not understood. There was evidence to suggest that the action of the pyrogen on the hypothalamus might be mediated by prostaglandins, especially prostaglandin E_2 (Feldberg *et al.*, 1973). However, a prostaglandin antagoniser does not block the effects of leucocyte pyrogens (Cranston *et al.*, 1976).

The tolerance which builds up to repeated injection or inhalation of endotoxin appears to be mediated by an increased reticuloendothelial clearance of endotoxin from the circulation. Such a mechanism could be involved in humidifier fever, particularly when there is no pulmonary involvement.

Discussion

J. C. Gilson (former Director, MRC Pneumoconiosis Unit, Cardiff), in opening the discussion, commented on the striking discrepancy in concentrations of dust producing symptoms in different situations. These ranged from mg/m^3 in the sewage plant, as reported by Rylander, to extremely low concentrations at the local rayon factory. The time of exposure needed to produce a response could also be very brief.

J. Pepys replied that large doses might be needed to produce sensitisation whereas a response could be triggered off by a very small quantity of material.

If sensitisation was a consequence of infection (O. M. Lidwell) another variable in the dose-response relationship might be introduced, namely, the dose needed to initiate infection.

Again (J. Pepys) there may be a genetic factor involved as in farmer's lung; this may be of importance in humidifier fever so it would be worth investigating the HLA system and B lymphocytes. However, with 50% of the workers affected this was unlikely to be the case.

J. A. R. Friend commented on the apparently low level of contamination in his factory environment. In the compressor room there were 11 bacterial and 33 fungal colonies in a 12·5-l air sample. There was also the problem of investigating asymptomatic subjects who were serologically positive; why did such subjects not have symptoms, and should inhalation challenge testing be done on these? In discussion it was suggested that people should take home PFR gauges and thermometers.

Perhaps there was an infective phase (W. P. Stamm). When mice were infected via the nasopharynx with Acanthamoeba this organism caused an infection of the lung. When it infected man it was usually missed in pathological examination. Perhaps the organisms became encysted and subsequently excysted from the tissue. Stamm added that one potential source may be physiotherapy pools, which often contained high numbers of organisms.

A. Jones (Employment Medical Advisory Service, Cardiff) thought there was a need to look for episodes of sensitisation in the general community and more particularly in different industries and in agriculture. J. C. Gilson agreed but thought this was for the future when more was known about the disorders.

Conditions leading to the build-up of organisms under static conditions were then discussed. D. E. Hughes reported that microbial biomass production results from two interdependent factors, namely, growth (increase in size) and increase in numbers (reproduction). Most precise information of these has been obtained in homogeneous systems (eg, chemostats) but in nature heterogeneous (unmixed, static) conditions are more common. Humidifier water tanks are of this latter category. Several factors affect biomass yield and productivity, for example, (*a*) *water activity:* Fungi are less demanding than bacteria, algae, and protozoa. Microbial oxidation can produce water in apparently dry systems; (*b*) *food supply:* Microbes exhibit the widest flexibility in organic compound utilisation in the biosphere; some synthetics (eg, polymers) do resist biodegradation; (*c*) *temperature:* The optimum temperatures for microbial biomass production range from 0 to 80°C; the range for survival is far wider but depends on other conditions, especially water activity.

Hughes continued that on mixed substrates growth and metabolism lead to a succession of species and products. Microbial infections of air may arise from paint, plasterboard, paper, oils, food products, and condensates of volatile wastes, including human. Thus effective remedies may result from considering these complexities of the ecosystems and can often be both cheap and simple.

S. Ratcliffe said that Department of Health and Social Security policy was the use of steam injection for many reasons but warned that this could introduce contaminants from the water boiler if care was not taken. It could also increase the cost. There was a need to establish the size of the medical problem for the country as a whole before any steps were taken

as they would probably be expensive nationally. It was commented that some industries needed a high humidity which, when combined with cooling in summer, produced water-wetted surfaces, thereby increasing the hazard of bacterial build-up at these points. Perhaps (R. Stone, Johnson & Johnson, Portsmouth) there was a case for direct sprays.

J. C. Gilson said that while it was desirable to identify the causative organism the main aim was to ensure conditions in which susceptible individuals did not become sensitised.

J. Pepys saw a need for a standard battery of antigens which could be applied to the serum from whole factory populations; the presence of antibodies would then be evidence for a potential hazard requiring investigation.

J. E. Cotes (MRC Pneumoconiosis Unit, Cardiff) suggested that while for an occupational group a positive reaction was evidence for a potential risk, there need not be a material risk to individuals; this was the case, for example, for many farm workers and others. A threshold should be designed at which action might be necessary.

A. Jones agreed and said that certainly action should be taken in the presence of clinical disease.

Other points which were made included the cost of steam humidification, which, on a national scale, would increase the country's fuel consumption by 15%. Other alternatives were to reduce the level of humidity in circumstances where the main requirement was for constant levels rather than for a high humidity. This was the case in the printing industry, where constancy was required for overprinting but a high level was not necessary except to the extent that the paper might otherwise be too brittle. As a general remedy there was a case for using clean water in humidifying systems, keeping it clean, and avoiding recirculation.

Comments

Many other points were raised, both in the meeting and at informal gatherings throughout the two days, that are worthy of comment.

There was general agreement that the disease was a 'Monday' illness and that this similarity with byssinosis could not be overlooked. Also the incidence was highest in the winter months, presumably due to the higher degree of air recirculation. Perhaps the Monday syndrome as seen in the UK was slightly different from some outbreaks reported in the USA; also there were certain differences between the UK outbreaks, for example, the expression of a pyrexial episode without respiratory distress in the local rayon factory compared with episodes with pulmonary involvement as described by Dr. Pickering. It is possible that the differences are related to length of exposure. The first symptoms described by Dr. Pickering were noticed in 1965 whereas in the local rayon factory the symptoms were noticed in 1975–76. Again, this may be a reflection of different antigen sources; the lack of complete reactivity between sera and antigens from the Aberdeen, Nottingham, and Leeds outbreaks suggested that further clarification was required. This was borne out by the reactivity of *N. gruberi* on gel diffusion with sera from the local factory and Acanthamoebae on immunofluorescence with sera from the other outbreaks. The similarities in the available nutrients in the environment at all four outbreaks could not be overlooked. The printing industry and the rayon industry both use regenerated cellulose for paper and fabric manufacture. Thus the possibility of a common microbial cycle existed, with a complex food chain operating up to protozoal level.

Another puzzling feature was the extremely low level of airborne material necessary to produce an episode in susceptible individuals. The quantity of respirable material in the local rayon factory office was almost the same as outside the factory; there was also little dust level variation between Mondays and other days of the week. This differs from classical extrinsic allergic alveolitis, where exposure is often well defined, for example, breaking hay bales, feeding cattle, or cleaning pigeon lofts, although it must be borne in mind that there is not a great deal of quantitative data available on these topics. Possibly the pyrexial episode of humidifier fever represents the first step in the sequence of events leading to a 'full' attack of extrinsic allergic alveolitis; alternatively, another mechanism other than immune complex–complement activation may be at work.

Finally, it was suggested that the term humidifier fever did not cover all cases (see contribution by J. A. R. Friend and J. Gaddie) and perhaps another name would be more appropriate.

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Topics presented

G. W. BRUNDRETT
Need for ventilation
S. RATCLIFFE
Design and construction of air conditioning systems
J. PEPYS
General aspects and mechanisms of pulmonary hypersensitivity reactions
J. A. R. FRIEND and J. GADDIE
Extrinsic allergic alveolitis due to cooling water contamination

C. A. C. PICKERING
Responses due to water from a vacuum ring pump
C. R. RYLANDER
Responses to airborne sludge
C. R. RYLANDER
Responses to airborne bacteria
O. M. LIDWELL
Microbial control measures
J. L. LONGBOTTOM
Serological testing in humidifier fever
D. HUGHES
Build-up of organisms under static conditions
J. H. EDWARDS
Sources of antigenic material
D. WARHURST
Amoebae and allergic lung disease
W. P. STAMM
Pathogenicity of free living amoebae
W. I. CRANSTON
Mechanisms of pyrogenic responses

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