Thorax (1976), **31**, 621.

Biological effects of proteolytic enzyme detergents[1]

A potentially serious respiratory hazard during the manufacture of enzyme detergents was first reported by Flindt (1969) and Pepys *et al.* (1969). In the eight years since the hazard was identified much effort has been put into investigating the extent and nature of the effects and removing their cause. A feature of the work has been the national and international exchange of medical and industrial hygiene information between producing companies and the help provided by academic departments of immunology and respiratory disease to assess the biological effects. These have included serial observations to monitor the efficacy of the rapidly improving dust conditions achieved by the engineers.

In 1974 Professor Hans Weill, of Tulane University, New Orleans, and Dr. John C. Gilson, Director of the MRC Pneumoconiosis Unit, suggested that 1976 would be an opportune time to review the scene to obtain a general picture of progress and of the lessons learnt which might have applications elsewhere. The opportunity given by such a meeting was welcomed by the companies in the USA and in Europe making the enzymes and the enzyme detergent products. Topics covered included: making the enzymes and the detergents; toxicological and immunological studies in animals; clinical features of the effects in small groups and in individuals; immunological surveillance of exposed working groups; and cross-sectional and longitudinal studies of respiratory symptoms and lung function of production workers in several countries. There was an excellent opportunity, therefore, for engineers, epidemiologists, immunologists, occupational physicians, respiratory physiologists, and statisticians to contribute to the general assessment of current knowledge in this field. Papers prepared for the meeting will be appearing in appropriate journals. A list of the papers precirculated is given (p. 631) to assist in tracing the published articles later. A majority of the work presented was unpublished.

[1]Report of a symposium held on 4–5 May 1976 at the MRC Pneumoconiosis Unit, Cardiff, sponsored by the Medical Research Council, UK, and the Soap and Detergent Industries Association (SDIA), UK. For the list of participants with their affiliations and the titles of papers presented, see p. 630. The report was prepared by J. C. Gilson, C. P. Juniper, R. B. Martin, and H. Weill with the assistance of Rapporteurs—D. R. Davies, J. H. Edwards, C. P. Juniper, W. E. Parish, and C. E. Rossiter.

PRODUCTION AND DUST CONTROL

Van Velzen (Gist-Brocades, Netherlands) described the manufacture of the enzyme by a fermentation process, using *Bacillus subtilis*. The purification steps need extremely careful quality control. This is achieved by a batch process of manufacture. After purification (germ removal), a concentrated enzyme powder is produced by precipitation, filtration, and drying. To ensure a clean environment, the relatively dusty powder is handled in a closed system. As in the detergent industry, great attention is now paid to engineering detail where spillages or emissions of dust may occur; for example, an ingenious pneumatic double seal is used in the bagging of the powder. In the detergent factory the heat-sensitive enzyme has to be incorporated in a powder base which is manufactured by spray drying in a large tower at elevated temperature. The manufacture of the powder base was described by Davies (Lever Brothers, UK) to give an indication of the scale of the manufacturing process (tons per hour). After addition of the heat-sensitive enzymes and other chemicals, the product is mixed and then put into cartons in the packing room. This was previously a labour-intensive area where exposures readily occurred. Great attention has been and is now continuing to be paid to reducing these exposures. The dustiness of the product has been reduced by 'encapsulating' the enzymes. It was glued to a coarse ($>150\ \mu$) granule of phosphate base by a tacky non-ionic detergent (an ethoxylated linear aliphatic alcohol). The enzyme manufacturers had now made further improvements by forming 'marumes' and 'prills'. These are beads containing the enzyme embedded in non-dusty matrix. The 'prill' is produced by spraying a molten mixture of the enzyme with an organic material (non-ionic) into a tower in which the droplets cool to form regularly shaped non-dusty solid beads. By this means the enzyme is incorporated into a less friable matrix than in the earlier methods of encapsulation.

Bruce (Procter & Gamble, UK) described the advances in dust monitoring. The SDIA had developed a high-volume (700 l/min) Galley sampler with a performance similar to the MSA Fixt Flo. They also used a Casella personal sampler (2 l/min). At low dust concentrations the instruments agreed well; at high levels ($>0{\cdot}2$ Gu/m^3; Gu=Glycine units, see Glossary of Terms, p. 628) the personal sampler gave values of up to 10-fold greater. The reasons for this were not yet clear. A continuous sampler was developed; this is useful in detecting peak emissions of enzyme. The use of an Anderson sampler had shown that about 50% of the dust collected was respirable ($<7\mu$), and 70% of the enzyme dust was in this respirable fraction. However, there was some difficulty in interpreting the results of an Anderson sampler with a dust consisting of fragile particles which might be fractured into smaller pieces during passage through the instrument.

Davies used the results of dust assessments from one factory from 1969 (when the first measurements were made) to 1975 to show the secular trend of improvement achieved by following the SDIA recommendations (SDIA, 1969). During the six years there had been a six-fold reduction in the average atmospheric dust concentration in the packing rooms of the four UK detergent factories (1200→200 μg/m^3), and in the same period the amount of associated enzyme dust had been reduced by a factor in excess of 30 (2–3 Gu/m^3) in 1969 to 0·05–0·1 Gu/m^3 in 1975. Further improvements had been achieved by the use of 'marumes' and 'prills' (SDIA Medical Review 1969–75). Atmospheric enzyme levels were now at the present limits of analytical detectability.

TOXICOLOGY

Cambridge (Unilever, UK), described the effects of heavy exposures in guinea-pigs and rats to aerosols of solutions of enzyme preparations. The effects were dose-related. Guinea-pigs exposed to 80 000 Gu/m^3 for one hour (enzyme activity 1100 Gu/mg) showed respiratory distress and hypothermia with some fatalities. Histologically, intra-alveolar haemorrhage with eosinophil and neutrophil infiltration was seen. At a quarter of the dose for half the time only eosinophil-neutrophil infiltration was seen. Similar effects were observed in animals exposed to dry enzyme powder. The rat was much more resistant to the effect of exposure to enzyme aerosols; even at the highest concentration alveolar haemorrhage was much less marked. The haemorrhagic response induced by high concentration of enzymes could also be demonstrated on the exposed vascular bed of the rat cremaster muscle preparation. Heating the enzyme preparation prevented pulmonary and cremaster muscle injury.

In the guinea-pig repeated weekly exposures to aerosols at a level which did not induce pulmonary damage at a single exposure resulted in systemic sensitization after the third or fourth exposure. Mediation of the immune system was shown by an immediate skin reaction, positive Schultz-Dale reaction, and passive cutaneous anaphylaxis reactions (PCA). The latter reaction established that the predominant circulating antibody was IgG1a. With continuing exposures at weekly intervals, the respiratory response decreased and was virtually absent after 20 exposures. This was associated with an increase in the total level of specific enzyme inhibitor in the serum.

The difference in responses of isolated normal or 'sensitized' guinea-pig lungs, when challenged with enzyme preparations, confirmed that the bronchospasm induced in normal lung was associated with the release of histamine, whereas the effect on sensitized lung was associated with a greater release of histamine and SRS-A. *In vivo* studies using specific antagonists confirmed these findings.

The effect of detergents on the immune response was seen only at high detergent levels. When guinea-pigs were exposed to very high levels of a detergent aerosol (anionic or non-ionic; 70 mg/m^3) in the presence of an enzyme aerosol, which alone did not produce sensitization (6000 Gu/m^3), a 40% incidence of sensitization occurred. Exposure for a total of 3500 hours (18 h/day, 4 days/week, for 1 year) to dust eluted from enzyme washing powder product, at levels of about 2 mg/m^3, with enzyme activity 1 Gu/m^3, produced sensitization in only 1 of 16 guinea-pigs.

Ritz (Procter & Gamble, USA) reported on the relative anaphylactogenicity and immunogenicity of a number of commercial enzyme preparations—Alcalase, Rapidase, and Monsanto DA-10—together with an experimental Alkaline Protease. Guinea-pigs were given enzyme intratracheally weekly or biweekly in the range of 10 ng–10 μg (protein nitrogen) per dose. The pulmonary response was graded in severity on a five-point scale. Serum samples were examined by PCA for the presence of IgG1 and by immuno-

diffusion for precipitating antibodies against the enzyme. Dose response relationships were observed for each enzyme preparation, symptoms of anaphylaxis being produced in general at about 1 μg dose. At 10 μg, all animals exhibited symptoms often after the second dose, and there was little difference between the preparations. However, in terms of immunogenicity Alcalase induced lower levels of IgG1 and precipitating antibodies than did the other enzymes. Augmentation of the anaphylactic and immune responses to Alcalase by anionic and non-ionic detergents was seen at high detergent levels.

Brown (Procter & Gamble, Belgium) had studied in detail in Cynomolgus monkeys the effect of inhalation of enzyme and detergent alone, and in combination at varying doses. The enzyme was a mixture of Novo-Alcalase and Milezyme 8X. The experiment lasted for six months and exposures were to a highly (95%) respirable dust. The effects were studied by behavioural changes, clinical signs, pulmonary function tests, and/or histopathological alterations. Extensive haematology, clinical chemistry, and urine analysis were also made.

At levels of 1 mg/m^3 detergent dust with 200 μg/m^3 enzyme, no histological, pulmonary function, or biochemical effects were observed. Higher dust levels, 10–100 mg/m^3, produced functional and histological effects but were primarily related to the detergent level. The histopathologic effects produced by exposure to high levels of detergent (10–100 mg/m^3) were similar to those seen after exposure to known lung irritants (O_3, SO_2, and NO_2) but were partly reversible on cessation of exposure. Lung function tests suggested bronchiolar constriction; the histology showed some alveolar fibrosis and bronchiolar epithelial hyperplasia. Pulmonary function was reversible after a period of no exposure. An immunological response (precipitating antibodies) was produced at all levels of enzyme dust exposures.

These varied experiments have defined a number of the responses, toxicological and immunological, produced by enzymes and detergents and their interactions. The destruction of lung tissue with the development of emphysema, such as is seen with the enzyme papain, was not a feature. Dolovich (Ontario) suggested that this was due to the detergent enzyme having no elastase activity which was necessary for the production of emphysema. Parish (Unilever, UK) commented also that the elasticity of the guinea-pig lung was greater than that of human, rabbit, or horse lung, so that cellular destruction in the guinea-pig might produce less emphysema. Wagner (PU, UK) said that the marked focal peribronchial reaction, seen in the very high levels of detergent, was similar to that seen in the earlier response to some inorganic dusts such as asbestos.

Repeated low doses of enzyme, except that of the very lowest dose, produced sensitization mediated by IgG1a. Tolerance was acquired after repeated exposures, probably due to the presence of other specific antibodies to the enzyme blocking the IgG1a mediated response. Cambridge said that, in general, the enzymes were less allergenic than egg albumin but more so than trypsin. All studies had shown a potentiating effect of the enzymes by the detergents. This had been known for many years (Parish) but the mechanism of potentiation was not understood. Little was yet known about the effect of particle size on the production of sensitization.

In extrapolating the results of animal studies to man, Parish emphasized the marked species difference in the 'shock organ'. The immediate allergic responses affected different organs, for example, in the guinea-pig the lung, and in some monkeys the gut. It was also necessary to recognize the big differences in the exposures as compared to man. In a majority of the animal studies, these had been relatively short term and at high concentrations.

IMMUNOLOGY

In the Third Session the extensive knowledge now acquired about the immunological effects based on clinical studies of individual cases and of groups of enzyme detergent workers was discussed. The only proven allergic response in man to the enzyme detergent dusts is an IgE-mediated asthma, or rhinitis, and very occasionally conjunctivitis. The enzymes, like common environmental antigens, for example, grass pollens, stimulate formation predominantly of IgE antibodies.

The enzyme-induced allergy or allergic disorders are almost limited to factory workers exposed to the dusts usually for fairly long periods. An exception was one sensitized housewife (case presented by Dijkman, Netherlands) who was exposed to antigen contaminating the clothes and the dusty hair of her husband, an enzyme detergent factory worker. The incidence of sensitization depends upon the amounts of enzyme antigen in the work rooms, duration of exposure, and, to

some extent, the susceptibility of the workers. When dust levels are high all persons, atopic and non-atopic, are at risk, though not all become sensitized. At low dust levels atopic persons are more susceptible than non-atopics. If only traces of the dust occur, as in some factories now, very few people, if any, become sensitized.

Detection of specific hypersensitivity is by the prick test and RAST (see Glossary of Terms); a positive skin test or RAST may occur before clinical symptoms are apparent. Clinical effects of the dust are more readily interpreted with the help of the results of immunological tests.

For reliable prick test results, a standard gentle procedure and concentrations of antigen that do not induce antigen-nonspecific irritation are essential (Pepys, London). The crude enzyme preparations contain components of culture media, cell debris, and some bacterial spores, so a standard skin prick test antigen containing all the enzyme allergens is essential. This was prepared by Unilever Research and made available to the SDIA and internationally (How, Unilever, UK). When using this antigen there was 99% agreement between negative skin tests and a negative RAST (How). Zachariae (Denmark) reported the use of skin prick tests with crystalline Alcalase in Rumania before enzyme detergents were in use. Six per cent of atopic and other patients (280) were positive, but only one had positive RAST. He thought the skin test might be non-specific in some instances, and that RAST was useful to confirm an allergy. In a study by Pepys, several thousand atopic persons were routinely tested with enzyme antigen when attending an allergy clinic, whether or not they used enzyme detergents at home; they showed no evidence of allergy to these enzymes. A similar survey of over 300 housewives reported by Dijkman led to the same conclusion. The essential need for careful standardization of the prick test procedure and the antigen was repeatedly stressed.

Prick tests have now been repeated in many hundreds of people. There was good evidence that this did not lead to sensitization of those tested. Indurated (firm oedematous) skin prick test responses occurring 6 to 8 hours after test, and occasionally severe, were reported by Newhouse (London) and Martin (Procter & Gamble, UK). There was considerable discussion about this type of response. It had features comparable with an Arthus reaction, but there is no immunological proof. This response may not even be immunological. Bacterial substances are particularly likely to induce inflammatory responses resembling Arthus reactions in the absence of appropriate antibody (Parish). Some persons who show such reactions on one test do not do so 6 or 12 months later (Juniper, Unilever, UK; Parish). This was not typical of Arthus sensitivity in persons continually exposed to an antigen.

The results of RAST to detect IgE antibodies to enzymes carried out independently in different laboratories were very consistent (Pepys; Wilson, Procter & Gamble, USA; How). Stored frozen sera must be centrifuged to remove aggregates in order to obtain reproducibility of duplicate tests in the RAST for Alcalase (Pepys). There was a statistically significant correlation between the size of prick test wheals of 3 mm diameter or greater and the IgE antibody measured by RAST: [$r=0{\cdot}5$ (Wilson) and $r=0{\cdot}6$ (How)]. As would be anticipated, this does not apply when RAST counts are high. The correlation is not sufficiently high for prediction in the individual. Furthermore, important evidence was presented that the demonstration of, or increase in, serum IgE antibody may occur one or two years after appearance of positive prick tests (Wilson), and nearly 25% of skin test positive employees never did develop elevated IgE levels. This shows that monitoring allergy in factory workers by RAST only (Zachariae; Witmeur, Novo, Denmark) may miss early cases of hypersensitivity, but 'false positives' were avoided.

There was evidence about reversion of prick tests in those no longer exposed to the enzymes. In the United States none of those sensitized for two or more years to Monsanto DA-10 enzyme mixture but 7% of those sensitized for at least two years to Alcalase reverted to prick test negative (Wilson). In the United Kingdom, 36 out of 564 (6·4%) workers sensitized to Alcalase/Maxatase reverted to prick test negative (Juniper).

A six-year serial study in one US factory (Wilson) had shown that, starting in 1969, the proportion of workers becoming prick test positive within six months ranged from 0 to 39% dependent on the job, but by 1970 the reduction in dust levels and the less frequent production of the product had greatly reduced the incidence. No one starting work after 1970 had been sensitized. In this factory there was no statistically significant difference in sensitization rates of the atopics and non-atopics as measured by either skin or RAST tests.

In tests for antibodies in other immunoglobulin classes, no precipitating antibody was seen in

Ouchterlony double diffusion agar plates, though non-immunological complexes of enzyme with $\alpha 1$ anti-trypsin and $\alpha 2$ macro-globulin were formed by most sera (How). The very discriminating techniques, capable of detecting extremely small amounts of antibody, RID (see Glossary of Terms, p. 628), showed anti-enzyme antibodies which were identified as IgG in 43 of 65 sera, IgA in 21 of 65 sera, and a few with IgM by indirect radio-immunoelectrophoresis, IRIEP (see Glossary of Terms), (Pepys). The occurrence of IgG anti-enzyme by IRIEP was confirmed (How). An even more discriminating technique, a solid phase assay, also detected IgG antibodies to enzyme (Pepys), as did the 'sandwich' procedure used to test IgG fractions of sera (How).

IgG antibodies were detected only in the sera of persons exposed to enzyme dusts in factories and were found in almost all sera containing IgE antibodies (Pepys). They were usually present in very small amounts, possibly less than is required to mediate conventional Arthus reactions. They are evidence of exposure to the antigens, not of allergy, and probably reflect the natural formation of antibody as occurs in response to numerous environmental antigens.

It was strongly recommended that in order to achieve comparable results in all laboratories standard reagents should be available and standardized procedures used. Important technical points, for example, the effect on the *in vitro* techniques of storage of sera, should be published and reference sera made available to standardize the results. This would ensure more general conformity in investigations in different factories internationally.

CLINICAL

Juniper described the clinical pattern of disease arising from allergy to proteolytic enzymes produced by *Bacillus subtilis*, starting with the observations of Flindt and Pepys in 1969. The features of dyspnoea, wheezing, and cough, with minimal sputum, were accompanied by haemoptysis and chest pain in some patients. Conjunctivitis when it occurred was transitory and there were no sequelae. Dermatitis due to sensitization was not seen in detergent factories although a specific erythematous and desquamatory condition affecting the palmar surfaces of the hands was observed in workers producing the concentrated enzyme (Zachariae). The 1969 SDIA Code of Practice had recommended screening procedures and regular medical examinations. The specific skin prick test reagent described by How was effective and safe in use. The early clinical picture became modified over the next two years or so with the absence of haemoptysis and chest pain. The severity of asthma and dyspnoea diminished from an incapacity lasting days or weeks in 1968/69 to a milder process lasting hours and requiring minimal treatment. These changes coincided with environmental improvement. Analysis of case histories revealed three clinical types of asthma, immediate, non-immediate, and dual. Immediate asthma occurred 10 to 15 minutes after exposure and lasted 1 to 3 hours. In other subjects, asthma developed slowly at about 6 hours to a maximum at 8 hours and lasted up to 24 hours. Clinical features included sweating, pallor, and anxiety with marked tachycardia and dyspnoea. There was cough with minimal sputum. Generalized rhonchi were usually heard but the degree of bronchospasm was less than would be expected from the dyspnoea observed. Crepitations were not a striking feature, although occasionally present. The aetiology, particularly of the late asthmatic episode, was easily missed, so steps were taken to alert local practitioners. Asthma at work due to non-industrial antigens was also seen and could cause confusion. The atopics tended to have immediate asthma, whereas the non-atopics tended to respond 6 to 8 hours later. The time taken for recovery of respiratory function after an attack of bronchospasm, produced by proteolytic enzyme, depended upon the duration of stimulation and the dose. Examples were given of full recovery and of the way an individual may react to other irritant dusts immediately after an attack of allergic asthma. Attacks of nocturnal asthma on succeeding nights following the initial episode were described and the similarity to the picture seen with other antigens was noted (Pepys). Allergy due to this antigen is primarily type I, and the clinical picture had become progressively milder as the environment improved.

Over 2800 employees in UK factories had been reviewed under the SDIA recommendations for medical surveillance. No cases of fibrosing respiratory disease had been detected. Many thousands of serial chest radiographs had been reviewed without revealing any permanent occupationally related disease associated with the enzymes or detergent dust. One man developed an acute alveolitis with subsequent recovery. During the seven-year period there were no indications that serial skin prick testing caused sensitization. Thirty-six subjects who were prick test positive on

two or more occasions reverted to and remained prick test negative. Thirteen subjects who had immediate skin prick test responses also developed a reaction which began about 6 hours after the test. This non-immediate reaction was characterized by a firm oedema, was larger (10 to 20 cm) than the immediate reaction, and lasted up to 24 hours. These reactions could cause temporary inconvenience (Newhouse). The discussion revealed that their significance was not yet understood.

Ogilvie (Liverpool, UK) gave the results of a seven-year follow-up of the early heavily exposed group of 12 workers described by Flindt (Manchester, UK). Lung function studies were carried out in 1968, 1971, and 1975. At the initial examination abnormalities included increased residual volume, *increased* transfer factor (T_{Lco}), and *increased* diffusion co-efficient (K_{co}). Twelve were seen again in 1971 and nine in 1975. No significant deterioration occurred in any aspect of lung function. The residual volume and transfer factors tended to revert to normal; those initially high had come down, those low had risen. This important study revealed no evidence that relatively heavy exposure to proteolytic enzymes in detergent powders caused, in those removed from further exposure, any progressive bronchopulmonary dysfunction due to airways obstruction, emphysema, or fibrosis over the seven-year period. In 1975, 10 of the original 12 who could be traced were at work. Only one of the nine tested had any respiratory symptoms. He had a history of bronchitis before exposure to the enzymes. The chest radiographs were normal. The interpretation of the transfer factor changes was discussed. It seemed possible that some subjects had had a predominantly asthmatic and others an alveolar reaction. It was concluded that if proteolytic enzymes caused an asthmatic response with an increased diffusing capacity, an associated alveolar reaction could not be excluded solely by the finding of a normal diffusing capacity.

Dijkman described the effects in six male workers exposed and sensitized in 1969 to detergent containing proteolytic enzymes. Nasal irritation was a presenting symptom, followed by wheezing which in three men was initially nocturnal only. Skin prick tests were carried out with common allergens which revealed slight reactions in three subjects, and to the enzyme reagent in five. In these five, bronchial challenge with the enzyme produced a dual asthmatic reaction, and in one only a late reaction. The immediate response subsided substantially within 4 hours, to be followed at approximately 5 to 8 hours after inhalation by a 'late' reaction. This 'late' reaction was accompanied by fever and leucocytosis lasting many hours or days, but there was no evidence of alveolar involvement. Immunodiffusion studies failed to demonstrate the specific precipitins to extracts of proteolytic enzymes from *Bacillus subtilis*.

In the discussion it was clear that the mechanism of production of the non-immediate reaction to the enzyme (and other antigens) was not fully understood, but that it did not necessarily imply a cellular response at the alveolar level.

EPIDEMIOLOGY

At the Session on epidemiology, six papers were presented, mainly concerned with continuing medical surveillance of workers engaged in producing proteolytic enzymes and in manufacturing enzyme detergents. Witmeur reported on experience in the production of the enzymes. Definite respiratory symptoms were present in 2% of the 554 workers examined in May 1975. Positive RAST tests to Alcalase occurred in 3% of workers in 1970 and in 2% in 1974, but the rates were much higher in the atopic workers (up to 27%). There was no relation between forced expiratory volume (FEV_1) levels and duration of employment or level of exposure to enzymes. In 172 workers examined at intervals up to six years, although the atopics had lower levels of FEV_1 there was no excess decline over the period in any group of workers and no relation to exposure.

Lainhart (Procter & Gamble, USA) reported a study of prick test results and lung function of 4578 people in four European plants making enzyme detergent products over a six-year period. There had been a decrease in sensitization rates for new employees from 5% in 12 months in 1970 to 3% in 1974. Although significant differences did occur between plants, there was no evidence that lung function (FEV_1, FVC) was reduced or declined faster among atopics, those with a positive history of response to exposure, or those prick test positive. The survey was based on past records. It revealed differences which may well have arisen from the way the information was first recorded and collected. It showed the potential value of systematic surveillance records in a large multinational company. In future the methods of recording will be better standardized.

Pham (Nancy, France) described a detailed cross-sectional comparison of two detergent

enzyme factories by studies of representative samples of 130 and 150 production workers. Although the proportion with bronchitis (9%) or who were prick test positive (11%) were very similar in the two plants, asthma (7% and 1%) and rhinitis (18% and 3%) were much more prevalent in one plant. Although there were some significant changes in lung function after acetylcholine, particularly in intermediate flow rates and FEV_1, there was no relation of lung function changes to skin test positivity or duration of employment. Pham recommended that acetylcholine provocation tests should be added to the tests routinely carried out in the industry to improve sensitivity of functional measurement.

Weill (New Orleans, USA) reported on longitudinal studies of a representative sample of 230 workers exposed to detergent enzyme dust in four US plants; these studies started in January 1970 shortly after the enzyme was first added to the detergent. There had been a marked reduction in dust exposure since 1970, partly because of improved environmental control, as in Europe, but also because enzyme-containing products were no longer made on all working shifts. Positive prick tests were very common, ranging from 40% to 75% in the four plants. (This high rate was in part due to the method of selecting subjects to include those with higher exposure.) Definite correlation exists between atopic status and prick test results; between exposure to enzyme dust and sensitization rate; between respiratory symptoms and atopicity but not additionally with smoking; and between job-related symptoms and atopicity, prick test results, and dust exposure.

Lung function changes have been assessed from the start of the study, ie, after some exposure. After initial decline before 1972, TLC, FEV_1, FVC have all increased during the five years of study. The transfer factor had fallen slightly but still exceeded 100% predicted in three out of the four plants in 1975. The net effect is that the final values for lung function all fall well within the normal range.

Newhouse had studied since 1969 at six-monthly intervals a group of men who had already been heavily exposed to the enzymes. Fifty-five were prick test positive and 47 prick test negative. Both groups had worked in the same departments and had similar lengths of exposure. High IgE levels were found in men with very active skin reactions and clinical symptoms. Over the period the mean FEV_1 of the prick test positive and negative groups were similar and declined at similar rates, but the mean FEV_1 of the prick test positive group was initially and throughout significantly lower than the predicted level. The FVC results were above predicted for both groups, whereas the FEV_1/FVC ratio was lower in the positive group. These subjects were a subgroup of the total population reported by Juniper and by Lainhart.

Juniper presented the SDIA Medical Review 1969/July 75 (SDIA 1976). He emphasized that this was a study of the whole industry in the UK (2865 people). Spirometry and skin prick testing were carried out on the whole population at six-monthly intervals. With falling dust levels conversion to skin prick test positive had also fallen from the original high values of 85% in atopics and up to 35% in non-atopics. Atopics were later excluded from working with these products and the rate for non-atopics had fallen to around 20 %.

The improved environment had led to a reduction in the number of workers transferred for medical reasons from enzyme dust exposure—an average of 25 per year for 1968 to 1971 to less than 10 per year for 1972 to 1975.

Serial studies of lung function in 2000 workers showed that there was no change in FEV, expressed as percent of the predicted, in relation to either reaction to the enzyme prick test or to the degree of contact with enzymes. The same findings applied to the FEV/FVC ratio. The rate of decline of FEV was about 45 ml per annum in all groups; it was similar for other groups within the industry not involved in enzyme detergent production, for new entrants to the industry, and for workers in unrelated industries in the same geographical areas.

The discussion on the studies of lung function was opened by Cotes (PU, UK). He said that the findings presented showed that the present risk to health of current exposure levels of proteolytic enzymes was likely to be small. One of the problems was, were the best tests of lung function being used? He suggested that FEV_1 was the most relevant and reproducible test, but some additional measures of flow rate needed to be studied in more detail, eg, FEV_{25-75}. There was support for this, especially as the flow rates could now be readily measured at the same time as the FEV. He also showed that, even for the most repeatable tests of lung function, measurement error is about 5% and prediction error about 15%, so that reference values have little useful-

ness except in the detection of marked disease. To assess accurately a rate of decline, it would be necessary to measure 100 people for five years, and even then this may detect only a 50% change in rate of fall of lung function.

He referred to the reports from Australia that the elastic recoil pressure in the lung might be reduced in those with heavy exposure in the past. Musk (Australia) confirmed that a difference, which was apparently related to the past intensity of exposure, had been observed in a follow-up of these subjects. No new information on this aspect was reported from the studies in Europe and USA.

General discussion centred round three main topics. First, lung function—it was eventually agreed that generalized statements about lung function levels in terms of prediction relations could be misleading because of instrumental, operator, and naturally occurring intergroup differences. The observations of Ogilvie and Weill on the fall of transfer factor from above normal to normal levels were thought consistent with clinical observations on ordinary cases of asthma responding to treatment, but good documentation of this change was not produced, although high values for transfer factor during asthmatic episodes have been previously reported. The discussion also brought out the difficulty in ensuring, when using tests of lung function in the long term, that technical changes did not introduce a bias.

There was less eventual agreement about terminology for prick test reactions. 'Arthus-like' was objected to strongly, particularly in close temporal association with a discussion on alveolar changes, although the same discussant confessed to the use of 'non-immunological Arthus-like' in print. Suggestions were made for the use of descriptive terms such as 'immediate', 'non-immediate', eg, '6–8 hour' reactions. This was clearly a topic needing further research leading to better definitions.

A strong demand was made for the separation and reporting of results on the few 'responders' to detergent enzymes. The general picture of fewer workers becoming sensitized, and the absence of any average deterioration of lung function related to exposure in large groups, was reassuring but there were still a few workers who had to be removed from further exposure. Little had been said about the extent of their disability in a wider sense, for example, possible increased sensitivity to common antigens and other pollutants such as cigarette smoke. It was, however, argued that results on such people had been presented in the papers. It was unfortunate that lack of time prevented further discussion of this, which might have led to further mutual understanding.

CONCLUSIONS

A number of general points came out of the meeting:

(1) The early detection and publishing of the potentially serious hazard from a new industrial process led to rapid and effective collaborative effort to identify the nature of the problem and bring it under control.

(2) The engineering control of the dust and the medical surveillance of the employees have prevented much serious disability. It has also enabled those with a positive skin prick test to continue at work without developing respiratory symptoms.

(3) When lung function and immunological tests are used for serial observations in large groups of individuals in several factories and in different countries, the validity of the findings is critically dependent upon precise standardization of the tests. In the future much more attention has to be given to this aspect.

(4) The skin prick test, when performed correctly, is a safe, sensitive, specific, and useful measure for biological monitoring of the response level to the enzyme. This observation could be of use in other situations.

(5) The enzymes used in detergents can produce an IgE mediated asthma which is dose related. Exposure levels during the manufacture of the enzyme and the enzyme detergents can now be kept so low that very few people will become sensitized. Occasional cases of sensitization are, however, likely to occur due to a combination of circumstances, such as accidental spillage and highly susceptible subjects.

(6) Knowledge acquired over the last eight years indicates that respiratory disease other than asthma is unlikely to arise from the use of these enzymes, but continued surveillance of those exposed is still required.

(7) The risk of respiratory and skin test sensitization to the enzyme detergents during their domestic use is extremely small.

GLOSSARY OF TERMS

GLYCINE UNIT

This is a measure of the enzyme activity. It is measured by the release of amino acid from a

standard protein substrate (a substituted casein). The amino acid is estimated colorimetrically. A number of units are in use and are related as follows:

1 Anson Unit=623 000 Glycine Units= 243 000 Delft Units.

1 μg of 1·5 Anson Units of protease activity as received from the enzyme manufacturers is approximately equivalent to 1 Glycine Unit/mg of enzyme concentrate.

IgE

Ig designates immunoglobulin, or globulin with antibody activity. IgE is the reagin, the antibody conferring immediate-type hypersensitivity and mediating asthma, hay fever, and the wheal and flare of the prick test. It is present in extremely small amounts in the serum and cannot easily be detected by the conventional precipitin and agglutinin techniques. IgE is usually detected *in vitro* by RAST.

GUINEA-PIG ANAPHYLACTIC ANTIBODIES

Two classes of antibody cause immediate-type allergy in the guinea-pig. One is a subclass of IgG, designated IgG1 which occurs in two forms, IgG1a and IgG1b. These are the antibodies usually formed after sensitization in the laboratory. IgG1a is mentioned in this report. The other class of antibody is IgE, which has properties similar to those of human IgE.

IgG AND IgM

Immunoglobulins G and M are present in much larger amounts in serum than IgE. They are precipitating antibodies and can activate complement and may mediate Arthus reactions. They (particularly IgG) are associated with extrinsic fibrosing alveolitis, eg, farmer's lung. Their presence is evidence of exposure, not necessarily of disease, as persons may have precipitins without disease.

IgA

Immunoglobulin A is present in serum and in a modified form on mucosal surfaces. It is believed to protect by masking or covering the determinants of antigens, preventing allergic responses. It is not known to mediate allergy.

ARTHUS REACTIONS

These are skin test responses occurring 4 to 8 hours after antigen challenge, either alone or as a dual response following a wheal and flare. They may be indurated, erythematous, with a surrounding zone of oedema, or more extensive areas of soft ill-defined oedema, with or without central induration. The reactions vary greatly according to the degree of hypersensitivity and concentration of antigen. Intradermal tests may induce severe congested or haemorrhagic reactions.

RAST

This radio-allergosorbent test was devised to detect the very small amounts of IgE antibody in sera. Antigen is coupled to inert particles or discs, and antibody in the serum being tested binds to the coupled antigen. The antigen is then treated with anti-IgE globulin labelled with a radioactive isotope. The amount of radioactivity bound, usually expressed as counts per minute, bears a direct relation to the amount of IgE antibody in the test serum that is bound to the antigen. In the case of Alcalase, aggregates occur in the serum stored for more than one to two years. These may combine with the enzyme coupled to the test substrate. The aggregates contain IgE and cause poor reproducibility of RAST. Removal of aggregates by centrifuging overcomes this.

OUCHTERLONY DOUBLE DIFFUSION PRECIPITATION

This test for precipitating antibody is carried out in thin agar gels, in which antibody in one hole or well diffuses into the gel to meet antigen diffusing from another well. If the antibody reacts with the antigen, lines or bands of precipitation occur where the antibody meets the antigen in sufficient concentration. This is a standard procedure to detect precipitating antibodies as are found, for example, in patients with extrinsic allergic alveolitis.

RID (radioimmunodiffusion)

The procedure is the same as that above, except that the antigen is labelled with a radioactive isotope, and the precipitation lines are detected by exposure to a photographic plate. This technique detects amounts of precipitating antibody too small to be seen macroscopically.

IRIEP (indirect radioimmunoelectrophoresis)

The test serum is separated in an agar gel, and the positions of the various immunoglobulins are detected by antisera to each of them (eg, anti-IgG). Antigen labelled with a radioactive isotope

is then added to the plates to see if any of the precipitated globulin (eg, IgG) is antibody for the particular antigen, and binds it. This technique detects very small amounts of antibody.

SOLID PHASE ASSAY TEST FOR ANTIGEN UPTAKE BY IgG ANTIBODIES

This is a procedure for the detection and measurement of extremely small amounts of IgG antibody, and of antibodies in the IgG subclasses or other immunoglobulin classes (Jacoby and Pepys).

'SANDWICH' RADIOIMMUNE ANALYSIS

This technique detects any antibody in a serum that binds to antigen. If the serum is separated into its various immunoglobulin fractions, it is possible to detect antibody in each class. It is a complicated procedure and open to technical errors not occurring in the solid phase assay test.

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LIST OF PAPERS PRESENTED

A. G. VAN VELZEN
Production of detergent enzymes

D. R. DAVIES
The manufacture of biological detergents

C. F. BRUCE
Dust monitoring and analysis

D. R. DAVIES and C. F. BRUCE
Environmental improvements

G. W. CAMBRIDGE
The pathology and pharmacology in laboratory animals of the allergic and proteolytic responses following inhalation of *Bacillus subtilis* proteases

H. L. RITZ and E. R. FLETCHER
Immunological responses in the guinea-pig

NANCY M. BROWN, WILLIAM B. COATE, WILLIAM M. BUSEY, WARREN H. SCHOENFISCH, and ELVIN A. NEWMANN
Inhalation toxicity using Cynomolgus monkeys

C. P. JUNIPER
Allergy due to *Bacillus subtilis*

COLIN OGILVIE
Seven-year follow-up of an early exposed group

J. PEPYS
Clinical immunology and methods of assessment (paper not precirculated)

E. ROYCE WILSON and PAMELA JONES DANNEMAN
Longitudinal study of IgE and skin test response related to exposures

M. J. HOW
Skin tests and serological tests in the diagnosis of allergic reactivity to *Bacillus subtilis* enzyme preparations

H. ZACHARIAE
Prick- and radioallergosorbent tests (RAST) to Alcalase in a population not exposed to enzyme detergents

O. WITMEUR
Medical experience in enzyme production

WILLIAM S. LAINHART and E. ROYCE WILSON
Six-year retrospective study of four enzyme detergent production plant employees

Q. T. PHAM
Respiratory manifestations and skin reagency among workers exposed to enzymes in the household detergents industry

HANS WEILL, JOHN DIEM, HENRY GLINDMEYER, and CARMEL WAGGENSPACK
Longitudinal studies of workers exposed to detergent enzyme dust in four United States plants

MURIEL L. NEWHOUSE
Longitudinal studies of prick test and lung function

C. P. JUNIPER
The medical review of workers in the enzymatic washing product industry in the United Kingdom: 1969 to July 1975
SDIA, April 1976

J. H. DIJKMAN
Clinical investigations in *B. subtilis* allergy

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