The increased prevalence of ANA appears to be related to the development of asbestosis and not to heavy exposure of asbestos dust alone.

The absence of ANA and rheumatoid factor in certain other fibrosing lung diseases suggests that the appearance of these autoantibodies is not the nonspecific consequence of any form of pulmonary fibrosis.

Studies *in vitro* have shown that in asbestosis patients with circulating antinuclear antibody evidence of lymphocyte sensitization can also frequently be found, though of lesser degree than is seen in patients with nonoccupational cryptogenic fibrosing alveolitis who present with a very similar clinical appearance.

Whether ANA can be used to identify individual susceptibility before the development of disease, or whether it influences the evolution of the pathology, will have to be determined from follow-up studies.

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Immunological and Clinical Findings in Workers and Consumers Exposed to the Enzymes of *Bacillus subtilis*

Allergic sensitization to enzymes of *Bacillus subtilis* of heavily exposed workers engaged in the manufacture of 'biological' detergents was reported by Flindt (1969) and Pepys *et al.* (1969). A number of other reports have been made of sensitization of factory workers (Greenberg *et al.* 1970, Newhouse *et al.* 1970, McMurrain 1970, Mitchell & Gandevia 1971) but few reports of sensitization of consumers (Belin *et al.* 1970, Shapiro & Eisenberg 1971, Falleroni & Schwartz 1971, Bernstein 1972).

Further investigations of the allergic effects of these and other enzymes have been made as follows (Pepys *et al.* 1973): (1) Skin and serological tests were made with the enzyme preparations, in asthmatic outpatients attending the Brompton Hospital for allergic investigation and regarded as possible consumers, and in a group of biological detergent factory workers (Greenberg *et al.* 1973) examined repeatedly. (2) The relevance of atopic status to sensitization was examined. (3) The nature and significance of antibodies in the different immunoglobulin classes to the enzymes was examined.

Prick Test Reactions

In asthmatic outpatients: Prick tests with purified *B. subtilis* proteinase (10 mg/ml) (Koch-Light) were made in more than 2500 subjects, with only two giving weakly positive and not clinically relevant Type 1 reactions. These tests were initiated in 1969 and about 800 new patients were tested each year. Of these subjects 80% were atopic, and about one-half of them gave Type 1 prick test reactions to up to three common allergens in a battery of 21 and the other half reacted to 4 or more allergens. These atopic subjects and particularly the more highly atopic group, are the most likely to be sensitized by exposure to allergens, thus providing in a sense a biological monitor of the presence and potency of environmental allergens.

In 506 of the above group, detailed questionnaires were completed and prick tests were made (all at 10 mg/ml) with the purified proteinase, and with the commercial *B. subtilis* enzyme preparations, Alcalase, Maxatase and Monsanto, with *B. subtilis* amylase, with a number of different enzyme preparations from *Aspergillus* spp. and with papain and bovine ribonuclease. In this group, 19% had not used biological detergents, 45% had used them for less, and 36% for more, than 6 months.

No reactions were obtained by the *B. subtilis* preparations or by papain and bovine ribonuclease. Positive reactions to the *Aspergillus* spp. enzymes where present corresponded with the known presence of allergy to *Aspergillus fumigatus*.

These findings contrast with those of Bernstein (1972) who reported positive intracutaneous skin tests to the *B. subtilis* enzymes in 27% of highly atopic subjects as well as reactions in moderate and low atopic subjects in an allergy clinic, and also similar high incidences of positive reactions to papain and bovine ribonuclease.

In factory workers: Out of 65 factory workers, 26 gave consistent Type 1 reactions on prick testing repeated at 5 intervals over a period of 18 months. In the 65 workers, 18 were regarded as atopic because of positive prick test reactions to common allergens, and 15 gave Type 1 reactions to *B. subtilis* enzyme preparations, compared with the remaining 47 nonatopic subjects, of whom 11 gave Type 1 reactions. The much higher incidence of sensitization to the enzymes in the atopic workers corresponds with previous reports by Greenberg *et al.* (1970), Newhouse *et al.* (1970) and Weill *et al.* (1971). The use of prick tests with common allergens for the identification of atopic subjects can therefore be of practical use for the reduction of Type 1 sensitization by exclusion of such subjects or by protecting them from exposure.

Radioallergosorbent Tests (RAST) for IgE Antibody

The RAST was used for measurement of specific IgE antibody against Alcalase and Maxatase by the methods of Wide *et al.* (1967) and Wide (1969). In order to assess the clinical and biological significance of the measurements of radioactivity obtained in tests, groups of sera were tested, including cord-blood sera, presumed to be negative since they do not contain, except rarely, IgE antibody against common allergens and would not be expected to have IgE to the *B. subtilis* enzymes; the sera of the asthmatic outpatients, all prick test negative and graded as nonconsumers, light, and heavy consumers; and the sera of prick test negative and positive factory workers.

The division into light and heavy consumers was made in one group, included in a first series of tests, according to whether the subject had used biological detergents for less or more than one year. In another group included in a second series of tests the division was made according to whether they had used these materials 25–150 times over a period of 6 months to 2 years (light consumers), or 200 times or more over periods of 6 months to 2 years (heavy consumers). The RAST results were closely similar in both groups of light consumers and were also closely similar in both groups of heavy consumers.

The RAST results showed that the radioactive counts were lowest in the 20 cord-blood sera. Increasingly and significantly higher counts were obtained in relation to the preceding group with the sera of 81 nonconsumers, 78 light and 84 heavy consumers. The sera of the 39 skin test negative factory workers had values only slightly higher as a group than the heavy consumers. The 26 skin test positive factory workers had much higher values than the others, with two-thirds having radioactive counts above 600/½ min, compared with none of the skin test negative factory workers and 6 out of 243 skin test negative consumers.

Correlation of RAST Results, Prick Tests and Atopic Status with Ventilatory Impairment

Of the 65 factory workers, 11 showed consistent ventilatory impairment on repeated examination over a period of 18 months. All gave Type 1 reactions to prick tests with the enzyme preparation, 7 were regarded as atopic because of Type 1 reactions to common allergens, and the RAST count was greater than 600/½ min in 8 cases. These subjects therefore showed the clinical significance of the association of atopy, Type 1 reactions to the enzymes and RAST counts above 600/½ min as obtained in this investigation. These findings differ from those of Mitchell & Gandevia (1971) who found no correlation between the skin test positive reactions and clinical symptoms, though they found more positive skin test reactions in the atopic subjects. Their patients had been very heavily exposed in contrast to those in the present study, and this may account for the differences, the heavy exposure perhaps producing sensitization of nonatopic subjects without the participation of the IgE antibody.

Radioimmunodiffusion (RID) Tests

The production of precipitates by the combination of the *B. subtilis* proteolytic enzymes with the α_2-macroglobulin and α_1-antitrypsin inhibitors in the serum makes it necessary to use special methods for the demonstration of specific antibody in the IgG, IgM and IgA classes. RID tests in which very small amounts of isotopically labelled (^{131}I) enzyme preparations were diffused against the sera did not give reactions with the enzyme inhibitors. Positive antibody reactions were obtained in 44 out of the 65 sera. No reactions were obtained with 36 blood-bank control sera, or 72 asthmatic outpatient sera of which equal numbers were derived from the nonconsumer, light and heavy consumer groups. Of the 26 prick test positive subjects, 21 were RID positive, compared with 23 of the 39 prick test negative subjects.

Radioimmunoelectrophoretic (RIEP) Tests

In this test the test serum was electrophoresed and reacted with an antiserum against whole human serum. This precipitated the different serum proteins at their characteristic electrophoretic positions in the gel. This preparation was then tested by addition of the isotopically labelled (^{131}I) enzyme preparations. These gave reactions in all cases with the precipitated α_2-macroglobulin and

α_1-antitrypsin. Reactions were also obtained with the IgG in 43 and IgA in 21 cases, showing the presence of specific IgG and IgA antibody against the enzymes. No IgA reactions were obtained without an associated IgG reaction. It was not possible to show IgM antibody. In tests on 18 cord-blood sera, 30 blood bank sera, 42 non-consumers, 48 light and 48 heavy consumers, reactions were produced with the α_2-macroglobulin and α_1-antitrypsin but not with IgG or IgA.

Conclusions

The findings show that at least two-thirds of the factory workers had been adequately enough exposed to the *B. subtilis* enzymes for the stimulation of IgG and IgA antibodies, which were not found in the consumers. The presence, however, of IgE antibody to the enzymes seems more relevant to the clinical manifestations, and since this is more readily produced by atopic subjects, this group needs particular attention. The possible contribution to the clinical findings of the sensitivity of the atopic subjects to common allergens must also be taken into account in trying to assess the influence of the patient's occupation, particularly where exposure to the enzyme preparations may be at a very low level.

The absence of prick test reactions to the enzymes in the large numbers of consumers tested, many of whom were highly atopic and therefore likely to be readily sensitized, shows that at the present time clinical sensitivity has not been produced by the encapsulated enzyme preparations in use in the UK, containing twice the concentration used in the USA. This contrasts with the high incidence of sensitivity to the enzymes in allergic subjects reported by Bernstein (1972) in Cincinnati, though there are few other reports of clinical sensitivity, and in some of these nonencapsulated enzyme preparations were in use. It may be that the patients tested by Bernstein (1972) were heavily exposed to the *B. subtilis* enzyme preparations and indeed to papain and bovine ribonuclease as well, all of which gave a high incidence of reactions, especially in the highly atopic subjects.

The report by Stenius *et al.* (1971) that in tests with a nonspecific wealing agent, the histamine liberator 48/80, the sizes of the weals elicited correlated with the levels of total IgE suggests that in patients with high IgE levels the mast cells may be more sensitive, and so primed by exposure to the common allergens that nonspecific wealing agents could then elicit stronger reactions. This is supported by the findings of Vane (1971) that the lungs of sensitized guinea pigs liberate greater amounts of tissue mediators than those of nonsensitive guinea pigs in response to nonspecific stimuli.

The demonstration that levels of IgE antibody to the enzymes were higher in those subjects with greater use of the biological detergents, though below those of prick test and clinical reactivity as judged by the reactions of sensitized factory workers, makes it necessary to continue the observations on consumers. Nothing is known in the population in general about whether there are levels of IgE antibody to common allergens, below those which correlate with skin test and clinical sensitivity, but which can be correlated with differences in exposure, nor whether these lower levels are of clinical importance.

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Clinical Findings in Workers Exposed to the Enzymes of *Bacillus subtilis*

Over the past thirty years the commercial applications and tonnage of enzyme preparations have increased, yet few cases of pulmonary sensitization in workers manufacturing enzymes have been reported (Simon & Pollak 1969, Zweiman *et al.* 1967).

Within a year from when a factory started the compounding of washing powder with a commercial derivative of *B. subtilis* containing proteolytic