

PULMONARY DISEASE DUE TO INHALATION OF DERIVATIVES OF *BACILLUS SUBTILIS* CONTAINING PROTEOLYTIC ENZYME

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Summary An investigation was made of chest illness among certain workers in a factory using a preparation containing proteolytic enzyme derived from *Bacillus subtilis* in the manufacture of detergent products. There had been asthmatic manifestations in some individuals and in others there were symptoms suggestive of a more peripheral pulmonary reaction. Although primary irritant effects may have occurred, the severe and sometimes prolonged breathless attacks in most of those investigated were thought to have been due to allergic mechanisms. Supporting evidence of allergy to the enzyme was obtained from immediate and late reactions to inhalation and skin-prick tests. Serum-precipitins were present in some of the affected individuals and also in unexposed controls. The findings indicate that, in addition to causing acute illness, inhalation of this material may lead to irreversible impairment of lung function, and that insidious lung damage could occur without episodes of overt illness. Rigorous preventive measures are therefore recommended.

Introduction

DURING the latter part of 1967, while I was working as an industrial medical officer, it was thought that chest illness among certain workers engaged in the manufacture of detergent products might be associated with the handling of proteolytic derivatives of *Bacillus subtilis* in powder form. The material had first been used in the factory on a very limited scale in the latter part of 1966, greater numbers of people being associated with its increasing use during the ensuing months. Some individuals had attended the industrial health centre complaining of chest symptoms, others had been off work and certified as having conditions such as bronchitis, bronchial spasm, asthma, or influenza.

Since there was a large population involved, with a high labour turnover, it was thought better to find out first whether there was a disease entity and, if so, its nature, rather than conduct a comprehensive survey. Selected individuals who had handled the enzymatic material, or who had worked in buildings where such material was handled, were investigated. A few were chosen who had not complained of symptoms, but the majority either had been off work or had attended the factory health centre on account of illness which might have been related to enzyme handling. An occupational and clinical history was taken, followed by clinical examination. Chest radiographs, blood and sputum examinations, lung-function tests, and immunological investigations were also carried out. In addition, atmospheric and product sampling was arranged.

Speed was necessary, not only on account of the potential seriousness of the situation, but also because discontinuance of the existing process reduced the likelihood of fresh cases occurring. This meant that investigations had to be made on patients who had recovered, or were recovering, from the acute phase of their illnesses. Thus, not only was there a diminishing possibility of

finding conclusive evidence by such measures as radiological and lung-function examination, but also there was not unlimited time in which to obtain suitable specimens for haematology and precipitin tests.

An association was found between enzyme handling and illness, with indications as to the mechanism of certain clinical effects. In view of the findings, and the widespread and increasing use of the material, it has been thought best to make a preliminary statement to assist others in the recognition and prevention of the disease.

Symptoms

The most significant presenting symptom was breathlessness, which might or might not be associated with wheezing or other manifestations of respiratory obstruction. The breathlessness was usually of acute onset and was sometimes very severe, lasting for periods ranging from several hours to several days, and persisting, less severely, in a few patients for at least several months. At its worst, the breathlessness in some patients had been such that they were unable to get out of bed; a striking feature of the histories of those more severely affected was the statement, by robust and phlegmatic individuals, that they thought they were going to die during the worst phases of an attack.

Sputum production was uncommon and, when present, was scanty and mucoid. Sometimes there was intractable unproductive cough, to which patients were liable—especially in cool or otherwise irritant atmospheres—for periods ranging from a few days to several weeks. Chest pain was a feature of the illness in some, as was malaise and general weakness, and a few patients believed they had had febrile episodes. Most had a good previous sickness record, and no previous history of serious chest illness, or of asthma, eczema, or hay fever. Many had worked for several years in atmospheres containing dust from the non-enzyme ingredients of the products without apparent ill-effects.

A few patients became ill after what was alleged to be a first exposure to enzyme dust, especially if this was relatively heavy, as, for example, when working beside a dosing point which was not totally enclosed. Under such circumstances symptoms might occur within a few minutes of exposure. In the majority, however, previous exposures had occurred without symptoms and when these first occurred there was usually a delay of about eight hours. This meant that a person could feel well up to the time of finishing work, but when he started to walk for the bus home he might find that he could manage a distance of only about 100 yards (90 m.) on the level before breathlessness forced him to stop for a rest. Since he might have worked near the material only at the beginning of the shift, and had noticed no symptoms at that time or on previous occasions, the possibility of a causal link was not always apparent to him. Sometimes this association could be established only after prolonged and careful questioning of both the patient and plant management about an incident that had occurred several weeks before.

Because of the delay between exposure and symptoms, few patients had initially attended at the factory health centre. Occasionally the night-shift nurse had had to send men home by ambulance on account of breathlessness associated with tachycardia. Others had been seen by the industrial medical officer, who had noted asthmatic symptoms and signs which, although sometimes alleviated by rest and symptomatic treatment, had also led to sickness absence. However, the majority did not become ill at

work, and they had first sought medical advice from their general practitioner who had even less reason than the patients to correlate the symptoms with working conditions. It should be noted that most of the illness occurred in an industrial area in winter. The diagnoses on medical certificates had included bronchitis, bronchial spasm, and influenza. Two patients had been certified as having asthma. In one, the doctor had noted that the onset of asthma had coincided with the patient's marriage a few months earlier; the other had been referred to a chest physician who had prescribed symptomatic treatment and breathing exercises. Another patient, with a previous history of bronchitis, had been referred for a cardiological opinion.

Investigations

Assuming there was an association with a substance in the working environment, it was apparent that the incidence of illness was not that to be expected had the effects been due solely to primary irritation. Most of those who became ill had had similar or greater exposure to the material on several previous occasions without developing symptoms. A further pointer to possible sensitisation was that, although the severity and nature of the symptoms varied, there seemed to be an "all or none" incidence of overt illness among people with comparable exposure. There was, however, an indication that, if exposure were heavy enough or sufficiently repeated, illness might be expected sooner or later.

The management of the firm concerned had been aware of the possibility of proteolytic effects on the moist skin, or on mucous membranes, and precautions had been such that the incidence of non-pulmonary effects from this cause had been negligible. Skin reactions had been confined to slight primary irritant effects on the back of the neck, or where the sleeves chafed at the wrists of those handling the neat powder, and there had been no reported

cases of epistaxis. However, whether or not primary irritant effects were occurring in the respiratory tract, on clinical grounds it seemed likely that there was an allergic factor in the chest illness in some cases.

Some of the people with no previous history of asthma, or of significant exposure to recognised allergens, had presented with symptoms and signs which could have been due to extrinsic allergic asthma. In other cases, the patient's description of breathlessness did not suggest a predominantly obstructive condition, there being excessive breathing but no complaint of wheeze or apparent difficulty in ventilating the lungs. This led to consideration of the possibility of a more peripheral lung reaction as found in extrinsic allergic alveolitis.

Although only a few patients had had chest radiographs during the acute phase of their illness, it was noteworthy that the few positive findings reported then, or in the period after return to work, were confined to those whose illness had been less typically asthmatic. Radiological reports had mentioned areas of increased reticulation, or the presence of nodular shadows. An area of decreased translucency, as of pneumonitis, had been noted in one case, and this had cleared after the patient's clinical recovery. Crepitations could be heard in the right lower axillary region of another patient whose X-ray had shown a diffuse hazy area in the left middle lung-field and the middle and lower zones of the right lung-field. Pulmonary-function tests showed a restrictive defect in this second patient.

Obstructive lung-function changes could be confirmed by spirometry in other patients. No tests of diffusing capacity had been made during the acute phase of any of the illnesses and there was no evidence of a residual diffusing defect in the twelve patients on whom fuller lung-function tests were performed after their clinical recovery. However, in the absence of such tests during

TABLE 1—IMMEDIATE SKIN REACTIONS TO INITIAL PRICK TESTS IN INDIVIDUALS ASSOCIATED WITH ENZYME HANDLING

Case no.	Symptoms				Reactions to test solutions (wheal diameter in mm. measured after 10 min.)							Control
	Breath- lessness	Cough	Chest pain	Sick- absence (spells)	Enzyme extract				Culture extract			
					Alcalase source		Maxatase source		Alcalase source	Maxatase source		
					10 mg./ml.	1 mg./ml.	10 mg./ml.	1 mg./ml.	10 mg./ml.	10 mg./ml.		
1	+	0	0	4	..	5	..	4	..	..	0	0
2	+	0	0	1	..	5	..	5	0	0	0	0
3	+	0	0	2	..	7	..	5	2	2	0	0
4	+	0	0	3	..	7	..	5	2	..	0	0
5	+	+	+	0	3	3	..	5	2	..	0	0
6	+	+	0	0	4	3	3	3	0	3	0	0
7	+	+	0	1	5	4	4	2	..	..	0	0
8	+	0	0	2	5	4	5	4	3	3	0	0
9	+	0	+	1	5	5	5	4	0	0	0	0
10	+	+	0	0	4	0	4	0	0	0	0	0
11	+	+	0	0	5	0	3	2	0	0	0	0
12	+	+	0	1	7	5	..	..	..	..	0	0
13	+	+	+	1	9	10	3	3	2	3	0	0
14	+	+	0	1	1	1	0	0	0	0	0	0
15	+	0	0	2	5	4	3	3	0	0	0	0
16	+	+	+	1	6	5	..	..	0	3	0	0
17	+	0	0	0	7	5	5	4	2	0	0	0
18	+	+	0	2	8	3	4	4	0	2	0	0
19	+	+	0	2	6	5	7	6	0	2	0	0
20	0	0	0	0	4	2	..	..	2	2	0	0
21	+	+	0	2	0	2	2	2	..	..	0	0
22	+	+	0	1	0	0	0	0	0	0	0	0
23	+	0	0	0	0	0	0	0	0	0	0	0
24	0	+	0	0	0	0	0	0	0	0	0	0
25	+	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	+	0	1	0	0	0	0	0	0	0	0

.. = not tested.

the acute phase of any illness, or on a larger number of individuals, this finding was of limited value.

Total and differential blood-counts, blood-film examinations, and haemoglobin, erythrocyte-sedimentation-rate, packed-cell-volume, and plasma-protein estimations were made on twenty-six individuals, all of whom had been exposed to the material and 23 of whom had experienced symptoms. Blood-eosinophilia was found in two people who had recently returned to work after asthmatic illness. There was no other significant haematological finding; the latex-flocculation test for rheumatoid factor was done and found negative in twenty-one cases. The presence of eosinophils was reported in the sputum of three of the five recovering patients from whom it was possible to obtain specimens, but clumping was not looked for, and the finding was not quantified in a way that would give weight to the isolated results. However, two of the patients had had a predominantly obstructive type of breathlessness; and one of them, who had previously had pollen asthma at the appropriate time of the year, was found to have blood-eosinophilia.

Immunology

The immunological investigation was made in collaboration with the department of clinical immunology at the Institute of Diseases of the Chest, University of London, Brompton Hospital, London S.W.3.

Preparations for skin testing were made from enzymatic material and also from bacterial cultures of the associated *B. subtilis* spores. After preliminary tests on individuals who had no history of exposure to the material, skin-prick tests were made on twenty-eight exposed workers. Results of these tests showed a high proportion of positive immediate responses in those with a history of symptoms which could have been attributable to inhalation of enzymatic material. Twenty out of twenty-five of this group gave wheal reactions within 5 minutes to enzyme solutions at a concentration of 1 mg. per ml. These reactions ranged in size from 1 mm. to 10 mm. Wheals were also obtained in one individual who gave a history of exposure but had had no overt illness. The bacterial extracts gave rise to less intensive wheal responses. Material derived from two different enzyme suppliers gave the same skin-testing results, irrespective of which preparation had featured in the patient's history of exposure, and it was found that skin responses to enzyme extracts were unaffected by heating them at 100°C for 5 minutes or at 56°C for an hour. The initial results are shown in table I. The results of tests at the Brompton Hospital on a control population are shown in table II, from which it appears that even at concentrations of 10 mg. per ml. wheals of greater than about 1 mm. are not normally obtained.

Fourteen of the group of factory employees with positive skin reactions had been off work with chest illness during the period in which the enzymatic material had been handled, some on more than one occasion. The duration of these spells of sickness absence ranged from 3 days to 6 weeks, the average being 17 days. In those who were less severely affected and remained at work, symptoms had lasted for periods ranging from 2 hours to 6 months. Only one individual with a positive skin reaction had been unaware of symptoms.

Two of the eight individuals with a history of exposure but negative skin reactions had been off work with illness. One had had a spontaneous syncopal attack associated with pyrexia 8 hours after exposure to enzyme dust. He was

TABLE II—IMMEDIATE SKIN REACTIONS TO PRICK TESTS IN BROMPTON HOSPITAL CONTROL PATIENTS (NO WHEELS GREATER THAN ABOUT 1 mm. DIAMETER)

Test material	No. tested	No. with wheals
<i>B. subtilis</i> crystalline proteinase (Koch-Light) (10 mg./ml.)	73	3
<i>B. subtilis</i> crystalline proteinase (Koch-Light) (1 mg./ml.)	73	0
<i>Enzyme extract:</i>		
Alcalase source (10 mg./ml.)	81	7
Maxatase source (10 mg./ml.)	81	6
Alcalase source (1 mg./ml.)	51	0
Maxatase source (1 mg./ml.)	81	0
<i>Bacterial-culture extract:</i>		
Alcalase source (10 mg./ml.)	71	7
Maxatase source (10 mg./ml.)	30	1

later off work for 2 weeks with cough and profound malaise. He had been absent with "bronchitis" 5 weeks earlier and had previously had breathlessness after enzyme handling. The other patient had been off work for a fortnight; his illness had developed half an hour after working with enzyme powder, starting with a cough and associated with a feeling of chest tightness for the first 3 days. Another person, whose skin reactions were negative, had noticed shortness of breath, with chest tightness lasting for 4 hours, immediately after a period of 4 hours' exposure to the material. Another had been troubled by cough which had developed 2 hours after working with the material and which lasted for 3 days. Another had had an attack of breathlessness, without cough, lasting for 3 days and relieved by ephedrine after this time. One individual had had an upper-respiratory-tract infection with purulent bronchitis, thought to be unrelated to enzyme handling; and the other two had had no symptoms.

In view of the proteolytic nature of the material, only prick tests were performed at this stage. Late skin responses were not noted under these circumstances, but as it was not always possible to recall shift workers for re-examination it is not conclusively known in these cases whether late skin reactions occurred.

When it was established that skin tests were giving specific results, selected patients giving positive skin reactions were admitted to the Brompton Hospital for fuller investigation. Three were chosen who still had symptoms. In one case the clinical picture was predominantly asthmatic; in another it was suggestive of a possible alveolitis. The third patient had recently returned to work after a chest illness which had followed inadvertent exposure to the material. His history of breathlessness was not typically asthmatic, but spirometry showed that there had been an obstructive element which was steadily improving. The investigation of these three patients is reported more fully by Pepys et al. (1969). Each patient was found to give reactions characteristic of immediate (type I) reagin-mediated responses and late (type III) precipitin-mediated responses to inhalation of aerosols of enzyme extract. A feature of the late reactions was the marked obstructive component in the lung-function changes shown by a second fall in forced expiratory volume in 1 second (F.E.V._{1.0}). One of the patients also gave a late reaction on skin testing similar to an Arthus (type III) response, and there were immediate skin reactions to extracts of crystalline proteinase derived from *B. subtilis*. Precipitins to enzyme extract were demonstrated in the serum of these patients and also in the serum of a few other individuals with positive skin

reactions. However, as reported, precipitins could also be demonstrated in the serum of a greater proportion of an unselected sample of asthmatic and non-asthmatic hospital patients.

Discussion

As a result of the investigations it was concluded that the enzymatic preparations of *B. subtilis* ('Alcalase' and 'Maxatase') as used in unmodified form in factory conditions had caused sensitisation and allergic chest illness which could be mediated by both type-I and type-III responses. Similar reactions have been described in allergic bronchopulmonary aspergillosis by Pepys et al. (1968). It was possible that primary irritation contributed to chest pain and cough in some patients, one of whom had hæmoptysis, but the more severe respiratory symptoms in the majority seem to have been caused through the allergic mechanism mentioned.

Each gramme of the enzymatic material contained several million viable *B. subtilis* spores. Initial considerations therefore had included those of sensitisation to the spores, and the possibility of the effects of "infection" from the bacillus, as well as those of primary irritant effects or sensitisation from the enzyme. There was also the possibility of continuing sensitisation effects from incubation and colonisation of the organism in the lungs, or damage due to toxin production. *B. subtilis* is not usually thought of as a pathogen in normal living tissues of healthy patients and, although there was the hypothetical possibility that it might become so in the presence of tissue damaged by proteolytic agents, factors deriving from infection are not thought to have been relevant. Apart from any theoretical considerations, the reasons given at the beginning of this paper for suspecting the possibility of allergy rather than primary irritation as the cause of illness in most of these cases are also against the likelihood of illness deriving from infection. One fact in further support is that it was not possible to culture *B. subtilis* from the sputum of those individuals from whom it was possible to obtain specimens. It should be borne in mind, however, that these patients were recovering. Although in other circumstances spores are known to cause illness through sensitisation, and it was found that extracts from cultures of *B. subtilis* gave positive skin reactions in some people, these reactions were appreciably weaker than those due to enzyme extracts and could well have been due to the same enzymatic allergen.

The presenting syndrome, and the sequelæ, in people who have become ill through inhaling this material are likely to vary because of permutations between primary irritant effects and those due to the different allergic responses. Nevertheless, there is apparently a high degree of correlation between breathlessness as a symptom and sensitisation as revealed by skin reactions. The finding or exclusion of precipitins does not in itself seem likely to prove of routine clinical value. However, it seems that, provided control solutions are used to identify hyper-reactive or dermatographic individuals, immediate wheal reactions to prick tests with appropriately prepared enzyme extracts at concentrations of 1 mg. per ml. are an indication of allergy. Individuals who give this reaction may or may not have been aware of symptoms but, as in the case of budgerigar fanciers (Hargreave et al. 1966), the absence of symptoms in sensitised individuals is unlikely to preclude the possibility that insidious development of pulmonary damage has occurred or will occur if exposure continues.

The people who had been ill and were found to have positive skin-tests had been engaged in a variety of tasks, such as weighing out quantities of about half a kilogramme of the enzyme powder for experimental work, tipping larger quantities into exhaust-ventilated hoppers, maintenance work on plant in which the material had been conveyed, or packing end-products admixed with a proportion of the powder in unmodified form. Some had been engaged in unrelated tasks in adjacent parts of the plant.

Since the ætiological factor was not readily apparent to the medical attendants of most of these patients, or to the patients themselves, industrialists may be unaware of the magnitude of the risk associated with the use of this preparation. A factor of particular import is that with a sensitising agent the hazard, in terms of both numbers affected and seriousness of illness in those repeatedly affected, is likely to increase with time. Manufacturers who have succeeded in handling mildly irritant materials safely in the past may find they have to make radical reappraisal of their formulations and handling methods if they wish to continue to use these enzymatic preparations. These observations are confined to the consequences of inhalation of the powder. The fact that in the factory concerned the incidence of primary irritant effects on the skin was slight and skin-sensitisation effects were not observed cannot be taken to indicate that such effects could not occur under appropriate circumstances. What it does indicate is that conditions could be such as to lessen or eliminate effects on the skin and still lead to chest disease.

Prevention

Symptoms were very severe in some of the patients, especially in those with a pre-existing liability to asthma or bronchitis. For this reason, and in view of the capacity of late (type-III) responses to give rise to irreversible fibrotic changes (*British Medical Journal* 1967), the need for preventive measures cannot be too strongly stressed. Correlation of environmental conditions with the incidence of illness led to the general conclusions on prevention given below.

Where proteolytic derivatives of *B. subtilis* are handled as a dry discrete powder there is an appreciable hazard to health. When disturbed, fine airborne dust is liberated which is capable of contaminating areas beyond those immediately adjacent to the source, and which may not settle for several hours. Even then, further disturbance can result from inappropriate cleaning methods or plant alterations which will lead to the dust becoming airborne again, once more capable of causing illness.

Atmospheric concentrations can be high enough to cause illness and yet be undetectable by the subject. Enclosures and associated methods of dust control, must be rigorous, but engineering methods alone are unlikely to be wholly successful in eliminating the risk. The dry material, whether or not admixed with other substances, can only safely be agitated in unenclosed conditions if it is somehow incorporated in units which are heavy enough not to become readily airborne, and hence respirable, and which must be resistant to attrition. Unless in this form, the powder should not be conveyed or stored except in impervious containers, which must be robust. Unlined paper-sacks are too fragile to use safely under factory conditions. Where there is an unavoidable possibility of inhalation, simple oronasal filter-type masks—even those alleged to trap particles down to 0.5 μ —are insufficient.

Self-contained breathing apparatus, or hoods or suits with an independent air-supply, will be required. It must be appreciated that even weighing out small quantities of the material may be hazardous, and fume-cupboard precautions are recommended for laboratory tasks. Care must be taken in the disposal of the effluent from exhaust ventilation in such a way as not to create an additional hazard.

Those who are already using these enzyme preparations may have to test for evidence of sensitisation in employees and transfer those sensitised to other buildings unless it is found possible to create contamination-free working conditions.

In addition to the specific findings, the outcome of this investigation underlines the need for caution before the handling of any finely particulate organic material.

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ALLERGIC REACTIONS OF THE LUNGS TO ENZYMES OF *BACILLUS SUBTILIS*

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Summary Allergic sensitivity produced by the inhalation of high concentrations of enzyme preparations from *Bacillus subtilis* was found in three workers with mainly bronchial disease. They gave strong immediate reactions to prick tests and immediate, followed by late, mainly asthmatic reactions to inhalation tests. Precipitins were found in the sera of the affected subjects, but even more often in controls.

Introduction

THE development of respiratory disease has been reported by Flindt (1969) in workers producing biological washing preparations, containing proteolytic enzymes and also viable spores of *Bacillus subtilis*. Three affected workers have been investigated in hospital by skin, inhalation, and serological tests.

Clinical Features

Case 1
This man, aged 38 years, who was previously well, developed persistent rhinitis 1-2 days after working with the enzyme material. 4 months later cough and scanty mucoid sputum appeared insidiously, and 2 months after that he had an acute illness starting while at work with sore throat, painful cough, malaise, chest tightness, dyspnoea, and wheezing. Next day his

TABLE 1—PULMONARY-FUNCTION TESTS IN AFFECTED WORKERS

Pulmonary-function tests	Patient no.					
	1		2		3	
	Measured	Predicted	Measured	Predicted	Measured	Predicted
F.E.V. ₁ (l.)	2.3	3.45	1.58	2.6	2.3	3.7
V.C. (l.)	3.86	4.6	2.18	3.71	4.12	4.96
F.E.V. ₁ /V.C. (%)	60	>70	72	>70	56	>70
P.F.R. (l./min.)	360	600	270	525	380	625
Specific conductance (body plethysmograph) (1/cm. H ₂ O. sec.)	0.11	0.23	0.076	0.23	0.077	0.18
F.R.C. (helium-dilution method) (l.)	3.49	3.74	2.26	3.1	4.84	3.74
I.C. (l.)	2.71	2.94	1.58	2.76	2.66	3.09
T.L.C. (l.)	6.2	6.68	3.84	5.86	7.5	6.83
R.V. (l.)	2.34	2.08	1.66	2.15	4.3	1.87
R.V./T.L.C. (%)	37	31	36	30	57	27
CO transfer factor (single breath method) (mm. Hg/ml./min.)	32	30	23	23	29	33
P _v CO ₂ (rebreathing method) (mm. Hg)	44	46	..	..	46	46

F.E.V.₁ = forced expiratory volume in 1 sec. V.C. = vital capacity. P.F.R. = peak flow-rate. F.R.C. = functional residual capacity. I.C. = inspiratory capacity. T.L.C. = total lung capacity. R.V. = respiratory volume. P_vCO₂ = carbon-dioxide tension in mixed venous blood.

scanty sputum became pale-green, and 3 days later he stopped work for 3 weeks, for the first 9 days of which the dyspnoea increased, with five very severe nocturnal attacks. He lost 10 lb. (4.3 kg.) in weight. On return to work he still had mild effort dyspnoea without wheezing. Exposure to the enzyme material provoked immediate chest tightness. On examination rhonchi but no crepitant râles were heard.

Table 1 shows evidence of airways obstruction, the ratio of forced expiratory volume in 1 second to vital capacity (F.E.V.₁/V.C.) being 60%. Isoprenaline inhalation had no effect on the F.E.V.₁ and a subcutaneous injection of adrenaline (0.5 ml. of 0.1% solution) raised the F.E.V.₁ by 0.35 litre. The poor reversibility is supported by the Altounyan test (1966) which compares the rise in F.E.V.₁ after atropine inhalation with the total response produced by an additional inhalation of isoprenaline. The atropine was responsible for 66% of the small total rise of 0.3 litre, a finding thought to indicate that corticosteroid treatment would be unlikely to produce improvement. It was not used in this patient, but was ineffective in the other two patients, who also showed poor reversibility of the airways obstruction. There was no chest radiographic abnormality, leucocytosis, or blood or sputum eosinophilia; and, latex, differential agglutination titre (D.A.T.), and antinuclear factor (A.N.F.) tests were negative.

Case 2

This man, aged 45 years, was well until 12 months after starting work with the enzyme. Progressive effort dyspnoea with wheezing, chest tightness, and a dry irritating cough developed insidiously and kept him off work for 6 weeks. The symptoms improved after only 3 weeks at home. On return to work they recurred at the end of the 1st day, and later they built up gradually during work, and were worse later at night at home. He improved again on transfer to another department. On examination, basal crepitations and rhonchi were heard.

Table 1 shows slight obstructive and restrictive ventilatory defects. The airways obstruction was not apparent on spirometry but was reflected in a decrease in peak flow-rate (P.F.R.) and specific conductance. Isoprenaline, and corticosteroids, 30 mg. per day for 2 weeks and then gradually reduced, had no effect. There was no chest radiographic abnormality, leucocytosis, or eosinophilia; and latex, D.A.T., and A.N.F. tests were negative. The patient had a budgerigar for 10 years, but skin and precipitin tests to avian antigens were negative.

Case 3

This man, aged 30 years, was successfully treated for