

Original Articles

ACUTE BRONCHIOLITIS FOLLOWING PROVOCATIVE INHALATION OF
"ALCALASE"—A PROTEOLYTIC ENZYME USED IN THE
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A pulmonary reaction characterized by the development of bilateral inspiratory and expiratory fine râles was observed in 11 of 29 subjects on brief exposure to aerosol "Alcalase", a proteolytic enzyme used in detergent powders. The concentration of "Alcalase" producing the reaction varied from 0.01 to 10 mg/ml (tending to be lower in skin-sensitized subjects), within the range likely to be encountered under the working conditions obtaining at the time. The pulmonary reaction occurred alone in four subjects. In the remaining seven subjects, it was associated with, although temporally independent of, an immediate or late bronchial reaction, as indicated by the development of asthmatic symptoms and a decrease in FEV₁. Symptoms were absent or minimal in the absence of a bronchial reaction. Neither skin sensitivity nor symptoms on industrial exposure were related to the development of râles on challenge. The reaction, which presumably occurs in the bronchioles or small airways, raises the possibility of long-term effects,³ and raises special problems and obligations in medical surveillance during individual exposures and as a long-term procedure.

In 1968, asthma in an employee of a Sydney factory making enzyme detergents was attributed specifically to "Alcalase" on the basis of the history and the results of bronchial provocation tests to various preparations. After this experience, and the publicity given to papers by Flindt (1969) and Pepys *et alii* (1969), a minor "epidemic" of respiratory disease in the same factory was investigated (Mitchell and Gandevia, 1971; Simson and Simpson, unpublished data). With the collaboration of management, unions, insurance companies and the individuals concerned, 29 selected subjects were studied in more detail in hospital, partly to define more precisely the mechanisms involved, and partly to elucidate certain problems posed by the initial survey, notably the significance of skin sensitivity to "Alcalase" and the often delayed onset of clinical symptoms after exposure. In response to small provocative

doses of an "Alcalase" extract given by aerosol, asthmatic reactions, with onset (i) immediately, (ii) after four to five hours or (iii) during the following night, were precipitated in 18 of the 29 subjects; recovery occurred with or without specific treatment (Mitchell, 1970). However, seven subjects who showed an asthmatic reaction and four who did not do so all developed bilateral fine râles after the provocative inhalation of "Alcalase". This reaction involving small airways is the subject of the present paper.

SUBJECTS AND METHODS

The 29 subjects were selected from an exposed work force of approximately 100 on a variety of defined grounds, including clinical severity of symptoms on exposure, positive results to skin tests with and without a history of symptoms on exposure, and symptoms on exposure with negative results to skin tests. The essential purpose of admission of the patient to hospital was to perform bronchial provocation tests in the course of serial clinical, functional and radiographic assessments of broncho-pulmonary status. Skin sensitivity to "Alcalase" and bronchial reactivity to histamine were also estimated.

Skin tests were performed using an extract of commercial "Alcalase" prepared in our laboratory, and giving comparable skin reactions to those obtained with "Alcalase" extracts from other sources. For prick tests, a solution of a concentration of 1 mg/ml was used; the results were read at 20 minutes and recorded as negative when no palpable weal was present, one-plus when a weal of up to 4 mm diameter was present, two-plus when a reaction was greater than this, and three-plus when pseudopodia were also present. For intradermal tests, 0.01 to 0.02 ml of the extract in a concentration of 0.05 mg/ml was used. The results were read at five hours and recorded as the mean diameter of induration in millimetres.

Ventilatory capacity tests were performed in the conventional manner with the subject seated. Each subject was trained to record his ventilatory capacity as the one second forced expiratory volume (FEV₁) and the forced vital capacity (FVC), using a McDermott direct-reading dry spirometer. This was performed hourly whilst he was awake, generally from 6 a.m. to 10 p.m.; the higher of two satisfactory estimates was accepted in each instance. Each subject was requested to record further values should he wake, for any reason, between 10 p.m. and 6 a.m.

Bronchial reactivity to histamine was measured by estimating the percentage change in FEV₁ following one minute's inhalation of an aerosol of histamine phosphate, administered as 2 mg of histamine base per millilitre of

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³Since this was written, loss of elastic recoil has been demonstrated in a group of more heavily exposed subjects (Gandevia and Mitchell, 1971).

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solution from a De Vilbiss 40 nebulizer; 0.25 ml of aerosol was generated in one minute using compressed air at a flow rate of 8 l./min. The FEV₁ value used in this calculation was the lowest reproducible reading obtained either one and a half or three minutes after the inhalation.

After 24 to 48 hours of control observations, bronchial provocation tests were carried out under medical supervision with an "Alcalase" extract administered as an aerosol from a De Vilbiss 40 nebulizer; again, 0.25 ml was generated during the exposure period of one minute. The initial concentration of extract was arbitrarily chosen from a series ranging in tenfold steps from 0.01 to 10 mg/ml according to the severity of symptoms noted on occupational exposure and the observed degree of skin reactivity. In the absence of clinical or functional evidence of a reaction over the ensuing 24 hours, a second dose was given, using either the same or a tenfold increase in concentration. At the dust concentrations prevailing in the plant at this period, the doses used were within those likely to be encountered in a day's work, and below those possibly encountered in the course of various "accidents". After provocation, estimations of ventilatory capacity were recorded every five minutes for half an hour, half-hourly for the next four hours, and then hourly as previously described.

After the provocative inhalation of aerosol "Alcalase", auscultation of the chest was carried out at specified intervals—namely, 1, 2, 4 and 20 hours later—but also at intermediate times and for longer periods as indicated in individual cases. The adventitious sounds heard, other than developed wheeze and rhonchi in those subjects who developed asthma, were fine, bubbling, inspiratory and expiratory sounds. These were most readily heard, and sometimes heard only, at the lung bases laterally, but they could be numerous and widespread over the whole of both lungs, in which case the findings resembled those of pulmonary oedema. In all cases, râles were heard independently by at least two observers. No râles were detected in any of the subjects prior to the provocative inhalation.

The steady-state diffusing capacity (transfer factor) for carbon monoxide (Bates *et alii*, 1962) and the ventilatory response to a five-minute stepping exercise (Gandevia, 1962) were measured before and 24 hours after challenge. In four subjects, the static volume-pressure properties of the lungs (Finucane and Colebatch, 1969) were studied during and after recovery from bronchiolar reactions.

Chest radiographs were taken before and 24 hours after aerosol challenge, with particular care to ensure films of comparable radiographic quality. The paired films were read by two observers without knowledge as to which film was taken in the control period.

RESULTS

Bilateral râles developed in 11 of the 29 subjects. In the absence of a bronchial (asthmatic) response, no symptoms developed as a rule; even widespread râles were associated only with some cough, occurring especially at the end of a deep inspiration. The râles usually occurred within two to four hours and lasted for 12 to 24 hours, sometimes for two or three days, and in one subject (Case 1) for two weeks.

Table 1 sets out the relevant information concerning the 11 subjects. Five gave negative immediate and negative or slight five-hour skin responses. Six gave positive immediate skin reactions, and four of these also gave moderate five-hour reactions. Bronchial reactivity to histamine was abnormally high, as indicated by a decrease in FEV₁ of more than 10%, in two of the group with negative skin responses, and one of those with positive skin responses. Symptoms on exposure at work also occurred in subjects with and without dermal sensitivity. The concentration which produced râles tended to be higher in those without skin reactivity than in those with it, but this may be partly a reflection of the caution taken to use small doses in those considered more at risk of developing significant asthmatic symptoms. Six subjects (Cases 1, 2, 4, 6, 8 and 11) had failed to show any reaction to concentrations one-tenth of those recorded in

Table 1. Seven subjects showed bronchial reactions to the provocation tests, manifest as marked and unequivocal decreases in FEV₁, either immediately (Cases 3, 4, 6, 9, 10, 11) or after a lapse of four to six hours (Cases 6, 8, 9, 10, 11), or both.

Thus, within the limits of a necessarily small series, the development of fine râles in response to aerosol challenge with "Alcalase" was unrelated to skin sensitivity, to bronchial reactivity to histamine, to symptoms on occupational exposure or to bronchial reactions as reflected in changes in FEV₁. By contrast, in this selected group, the presence or absence of bronchial reactions tended to be related to the presence or absence of skin sensitivity, although the relationship is not absolute—a finding confirmed by observations in the other 18 cases in which râles did not develop (which are therefore excluded from the present paper).

TABLE 1
Airway Reactions to Aerosol Protease

Case Number ¹	Skin Reactions to "Alcalase"		Bronchial Reactivity to Histamine Concentration (FEV ₁ per Centum) ²	Provocative "Alcalase" Concentration (mg/ml)	Reaction to Provocative "Alcalase"	
	Immediate	Late (mm)			Bronchial ³	Bronchiolar ⁴
1	—	0	-9.6	10	—	+++
2*	—	3	-7.5	10	—	+
3*	—	0	-4.0	0.1	+	+
4*	—	6	-28.3	0.1	+	+
5	—	4	-29.0	0.1	—	+
6*	+	6	-4.6	0.1	+	+
7	+	2	0	0.1	—	+
8*	++	12	-7.0	10	+	++
9*	+++	60	-8.1	0.01	+	+++
10*	++	10	-35.0	0.1	+	+++
11	+++	40	-2.8	1	+	+

¹ Asterisk indicates symptoms on exposure to enzymes at work.

² Expressed as percentage change on initial FEV₁.

³ Regarded as positive if FEV₁ decreased by more than 10% either within 40 min (immediate) or later (usually after 4-6 hours or during the night).

⁴ Arbitrarily graded according to extent and duration of râles.

Diffusing capacity for carbon monoxide and/or ventilatory response to a known amount of exercise were estimated before and after challenge in eight of the 11 subjects; the results of neither test showed any significant change. The mechanical properties of the lungs were assessed during and after bronchiolar reactions in four subjects. Static lung compliance was not reduced, and the tidal compliance showed only the normal range of variation during deep or continued quiet breathing. These functional findings are interpreted as consistent with bronchiolar dysfunction, and as failing to indicate any evidence of alveolar involvement. Dynamic compliance was not estimated.

No unequivocal changes were detected in any of the paired chest radiographs taken before and after provocation.

Attention may be drawn to three illustrative cases. In Case 1, the subject had had intermittent exposure to enzyme-containing dust for a total period of perhaps four months during the course of two years, and had remained asymptomatic throughout. His skin responses were negative, and he showed no asthmatic response and developed no symptoms in response to the provocative aerosol in concentrations of 1.0 and 10 mg/ml. However, after the

higher concentration, he developed widespread fine râles persisting for several days (Figure 1); no change was observed in FEV₁ over this period. The dose to which this man was exposed was below that which he might have encountered in an accident at work, or during the course of a few days' work in the environmental dust concentrations obtaining at that time.

possibly alveolar (Pepys *et alii*, 1969) effects. That the râles reflect a reaction involving the bronchioles or small airways is suggested by the character of the sounds themselves. The adventitiæ are similar to those associated with the inhalation of chlorine and other irritant gases known to exert an inflammatory effect at bronchiolar level; they are not of the kind classically associated with respiratory

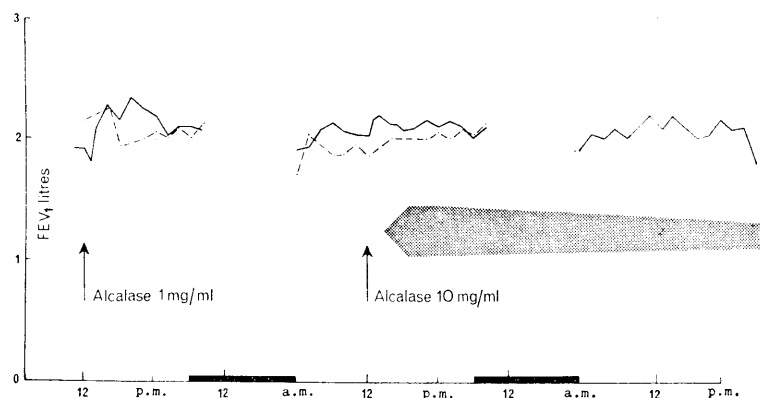


FIGURE 1: Case 1: illustrating the development of fine râles (represented by the shaded area) but no asthmatic reaction following the inhalation of “Alcalase” extract. Interrupted line: FEV₁ recorded prior to challenge. Continuous line: FEV₁ recorded after challenge. The black bars on the time axis represent period of sleep.

By contrast, the subjects in Cases 9 and 10 had had similar enzyme exposure, but were amongst the most strongly reactive of the entire series of 100 workers in terms of symptoms on exposure and of skin and bronchial reactions; they also developed widespread and relatively persistent râles (Figures 2 and 3). Any fine râles developing as a complication of severe asthmatic reactions at work could readily escape observation, or if they were

unit disease, such as fibrosing alveolitis (diffuse interstitial lung disease), in which fine, "dry". "crackling" or crepitant sounds are commonly confined to the inspiratory phase and usually concentrated towards the end of inspiration. The present sounds may occur early in inspiration, and are scattered irregularly through both inspiration and expiration. That the sounds are not alveolar in origin is further supported by the lack of significant change in

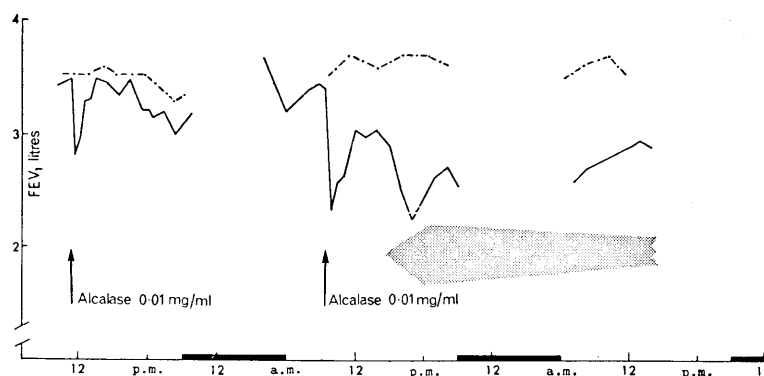


FIGURE 2: Case 9: illustrating the development of fine râles and an asthmatic reaction following the inhalation of "Alcalase" extract. Schema as in Figure 1.

noted, their relative independence of the asthmatic reaction, and hence their possibly different significance, could easily be overlooked. All three cases illustrate the fact that the râles are independent of changes in ventilatory capacity.

DISCUSSION

The finding of râles indicating a form of pulmonary reaction to an inhalation of aerosol "Alcalase" was unexpected; regular auscultation and serial lung function tests had been planned primarily to detect bronchial and

the diffusing capacity and the chest radiographs after provocation, and by the absence of mechanical evidence of alveolar instability at a time when the adventitious sounds were maximal. The lack of relationship between râles and FEV₁ also supports a bronchiolar level of reaction, in that it is consistent with the small contribution of the peripheral airways to total airways resistance (Macklem and Mead, 1967; Hogg *et alii*, 1968); it is evident that the reaction can occur in the peripheral airways independently of the large airways.

The evidence is not sufficient to establish whether the mechanism of the bronchiolar reaction is *chemical (irritative)* or immunological. Bronchiolar reactions after deliberate challenge occurred in subjects with no other manifestations of hypersensitivity, but so too did asthmatic reactions (Mitchell, 1970). The time course of the reaction is conceivably consistent with either type of reaction. The possibility that both mechanisms may be relevant in practice is suggested, first, by our impression that lower concentrations suffice to produce a bronchiolar reaction in sensitized subjects, and secondly, by the fact that whenever these adventitious sounds have been noted in the course of, or shortly after, occupational exposure, the affected subjects have given positive skin responses (personal observations; W. P. Nelson, personal communication). On the other hand, in cases occurring in industry, the subjects are obviously likely to present with symptoms, and hence with the probability of an asthmatic type of reaction, reduction in FEV₁ and skin sensitivity (Mitchell

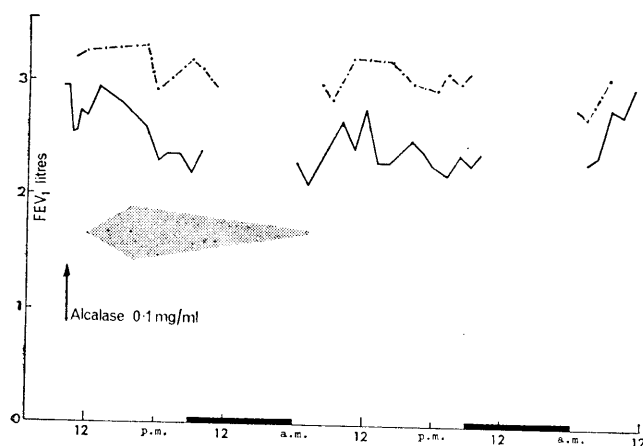


FIGURE 3: Case 10: illustrating the development of fine râles and an asthmatic reaction following the inhalation of "Alcalase" extract. Schema as in Figure 1.

and Gandevia, 1971). It must be stressed also that the development of fine râles is easily overlooked when they occur in conjunction with moderate or severe wheezing—an observation borne out by experience in industrial surveys during exposure. Even if they are observed, their temporal independence of the asthmatic phase, and hence their different significance, may readily escape detection.

The demonstration of a bronchiolar pattern of reaction to inhalation of a proteolytic enzyme raises the possibility of long-term sequelae. Papain, a proteolytic enzyme, admittedly with a different site of action in the protein molecule, produces bronchiolitis, and it has been shown experimentally to produce emphysema in several species of animals (Gross *et alii*, 1964; Park *et alii*, 1970). Our evidence on long-term effects of "Alcalase" in man is circumstantial and inconclusive,¹ but is sufficient to cause some concern, at least as regards employees working under previously existing conditions (in which environmental dust concentrations in excess of 125 µg of "Alcalase" per cubic metre might be encountered). We are aware of at least four subjects amongst a maximum of 200 employees, exposed for less than six months of the two years for which

enzymes had been employed in the industry, who have developed "chronic asthma", now indistinguishable from asthma of non-occupational origin. In all four cases it occurred for the first time in immediate relation to "acute" enzyme exposure, and all four subjects give positive skin responses. In subjects of the relevant age group, this represents a remarkably high incidence of new cases of asthma of this type. Bronchiolar disease and loss of elastic recoil due to emphysema may contribute to the development of asthmatic manifestations, notably by diminishing airway support. Evidence of progressive loss of elastic recoil is provided by studies of the mechanical properties of the lungs in two of the four subjects over several months during which no further exposure to enzymes occurred; in both cases, transpulmonary pressure at full lung inflation decreased and deflation (static) compliance increased; total lung capacity increased, but the relatively normal relationship between transpulmonary pressure and airways conductance was maintained, indicating no significant change in the larger airways in terms of size and distensibility. Both these subjects had been observed to show a bronchiolar reaction to provocative challenge. Whilst these findings may be coincidental, or incidental to the development of non-occupational disease, we believe that they warrant further investigation of men exposed to the former working conditions, a study currently being undertaken by one of us (C.M.).

These observations raise problems which in some respects are without a clear precedent. Subclinical bronchiolar reactions to an occupational inhalant have not previously been reported, whether as an irritant or a sensitization phenomenon. Clinical manifestations of bronchiolar involvement have been recognized after accidental exposures to irritant gases such as chlorine and phosgene, and bronchiolar involvement may well be responsible for some of the clinical signs of vanadium (Zenz *et alii*, 1962) and perhaps cadmium inhalation; all may be associated with the development of chronic obstructive airways disease. In these circumstances, and in the absence of adequate prognostic information, we believe that meticulous long-term surveillance should be obligatory for all workers at risk of inhaling proteolytic enzymes, and that this surveillance should not be confined to symptomatic or skin-test-positive individuals; nor should it be necessarily limited to the period of their employment in the industry. In our view, relaxation of a rigid follow-up system should take place only after reassurance has been obtained from prospective studies, and in the light of experience of the long-term results of the more stringent safety procedures already introduced by the larger and more responsible industrial concerns. These prospective studies should include more sensitive indices of the mechanical properties of the lungs than conventional and simple indices of ventilatory capacity.

Finally, in view of our findings, we consider that provocation tests employing active enzyme extracts are contraindicated in users, whether symptomatic or not. Our use of them was justified, not only by the urgent necessity to clarify a complex situation, but also by the fact that the doses used were less than those likely to be encountered at work at that time by the subjects being studied.

¹ But see later work, Gandevia and Mitchell, 1971.

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