

Follow-up of a group of workers exposed to dusts containing derivatives of *Bacillus subtilis*

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Summary

Six rounds of investigations at 6-monthly intervals of ventilatory capacity, skin sensitivity and respiratory symptoms were carried out together with serological studies on a group of workers intermittently exposed to *Bacillus subtilis* derivatives containing proteolytic enzyme while compounding washing powder. 369 sets of readings of ventilatory capacity were made on seventy-seven males and 180 sets of readings on forty-one females. Overall, with change of employment and improvement in industrial hygiene, ventilatory capacity measurements improved.

Subjects who became prick test positive, as a group showed a decrease in the ratio of observed to expected FEV₁ significantly greater than for the prick test negative group.

There was no significant excess of symptoms developing in the prick test positive group during the course of the survey though smokers and those with previous dust exposure had a higher number of symptoms than non-smokers and those not exposed to other dusts respectively.

A serological study of nine subjects initially prick test positive to *B. subtilis* who subsequently varied their skin reactions, showed them to have low levels of IgE and predominantly normal ventilatory capacities.

Introduction

A survey of 121 workers intermittently exposed to dusts containing derivatives of *B. subtilis* (Greenberg *et al.*, 1970) showed that forty-eight (40%) had become sensitized (prick test positive) of whom twenty-one (44%) had reduced ventilatory capacity as compared with ten (14%) who were prick test negative.

We now report an analysis of the results of six rounds of investigations carried out on this group of workers at 6-monthly intervals and describe their progress.

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Materials and method

Methods employed in the first round in May 1969 were previously described (Greenberg *et al.*, 1970). On the second round no symptomatic enquiry was made and only subjects giving a negative prick test reaction to *B. subtilis* derivative on the first round were retested for skin sensitization. Spirometry was carried out only on subjects *B. subtilis* derivative prick test positive or with unusual spirometry readings on the first round.

On the third and fourth rounds spirometry and symptomatic enquiry were carried out on all subjects, but skin testing was confined to those previously negative.

On fifth and six rounds spirometry, prick testing and symptomatic enquiry were carried out on all subjects. The symptomatic questions were those relating to the hazard, namely chest illnesses, cough, chest tightness or soreness, dyspnoea, wheeze, sputum and rashes.

For prick testing, in place of the battery of reagents used on the first round, the following combinations were used together with a control: Alcalase, Maxatase and Proteinase (second and fourth rounds), Maxatase and Proteinase (third and fifth rounds) and Proteinase (sixth round). The criterion for a positive reaction was a weal of 1 mm or greater diameter unless there was a reaction from the control when the test reaction had to exceed that of the control.

In the first five rounds of the survey blood was examined serologically for subjects selected on the basis of skin sensitivity. Initially tests for precipitating antibody were carried out by the agar gel double diffusion technique at the Institute of Diseases of the Chest (Pepys *et al.*, 1969), but on the sixth round, blood from sixty-four subjects was examined using a radio-immuno-electrophoretic technique (Pepys *et al.*, 1973).

Administration of questionnaires, skin testing and lung function were carried out 'blind' in the sense that individual examiners were unaware of the results obtained at previous examinations: records of previous surveys were not made available in the field. Similarly serological testing on the sixth round was carried out in the absence of knowledge of the field findings on subjects who were examined at random.

During the course of the survey conditions altered considerably: the overall levels of atmospheric dust were progressively lowered to conform to the SDIA code of practice (1971); in addition a considerable number of subjects ceased exposure.

The participation of workers in the survey was voluntary and conditional upon information concerning individuals being confidential: in the course of the survey when it was considered inadvisable for a worker to continue exposure to *B. subtilis* derivative the individual was informed of this opinion and permission sought for communication with employers, unions and general practitioners: the majority agreed to this and discontinued exposure, but a few affected subjects chose to continue exposure against advice.

Results and discussion

Response to questions on respiratory symptoms

A summary of responses to the questions about symptoms at each of five examinations is contained in Table 1.

In considering the results of symptomatic enquiry it must be borne in mind (a) that the number, order and wording of questions was not identical for all examinations (the questionnaire changed after the first round) and (b) that 'learning' may affect a subject's response when a questionnaire is repeated.

Table 1. Summary of responses to questionnaire at sequential examination

	May 1969	March 1970	September 1970	March 1971	October 1971	Total
Subjects examined (No.)	121	104	90	86	97	498
With symptoms (other than rash) (No.)	70	63	49	47	44	273
With symptoms (other than rash) (%)	57.9	60.6	54.4	54.7	45.4	54.8
Average number of symptoms for those examined	0.909	1.385	1.500	1.140	1.237	1.219
Average number of symptoms for those with symptoms	1.571	2.286	2.755	2.085	2.727	2.223
Rash (no. of subjects)	24	11	6	12	3	56

No symptomatic questions were asked in September 1969.

Table 2. Analysis of variance of regression fitted to all observations of responses to symptomatic questions

Source of variation	Sums of squares	Degrees of freedom	Mean square ratio
Overall slope	10.02	1	7.90 (0.01 > P > 0.001)
Deviations of means about the line of means	608.97	114	4.21 (0.001 > P)
Difference between, between individual slope and within individual slopes	12.69	1	10.01 (0.01 > P > 0.001)
Between slopes within individuals	170.00	115	1.17 (not significant P > 0.05)
Residual	328.31	259	
Total	1129.99	490	

Average slope 0.0105 (P < 0.05).

With these reservations and the assumption that each symptom has an equal weighting a crude form of 'Health Index' may be derived (Grogono & Woodgate, 1971) to study how subjects change with time.

Table 2 shows the analysis of variance tables derived if it is assumed that each subject progresses uniformly with time—an assumption that while not realistic for individuals is useful for describing the behaviour of a group—and the number of symptoms shown at each examination is regressed against the length of time since the first examination. While it might seem more desirable in a study such as this to regress against exposure to enzyme in terms of duration of exposure or time-weighted dust measurement, no other better measurement than time elapsing from first examination was available. The process in which subjects had been engaged had undergone changes in terms of improvement of environmental hygiene (measurements of dust load for individuals could not be derived, however), but remained an intermittent one. A study of the histories of enzyme exposure given by the subjects showed this to be a faulty way of obtaining evidence. Records of individual exposure were not obtainable from records and during the course of the survey certain of the subjects left the enzyme

department and some left the company but were nevertheless re-examined periodically. Due to sickness, holiday, cessation of employment or reluctance, certain subjects were unavailable for one or more periodic examinations.

Three observations may be made from Table 2:

- (i) There is a significant overall slope, 0.0143 ± 0.0050 ($P < 0.01$), suggesting that the population has an increasing number of symptoms.
- (ii) Individuals' means do not lie on a straight line, suggesting that some subjects have more symptoms than others.
- (iii) The slopes for individuals do not differ. The average slope is 0.0195 ± 0.0051 ($P < 0.05$) which suggests that on average each individual has an increasing number of symptoms as the study proceeds.

There is, however, no measure of the 'normal' progression of symptoms with age available to act as a control either for atopic or non-atopic subjects.

Table 3 summarizes the difference in the number of symptoms shown for the various groups. From it may be observed:

- (i) That smokers have more symptoms than non-smokers ($t_{114} = 2.16$, $P < 0.02$).
- (ii) Smokers are developing symptoms quicker than non-smokers ($t_{114} = 2.72$, $P < 0.01$).
- (iii) Subjects with other dust exposure have a higher number of symptoms than those with no other dust exposure ($t_{114} = 2.18$, $P < 0.05$).

Complaints concerning rashes

From Table 1 it is apparent that the numbers of complaints concerning rashes is decreasing with time. A higher proportion of females complained of rashes. This may relate to greater susceptibility, better recognition or to greater cumulative exposure to skin hazard at work and at home.

Apart from the figure for May 1969 which relates to cumulative experience, most complaints related to the winter months which is in keeping with a non-industrial factor in aetiology.

Measurements of ventilatory capacity (FEV_1 and FVC)

369 sets of readings were made on seventy-seven males and 180 sets of readings on

Table 3. Summary of different responses and progression in responses to symptomatic questions given by various groups

Group	No.	Mean $\pm$ SD	Average within individual slope $\pm$ SD
Atopic*	23	1.2692 $\pm$ 0.1647	0.0096 $\pm$ 0.0115
Non-atopic	93	1.1964 $\pm$ 0.0751	0.0108 $\pm$ 0.0056
Smokers	96	1.2754 $\pm$ 0.0772	0.0165 $\pm$ 0.0056
Non-smokers	20	0.9205 $\pm$ 0.1447	
		$(P < 0.05)$	-0.0185 ± 0.0116
			$(P < 0.01)$
Sensitized†	43	1.3880 $\pm$ 0.1210	0.0215 $\pm$ 0.0150
Non-sensitized	73	1.1071 $\pm$ 0.0821	0.0042 $\pm$ 0.0068
Other dust exposure	27	1.5000 $\pm$ 0.1533	0.0124 $\pm$ 0.0116
No other dust exposure	89	1.1266 $\pm$ 0.0761	
		$(P < 0.05)$	0.0099 $\pm$ 0.0056

* Prick test positive to one or more common allergens.

† Prick test positive to a *B. subtilis* extract at first examination.

forty-one females. Three subjects have been omitted from the regression analysis as they had only been examined once.

The mean value for the ratio of observed to expected values (Cotes, 1968) of $FEV_{1.0}$ in all observations was 91.47%. This is to be expected as the group of workers studied included 'unhealthy' as well as 'healthy' workers.

Mean values for FEV_1/FVC were 72.50% for males and 80.48% for females. The sex difference for FEV_1/FVC is accounted for by the more rapid fall with age in males (Cotes, 1968). On the assumption that in the course of the study a subject's $FEV_{1.0}$ and $FEV_1/FVC\%$ will decline at a fixed rate each individual's measurements of FEV_1 (Observed)/ FEV_1 (Expected)% and $FEV_1/FVC\%$ can be regressed against time elapsed since the first examination. The ratio of observed to predicted FEV_1 is independent of age and sex unlike that of FEV_1/FVC . Hence Table 4 shows the results of $FEV_1/FVC\%$ separately for males and females and is the result of straight line regressions through individuals' readings and through the readings of the whole population. The overall picture given by Table 4 is of a population whose ventilatory capacity measurements are improving. Females show a significant mean improvement in ratio of observed to expected FEV_1 of $0.1659 \pm 0.0606\%/month$ ($P < 0.01$ when tested against zero slope) and an average improvement in $FEV_1/FVC\%$ of $0.0300 \pm 0.036\%/month$ which although positive is not significant.

Males similarly show an upward trend in the ratio of observed to expected $FEV_{1.0}$ of 0.0388 ± 0.0303 , but this is not significant. The overall slope of observed to expected $FEV_{1.0}$ is 0.0784 ± 0.282 .

Table 5 shows the means and standard deviations of the means and the slopes of various groups. The effect of smoking on ventilatory capacity has been reported in several industrial surveys, for example coal (Higgins & Oldham, 1962) and cotton (Fox *et al.*, 1973). In this survey there was no significant difference between the results for smokers and non-smokers except between the FEV_1/FVC ratios for males, $P < 0.05$, but results for smokers are consistently below those for non-smokers. The most striking differences shown in Table 5 are those between subjects who were sensitized to *B. subtilis* derivative at the first examination and those who were not. This confirms the findings reported in the earlier part of the study (Greenberg *et al.*, 1970).

Of special interest is that the ratio of observed to expected FEV_1 for sensitized subjects is decreasing, slope -0.0231 ± 0.0429 , whereas for subjects not sensitized it is increasing, slope 0.1444 ± 0.0374 . The difference between these two slopes is statistically significant, $P < 0.01$.

The significant differences between observed to expected FEV_1 for males and females and between the ratio FEV_1/FVC for males in the groups atopic and non-atopic would be accounted for by the greater propensity for atopic subjects to become sensitized (Pepys, 1969).

No adverse effect on ventilatory capacity could be demonstrated when those who had previously been exposed to dusty industries such as mining were compared with subjects who had not had such exposure.

Skin tests

Table 6 summarizes the results of skin tests.

Interpretation of the prick test is subject to the same problems as the interpretation of any sign. A multiple observer technique, the facilities for which were not available on this survey, would have increased the confidence of diagnosis. While technique and

Table 4. Analysis of variance of regressions fitted to ventilatory capacity observations $\left(\frac{FEV_1(O)}{FEV_1(E)}\%, \frac{FEV_1}{FVC}\%\right)$

Source of variation	FEV ₁ (Observed)/FEV ₁ (Expected)% Males and females				FEV ₁ % Males				FEV ₁ % Females			
	Sums of squares	Degrees of freedom	Mean square ratio	Sums of squares	Degrees of freedom	Mean square ratio	Sums of squares	Degrees of freedom	Sums of squares	Degrees of freedom	Mean square ratio	Sums of squares
Overall slope	1727.21	1	40.38***	1068.27	1	53.84***	61.81	1	2.56 ^{N.S.}			
Deviations of means about the line of means	186846.85	116	37.65***	71074.14	75	47.76***	6879.58	39	7.97**			
Difference between, between individual slopes and within individual slope	6981.78	1	163.21***	33277.89	1	1677.25***	144.82	1	6.54*			
Between slopes within individuals	7819.84	117	1.56**	1829.95	76	1.21 ^{N.S.}	1559.47	40	1.76*			
Residual	13389.41	313		4265.77	215		2169.99	98				
Total	216765.09	548		111516.02	368		10815.67	179				

N.S. Not statistically significant ($P < 0.05$).* Significant $0.05 > P > 0.01$.** Significant $0.01 > P > 0.001$.*** Significant $0.001 > P$.

Table 5. Ventilatory capacity and its progression compared with various groups

Group*	Numbers		FEV ₁ (Observed) FEV ₁ (Expected)%		Males and females		FEV ₁ % Males		FEV ₁ % Females	
	Males	Females	Mean	overall ± SD	Slope ± SD	Mean ± SD	Slope ± SD	Mean ± SD	Slope ± SD	
Atopic	17	8	87.99 ± 2.20 (<i>P</i> < 0.01)†		0.1362 ± 0.0675	62.80 ± 2.72 (<i>P</i> < 0.001)	− 0.0330 ± 0.0637	80.34 ± 1.45	0.0469 ± 0.0180	
Non-atopic	60	33	92.47 ± 0.89		0.0621 ± 0.0230	74.14 ± 0.78	− 0.0386 ± 0.0232	80.51 ± 0.63	0.0257 ± 0.0410	
Smoker	67	31	91.23 ± 0.94		0.0635 ± 0.0299	70.86 ± 1.01 (<i>P</i> < 0.01)	− 0.0247 ± 0.0244	80.40 ± 0.66	0.0816 ± 0.0469 (<i>P</i> < 0.001)	
Non-smoker	10	10	92.75 ± 1.93		0.1530 ± 0.0185	75.94 ± 1.37	− 0.1361 ± 0.0671	80.70 ± 1.21	− 0.1028 ± 0.0469	
Sensitized**	31	14	85.48 ± 1.65 (<i>P</i> < 0.001)		− 0.0231 ± 0.0429	67.54 ± 0.91	− 0.0685 ± 0.0285	78.63 ± 1.05	0.0035 ± 0.0841	
Not sensitized	46	27	95.69 ± 0.78		0.1444 ± 0.0374	74.53 ± 1.41	− 0.0144 ± 0.0345	81.52 ± 0.45	0.0432 ± 0.0322	
Other dust exposure	25	2	94.68 ± 1.53		0.0426 ± 0.0577	73.30 ± 0.79	0.0020 ± 0.0330	82.07 ± 2.46	0.1881 ± 0.2690	
No other dust exposure	52	39	90.51 ± 1.00		0.0899 ± 0.0323	70.66 ± 1.27	− 0.0571 ± 0.0303	80.39 ± 0.60	0.0237 ± 0.0359	

* Three subjects only examined once have not been included in regressions.

† Figures in parentheses represent the significance of *t* tests between two groups. Where no figures are given *P* < 0.05.

** With respect to tests carried out at the first examination.

interpretation may be responsible for apparent variations in response, a study of nine of the fourteen subjects initially judged positive and on subsequent occasions negative, showed IgE levels of a low order in all cases (Table 7) with no effect on ventilatory capacity in eight and slight effect in one case. Whatever the problems of precision in the individual case, prick testing does identify two groups behaving dissimilarly in other respects than skin reactivity. This is shown in Table 5 where significant ventilatory capacity differences between prick test positive and negative groups are evident. Studies by Pepys *et al.* (1973) relate prick test positivity, high IgE levels and clinical effect.

Fears have been expressed that the increase in number of subjects sensitized in

Table 6. Summary of results of skin tests at each examination

	Date of examination						Total
	May 1969	September 1969*	March 1970*	September 1970*	March 1971	October 1971	
Total number tested	121	47	60	40	83	85	—
Number positive	48	1	17	3	29	26	—
Number positive for the first time	48	1	14	3	9	3	78
As a percentage of all subjects i.e. 121	40.0%	0.8%	11.6%	2.5%	7.5%	2.5%	64.5%
Number negative for the first time having been positive at the first examination	—	—	1	—	6	5	12
Per cent of those positive at the first examination	—	—	2.2%	—	13.0%	10.9%	26.1%

* At these three examinations skin tests were performed primarily on subjects who had not previously been sensitized.

Table 7. IgE levels, specific precipitins, atopic status and ventilatory capacity of nine subjects originally prick test positive, but who subsequently varied

Survey No.	Prick test: <i>B. subtilis</i> reagent						Atopy	Antibody		Ventilatory capacity October 1971
	May 1969	March 1970	March 1971	October 1971	March 1972	August 1972		IgE*	IgG	
2	3 mm		—	—	+	—	+	355	+	Improving
20	3 mm	+	+	—	—	—	+	295	—	Normal
45	2 mm	—		—	—		+	467	—	Normal
56	3 mm		—	—	—		—	364	+	Normal
62	5 mm		—	—	—		—	391	—	Normal
74	2 mm		—	—	—		—	337	+	Normal
77	2 mm		+	—	+	—	—	352	—	Normal
85	2 mm		+	—	+		+	536	—	Normal
95	4 mm		—	—	—	—	—	281	—	Normal

* RAST counts/ $\frac{1}{2}$ min.

time results from repeated 6-monthly prick tests. There have been no reports of this occurring in immunology clinics. Each prick test may introduce in terms of crude extract 3×10^{-8} g (calculated on the basis of the test introducing 3 millionths of a millilitre into the skin (Squire, 1952) of a 10 mg/ml concentration). This is inconsiderable compared with the dose of derivative that might be inhaled over the course of a day in terms of the current accepted levels (assuming 1 m³ of air inhaled hourly with only 50% retention of crude enzyme).

Conclusions

Subjects who were previously exposed intermittently to heavy concentration of *B. subtilis* derivative and became prick test positive and subsequently either continued to be exposed intermittently to low levels of dust or ceased exposure showed as a group a decrease in the ratio of observed to expected FEV_{1.0} over the period of the study significantly greater than that for the prick test negative group. There was no significant excess of symptoms developing in the prick test positive group during the course of the survey though smokers and those with previous dust exposure had a higher number of symptoms than non-smokers and those not exposed to other dusts.

As the atopic subject tends to develop Type I sensitivity more rapidly than the non-atopic subject, should he enter the industry, even under present conditions he should receive special consideration. Non-atopic newcomers to the industry ostensibly exposed to the new sanitary levels of dust may still become prick test positive though not overtly diseased (personal communication): the significance of this observation is not known.

For evaluating the safety of the current standard of hygiene (SDIA, 1971) a longer term survey of a different design is required.

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