Comparative study of the smooth muscle contractor activity of airborne dusts and of dustiness in cotton, flax, and jute mills

P. J. NICHOLLS and J. W. SKIDMORE

Welsh School of Pharmacy, UWIST, Cardiff CF1 3NU and Medical Research Council's Pneumoconiosis Unit, Llandough Hospital, Penarth CF6 1XW, Glamorgan

Nicholls, P. J. and Skidmore, J. W. (1975). *British Journal of Industrial Medicine*, 32, 289-296. Comparative study of the smooth muscle contractor activity of airborne dusts and of dustiness in cotton, flax, and jute mills. A bioassay technique using isolated guinea-pig ileum was employed to compare the smooth muscle contractor activity of various dusts from mills in which the prevalence of byssinosis was known. The activity of dust from a mill spinning a coarse grade of cotton was several times greater than that in dust from a mill processing a fine grade of cotton. There was a similar order in the difference of the prevalence of byssinosis in these mills. However, the activities of fine cotton, flax, and jute dusts were very similar to each other, in spite of marked differences in the prevalence of byssinosis in these mills. For cotton dust, smooth muscle contractor activity was associated with all particle sizes, although the lowest level of activity was found in the largest sized fraction (< 2 mm). Activity in the cotton dust extracts was not correlated with nitrogen, carbohydrate, or potassium content. However, about one-fifth of the activity of a cotton dust extract was associated with an insoluble particulate fraction. The possible chemical nature of the water-soluble contractor agent is discussed. It is concluded that, until the role of this agent in the pathogenesis of byssinosis has been established, the bioassay technique cannot be employed as a means of assessing the byssinogenic potential of cotton dust.

Several hypotheses have been advanced concerning the factors which may cause the symptoms of byssinosis and the changes of lung function reported in workers exposed to the dusts of cotton and flax (Schilling, 1956; McKerrow *et al.*, 1958; Bouhuys, Van Duyn, and Van Lennep, 1961; Schilling *et al.*, 1964; Elwood *et al.*, 1965; Elwood, 1965). That the effects are non-specific and are due simply to the dust has been shown to be unlikely by McKerrow *et al.* (1958). A second hypothesis is that there is a specific substance or group of substances in the dust which causes narrowing of the airway either by direct action on the smooth muscle of the bronchi and bronchioles (Davenport and Paton, 1962; Nicholls, 1962) or through a histamine-release

mechanism (Antweiler, 1960; Bouhuys, 1963a, b; Nicholls, Nicholls, and Bouhuys, 1966). A third hypothesis proposes an immunological mechanism (Massoud and Taylor, 1964) but this is not supported by the results of more recent investigations in normal subjects (Edwards *et al.*, 1970). Indeed, the reaction of the so-called byssinosis antigen with immunoglobulins is remarkably non-specific (Edwards and Jones, 1974). It has also been suggested that bacterial endotoxins present in cotton dust could be responsible for the symptoms of byssinosis (Pernis *et al.*, 1961). However, while the role of these bacterial products in the causation of mill fever is generally accepted, their ability to produce byssinosis-like symptoms has been questioned (Antweiler, 1961). A

more recent study has demonstrated that chronic inhalation of bacterial endotoxin by rabbits causes a hypersensitivity reaction in pulmonary tissue (Cavagna, Foa, and Vigliani, 1969) but the relation of this interesting phenomenon to the symptoms of byssinosis remains to be evaluated. Braun *et al.* (1973) have demonstrated, in cotton dust, proteolytic enzyme activity probably of fungal origin. These workers proposed that this activity is involved in the production of the signs associated with byssinosis.

It is also possible that these textile dusts of vegetable origin possess other biological actions in addition to those already described and that several mechanisms may operate in combination to produce the symptoms of byssinosis.

Roach and Schilling (1960) observed that the prevalence of byssinosis is much lower in mills processing fine cotton than in those using coarser grades, and that the difference could not be fully explained by differences in the dustiness of the working areas. Nicholls (1962) found that the smooth muscle contractor activity of cotton and flax dusts assayed on the isolated guinea-pig ileum, was greater than that of dusts obtained from jute and sisal mills. As classified byssinosis has not been found among jute mill workers (Mair *et al.*, 1960) although exposure to jute dust may cause a reduction in FEV_{1.0} in some individuals (Valić and Žuškin, 1971), it appeared that a comparison of the smooth muscle contractor activity of dusts from various types of mill would be worthwhile. Therefore, dusts were assayed from cotton, flax, and jute mills in which there was already knowledge about the prevalence of byssinosis. It was considered that correlation between smooth muscle contractor activity and the prevalence of byssinosis could possibly indicate (1) the relevance of the contractor activity to the appearance of the symptoms of the disease, and (2) that a bioassay based on this activity might provide a means of assessing the 'byssinogenic' potential of a dust. A study of some chemical differences in the dust was also made in an attempt to determine the chemical nature of the active agent in dust.

Methods

For the investigation, samples of airborne dust were collected during October 1960 (series A) in the cardrooms of two Lancashire cotton mills, one processing a fine cotton and the other a coarse cotton. The study of Roach and Schilling (1960) showed that the prevalence of byssinosis in carding engine operatives in these two mills was 22 and 63% respectively. Samples of airborne dusts were also collected during the following January in the cardroom of a flax mill and in the preparation room of a jute mill in Scotland. These were mills included in a study of byssinosis by Mair *et al.* (1960). These authors

found a prevalence of 9% in the flax mills and none in the jute mills in the sample examined.

After a period of three years (series B) the investigation with the cotton mills was repeated and extended.

Collection of dust samples

1. For bioassay As a minimum of 1 g dust was needed for bioassay, domestic vacuum cleaners fitted with filter paper bags were used to collect samples of dust from the air near the carding engines in the cotton and flax mills. In the jute mill the dust concentration in the cardroom was too low for samples to be collected in a reasonable time and therefore samples were obtained near the spreader in the preparation room. The vacuum cleaners were raised 1.2 m from the floor and sampled for the full working shift, the dust collected containing particles of all sizes. Daily samples were obtained from the Thursday of one week to the Wednesday of the following week.

2. Dust concentrations Simultaneously with the collection of the dust samples for bioassay, dust samples were collected using the Hexhlet/Soxhlet sampling machine to determine the contribution of three size fractions to the total and also to measure the concentration of the dust. The Hexhlet sampler collected, on its filter, dust particles which by virtue of their terminal velocities penetrated an elutriator designed to collect all particles with terminal velocities greater than that of a 7.1 μm unit density sphere. The Soxhlet, a Hexhlet on which the elutriator was replaced by a wire gauze with 2 mm apertures, collected on its filter the fraction smaller than about 2 mm and on the gauze the fraction larger than about 2 mm.

In the repeat investigation in the cotton mills, more sampling machines were used to collect, over a week, sufficient dust in each size fraction to permit bioassay.

Bioassay of smooth muscle contractor activity

Assay of the activity of the dust samples on the isolated guinea-pig ileum was made with extracts of the dust freshly prepared in Tyrode solution adjusted to pH 7.4 (Nicholls, 1962), extracts older than 3 hr being discarded. The assays were performed within six months of the collection of the dust samples. During this time there was no apparent change in the smooth muscle contractor activity of the dusts.

In one instance an aliquot of a freshly prepared extract of dust from the coarse cotton mill was filtered under suction through a cellulose membrane (Millipore) possessing a mean pore diameter of 0.2 μm . Both the filtered and unfiltered extracts were assayed biologically.

1. Standards of activity In previous work on the level of contractor activity in vegetable fibre dusts (Nicholls, 1962), the assessment was made in terms of histamine, that is, the activity of the dust was expressed as that amount of histamine producing the same degree of muscle contraction as that of the extract. However, as histamine is not identical with the contractor substance in cotton and jute dusts (Davenport and Paton, 1962), it seemed more appropriate to assay the activity of the dusts using a standard reference dust for comparison. The dust employed as a standard was obtained from the cardroom of the Dutch mill processing a fine grade of cotton. This dust was collected from the air near the carding engines using domestic vacuum cleaners as described above for

the other dusts at the same time as series A. The activity of this dust was arbitrarily assigned the value of 400 units of activity/g dust. This was the standard against which the cotton dust samples collected in series A were assayed.

For the assay of the cotton dusts collected in series B, the sample of 'Thursday' dust of series A from the mill spinning the coarse cotton was used as the standard for comparison. This dust contained 3400 units of activity/g as determined by assay with the standard Dutch dust.

During a comparison of the standard Dutch dust with that of flax dust obtained from a New Zealand flax mill in 1960, doubt was cast on the identity of the active contractor agent in these two dusts. It was found on a number of occasions that the sensitivity of the ileum became markedly lowered to the action of the flax while the sensitivity to cotton was unchanged. Accordingly, it was decided to employ this flax dust as the standard for assay of the flax samples. This dust had also been collected in a manner similar to that for the standard cotton dust sample. As flax and jute fibres have a similar anatomical origin, namely, bast fibres, the New Zealand flax dust was also employed as the standard for the assay of the jute dusts. When the activities of the standard Dutch cotton and standard flax dust were compared on guinea-pig ileum preparations that maintained their sensitivity to both materials, it was found that the flax dust was half as potent as the cotton. Therefore the flax dust was assigned the activity of 200 units of activity/g so that the values obtained in the assay of flax and jute dusts would be comparable to some extent with those of the cotton dusts.

Although the use of a separate standard for flax and jute reduces the reliability of the overall comparison of the bioassays, it was considered that the most important aspect of the study was the comparison between dusts from the coarse and fine cotton mills.

2. Procedure The assay was performed on the isolated guinea-pig ileum (Hartley strain) suspended in 5 ml aerated Tyrode solution pH 7.4 containing atropine sulphate and mepyramine maleate (both at 0.01 μ g/ml). Guinea-pig ileum was used as this preparation gave good responses to the unidentified smooth muscle contractor substance of dust extracts (Davenport and Paton, 1962). Atropine was employed to prevent the appearance of occasional spontaneous increases in muscle tone and

mepyramine was included to antagonize the effect of the small amounts of histamine present in the dust extracts. Not all guinea-pigs provide muscle preparations that are sensitive to the dust and in the present series only about 60% of the animals were found to respond to the extracts. The reason for this is unknown. Doses (volumes) of dust extracts were added to the bath at intervals of five minutes and remained in contact with the tissue for 30 seconds. In all cases the volume of fluid bathing the ileum was maintained at 5 ml.

Initially, a constant submaximal response of the tissue to a volume (x) of the extract of the standard dust was obtained. Then various volumes of the extract from the dust to be assayed (the unknown) were tested on the preparation until a volume (y) was found which produced a response equal to that elicited by the volume (x) of the standard. The volumes x and y of standard and unknown respectively were each tested on the preparation a further three times (x,y,x,y,x,y) to ensure that the responses were constant. These volumes of standard and unknown giving the same responses on the ileum contained equal amounts (that is, units) of the activity and from this the potency of the unknown was calculated. With this procedure the standard deviation of replicate assays on five different ileum preparations was 15% of the mean. For the results in the present experiment each assay was made on two ileum preparations and the figures expressed are the mean of the two values obtained.

The design of the assay was determined partly by the relatively small quantities of dust collected. In situations where larger amounts would be available, a more reliable approach would be to construct dose-response curves for each extract and determine the weight of dust which after extraction gives 50% of the maximal contraction.

Chemical analysis

Nitrogen was determined on total dust and dust extracts by the Kjeldahl procedure. For the dust extracts, total carbohydrate was estimated in terms of glucose by the anthrone method (Ashwell, 1957) and potassium, as potassium chloride, was estimated by flame photometry.

Results

Dust concentrations

Differences between the concentrations in the two

TABLE 1
COTTON, FLAX AND JUTE DUST CONCENTRATIONS

Mill	Series	Mean of dust concentration ¹ (mg/m ³) (SD)			
		<7.1 μ m	<2 mm	>2 mm	Total dust
Cotton	A	0.61 (0.14)	1.86 (0.22)	3.93 (0.58)	5.79 (0.67)
(Coarse)	B	0.54 (0.09)	0.82 (0.13)	0.48 (0.21)	1.30 (0.25)
Cotton	A	0.73 (0.08)	1.35 (0.25)	2.23 (0.35)	3.58 (0.32)
(Fine)	B	0.54 (0.07)	1.51 (0.39)	2.53 (0.43)	4.05 (0.51)
Flax	A	3.66 (1.67)	11.37 (4.37)	—	11.37 (4.37)
Jute	A	1.62 (0.14)	10.98 (1.71)	4.35 (1.71)	15.33 (1.42)

¹Each value is the mean of 5 samples

cotton mills (Table 1) were found only in the size fractions larger than $7.1\text{ }\mu\text{m}$; below this size the concentrations were essentially equal. Much higher concentrations were found in the flax and jute mills. At the jute mill, only a very small number of operatives were at risk at the sampling point. Comparison of the concentrations found in the coarse cotton mill in series A and series B indicates the efficiency of a dust suppression device that had been fitted in the intervening period; the total dust concentration had been reduced by some 80% but the reduction was associated only with the dust larger than $7.1\text{ }\mu\text{m}$.

Bioassay

All the dust samples examined pharmacologically possessed smooth muscle contractor activity. The levels of activity for the dusts from each of the four mills are given in Table 2 where it may be seen that the dust from the coarse cotton mill is much more active than the others. Smooth muscle contractor activity in dusts from coarse cotton mills has also been found to be generally higher than that in dusts from mills processing medium grades of cotton (Berry *et al.*, 1973). In series B, the investigation in the two cotton mills was repeated and extended to collect sufficient dust in the three size fractions to permit bioassay. The activity levels are given in Table 3 for the total dusts collected on this occasion and in Table 4 for the size selected samples. In this repeat investigation, the activity levels of the dusts from both mills were found to be much higher than on the first occasion. This may have been due to the change in dust standard employed for the assay. Since the stock of the Dutch cotton dust had been depleted, a sample of the dust collected in the first investigation was used as the standard in the second series. It is possible that this new standard may have lost some of its activity in the intervening period.

TABLE 3
SMOOTH MUSCLE CONTRACTOR ACTIVITY OF MILL DUSTS (SERIES B)

Day of collection	Mean contractor activity ¹ (units/g dust)	
	Coarse cotton	Fine cotton
Monday	21 420	9180
Tuesday	11 330	6089
Wednesday	10 200	7140
Thursday	10 200	6800
Friday	12 370	6089
Mean value ..	13 104	7060

For this assay, the Thursday sample of cotton dust collected from the coarse mill (see Table 1) was employed as standard. The activity of this standard was 3400 units/g

¹Each value is the mean of two determinations

TABLE 4
SMOOTH MUSCLE CONTRACTOR ACTIVITY OF SIZE SEPARATED FRACTIONS OF COTTON DUST

Size fraction	Mean contractor activity ¹ (Units/g dust)	
	Coarse cotton	Fine cotton
<7.1 μm ..	11 700	7200
<2 mm ..	12 800	6319
>2 mm ..	4828	4540

The standard cotton dust for the assay had a potency of 3400 units/g

¹Each value is the mean of two determinations

TABLE 2
SMOOTH MUSCLE CONTRACTOR ACTIVITY OF MILL DUSTS (SERIES A)

Day of collection	Mean contractor activity ¹ (units/g dust)			
	Coarse cotton	Fine cotton	Flax	Jute
Monday	5000	362	504	338
Tuesday	1440	462	640	516
Wednesday	3165	595	210	469
Thursday	3400	218	384	206
Friday	1670	600	576	252
Mean value	2935	431	463	356

The smooth muscle contractor activity of the dust extracts was compared with that of standard extracts possessing a defined potency (as units of activity) on isolated guinea-pig ileum. For cotton dust, the standard was a sample of cotton dust from a Dutch mill with an assigned potency of 400 units/g; for jute and flax, the standard was a flax dust from a New Zealand flax mill assigned an activity of 200 units/g

¹Each value is the mean of two determinations

However, whatever the reasons for the changed levels of activity, it is the comparison between the cotton dusts from the two mills at each time which is of main interest. Thus it may be observed that on both occasions the activity of the coarse cotton mill dust was greater than that of the fine mill dust. The ratios of the mean activities (coarse: fine) are 7:1 for series A and 2:1 for series B. These are of a similar order to the ratio for the prevalence of byssinosis in these two mills, namely, 3:1 (Roach and Schilling, 1960).

Of the three size selected fractions, it is the largest sized fraction that possesses the lowest level of activity for both the coarse and fine samples. As dust suppression in the coarse cotton mill reduced the dust fraction above $7.1 \mu\text{m}$ an increase, if anything, in the activity ratio of coarse: fine mill dust was to have been expected. The observed reduced ratio between the two dusts in series B may, therefore, be due to some change in conditions at a point in processing prior to carding.

The variation of the smooth muscle contractor activity between days was greater in series A. Some of this variation may have been due to 'sampling' of the 1g of dust required for assay from the total dust collected on each day. In series B the whole of the dust collected on each day was used for the assay. It is interesting to note that in both series the level of the activity in the Monday samples from the coarse cotton mill was greater than that on other days. Whether this has any relation to Monday symptoms is, of course, purely speculative.

The activity of an extract prepared from a sample collected in the coarse cotton mill was reduced by 19% after filtration through a micropore cellulose membrane. The material retained by the filter was scraped off and resuspended in a volume of Tyrode solution equivalent to the original extract. This

suspension produced a small contraction of the isolated guinea-pig ileum.

The contractor activity of flax and jute dust extracts was of a similar order to that found in the fine cotton samples (Table 2). The type of contractile response to the ileum produced by jute is different (slower in onset) from that elicited by cotton dust (Davenport and Paton, 1962) and the observations of the present investigation (see Methods section) indicates that the contractor activity of flax is different in nature from that in cotton dust. In addition, it is possible that the active substance in jute is not the same as that in flax. Therefore, the quantitative comparison of these two dusts is not very reliable. As size selected samples of these dusts were not collected, the activity of dust in the different size respirable ranges is unknown.

Chemical analysis

The results of the chemical determinations on the cotton dust samples (series A) are presented in Tables 5 and 6. There is no obvious trend of composition with day of the week and in certain instances there was a marked variation between samples collected simultaneously. The variation was greatest in the dust from the coarse cotton mill and may be associated with the sampling error previously referred to. The dust samples from this mill were much darker in colour than those from the fine cotton mill. This was taken as indicating that the dark dust contained a higher proportion of pigmented plant debris. However, although on average the analyses suggest that there are real differences in the chemical composition of the two cotton dusts, it is the dust from the fine cotton mill that contains more per g of each constituent determined. No correlation of the contractor activity with any of these chemical constituents was found (Table 7).

TABLE 5
CHEMICAL ANALYSIS OF COTTON DUST (SERIES A: COARSE COTTON)

Day of collection	Soluble potassium (mg)	Total nitrogen (mg)	Soluble nitrogen (mg)	Soluble carbohydrate (mg)	Contractor activity (units)
Monday ..	2.163	10.77	1.006	19.10	5000
Tuesday ..	1.699	7.84	0.835	11.68	1440
Wednesday ..	3.545	11.46	1.041	16.18	3115
Thursday ..	1.661	10.87	0.945	9.05	3400
Friday ..	1.728	12.26	0.757	14.56	1670
Mean value ..	2.154	10.65	0.9167	14.12	2935

All values are expressed / g of dust and represent the means of two determinations
Potassium, determined by flame photometry, is expressed as potassium chloride
Nitrogen was determined by the Kjeldahl method
Carbohydrate, determined by the anthrone reaction, is expressed as glucose

TABLE 6
CHEMICAL ANALYSIS OF COTTON DUST (SERIES A: FINE COTTON)

Day of collection	Soluble potassium (mg)	Total nitrogen (mg)	Soluble nitrogen (mg)	Soluble carbohydrate (mg)	Contractor activity (units)
Monday ..	7.713	11.49	1.123	18.23	362
Tuesday ..	2.740	12.46	0.781	9.93	463
Wednesday ..	2.997	14.64	0.911	14.05	595
Thursday ..	2.413	13.01	1.026	28.58	218
Friday ..	3.221	12.00	0.550	36.31	600
Mean value ..	3.916	12.72	0.8793	21.42	448

All values are expressed / g of dust and represent the means of two determinations

Potassium, determined by flame photometry, is expressed as potassium chloride

Nitrogen was determined by the Kjeldahl method

Carbohydrate, determined by the anthrone reaction, is expressed as glucose

TABLE 7
CORRELATION COEFFICIENTS BETWEEN SMOOTH MUSCLE CONTRACTOR ACTIVITY AND CHEMICAL
CONSTITUENTS OF COTTON DUST

Dust	Potassium	Total nitrogen	Soluble nitrogen	Carbohydrate
Coarse cotton	+0.2949	+0.2815	+0.8006	+0.5091
Fine cotton	-0.2566	+0.2654	-0.7011	-0.0526

Discussion

While it is clear that the dusts from the mill with a high prevalence of byssinosis possessed a greater amount of smooth muscle contractor activity than those from the mill with a low prevalence, it is less clear how this activity is related to the symptoms of the disease. It has been assumed that the substance contracting the ileum is responsible for the contraction of animal and human bronchial muscle seen *in vitro* with cotton dust extracts (Davenport and Paton, 1962; Nicholls, 1962). As seen from the size-separated fractions, the fine dust is active and this suggests that the contractor substance is able to reach the finer branches of the bronchial tree where it could cause bronchoconstriction. The amounts of dust required to produce effects on the isolated ileum and bronchi are of the order of 100 mg and even larger quantities are necessary to elicit the other pharmacological actions that have been observed (Antweiler, 1960; Davenport and Paton, 1962; Nicholls, 1962; Bouhuys, 1963a). However, it would take several weeks for a cardroom worker to inhale quantities such as these. Using doses which approximate more closely to the situation in the mills, Bouhuys and Nicholls (1966) have failed to observe any change in the airways resistance and pulmonary compliance of unanaesthetized guinea-

pigs when these animals were exposed to aerosols of extracts of cotton dust. This demonstrates that, in the guinea-pig, the smooth muscle contractor agent is unable to cause any acute byssinosis-like changes in pulmonary function. It is not known whether this is true in man as species differences in sensitivity to the contractor agent may exist. As the acute symptoms of byssinosis appear to be associated largely with a release of histamine in the lungs (Edwards *et al.*, 1970), then if the smooth muscle contractor is active in man, it must play only a relatively small role in this phase of the disease. However, this direct-acting contractor substance may be involved in the pathogenesis of the chronic stages of the disease. The active substance may be regarded as a noxious stimulus and regular inhalation of the dust may thus represent repeated micro-insults to the respiratory tract with consequent chronic sequelae. Thus assay of the smooth muscle contractor agent may provide an index of relevant dust activity.

Although on the basis of the bioassay, the comparison between the two cotton dusts reflected the relative prevalence of byssinosis in the respective mills, the bio-activity does not appear to be a reliable guide to assessing the hazard of exposure to such a dust in view of the uncertain role of this activity in producing the symptoms of byssinosis. In addition, as has been indicated earlier, the comparison with

the other vegetable dusts is not reliable. Even if it were, the assay results have not discriminated between the fine cotton dust, flax, and jute although the prevalence of byssinosis differed considerably in the respective mills using these fibres.

It is possible that, while in cotton and flax dusts the contractor activity with a quick onset of action on the ileum is associated with the production of the byssinotic state, the slow contractor activity of jute contributes only to the decrease of FEV_{1.0} seen in jute workers (Valić and Žuškin, 1971).

The results of the present experiments give no clue to the chemical nature of the contractor substance which still remains unidentified. Absence of correlation of activity with nitrogen or carbohydrate indicates that either the active substance is present only in small amounts and is overlooked because of the large amounts of inactive material or that the correct chemical determination has not been made. Davenport and Paton (1962) have suggested that the contractor activity may be associated with the presence of charged particles in the dust extracts. If this were substantiated, a simple relation of activity to a chemical constituent would not be expected. From the results of membrane filtration of the cotton dust extract, it is evident that a proportion of the smooth muscle contractor activity is associated with particulate matter. Such a pharmacological effect of particles may explain why aqueous extracts prepared from synthetic fibre dusts possess smooth muscle contractor activity (Berry *et al.*, 1973).

Several other proposals as to the nature of the smooth muscle contractor agent in cotton dust have been made, namely, an amphoteric substance of unknown structure (Davenport and Paton, 1962) and a polysaccharide complex (Nicholls, 1962). With reference to the latter suggestion, we have recently found that an aminopolysaccharide fraction, byssinosan, prepared from cotton dust by the method of Mohammed, El-Gazzar, and Adamyova (1971), is capable of contracting the isolated guinea-pig ileum in the presence of atropine and mepyramine. Antweiler (unpublished work presented at the first International Conference on Byssinosis, Manchester, 1962) suggested that various amino acid-like compounds in cotton dust contracted smooth muscle. The amino acids, serine, alanine, glycine, and proline produce a contraction of guinea-pig ileum in the presence of mepyramine (Lewis *et al.*, 1972). These free amino acids have been found in the dormant and germinating cotton seed (Lloyd and Nicholls, 1965) and it is possible that they are present in cotton dust. However, it is doubtful that their concentration is sufficiently high to elicit a response on the isolated ileum.

The complete chemical characterization of this active agent would appear to be an essential prerequisite of any further studies in animals and man

of the possible role of the smooth muscle contractor activity in the acute and chronic phases of byssinosis. Until this occurs, the empirical correlation reported here between smooth muscle contractor activity and the prevalence of byssinosis in two cotton mills is of limited value. Only when a causal connexion has been established between chronic inhalation of such an active agent and byssinosis would a bioassay technique be useful for the assessment of the byssinogenic potency of various dusts. Such a study extended to the other vegetable fibre dusts may also be of value in explaining respiratory effects after their inhalation (Valić and Žuškin, 1971).

P J N thanks the Medical Research Council for financial assistance.

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Received for publication 19 May 1975

Accepted for publication 23 July 1975