STUDIES ON COTTON DUST IN RELATION TO BYSSINOSIS

PART I: BACTERIA AND FUNGI IN COTTON DUST

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There is general agreement that byssinosis is associated with the inhalation of cotton dust. Good descriptions of the disease have been given by the Home Office Departmental Committee on Dust in Card Rooms in their report (1932), by Prausnitz (1936), and by Schilling (1950). An extensive and complete review of the literature on cotton dust in relation to affections of the respiratory tract has been prepared by Caminita, Baum, Neal, and Schneiter (1947). The dust affects the lungs, and the first symptoms are cough, a feeling of tightness in the chest, and breathlessness which develop gradually after working for several years in the dusty atmosphere produced by opening bales of cotton and by the carding machines which clean and comb-out the fibres. Some persons do not suffer from this disease after a life-time of exposure to the dust; those who do, find their symptoms are worse on Mondays after a week-end away from the mill. The disease is usually progressive, but some workers suffer only on Mondays during the whole of their working lives, without any progression. A change of occupation which avoids exposure to the dust gives freedom from symptoms, but a return to the dusty atmosphere of the mill after such a period of freedom results immediately in a return of symptoms. The pathological changes found in the later stages are those of emphysema. Where the disease progresses symptoms eventually persist throughout the whole week and finally there is incapacity for work, frequently with congestive heart failure.

The respiratory disability has been termed "asthma", and the disease has been known as "card-room asthma", "strippers and grinders asthma", or "cotton mill asthma", a terminology which has been used in a loosely descriptive sense but which nevertheless has had some influence in

suggesting, by analogy with some other types of asthma, that a factor of sensitization may be implicated in the aetiology of the disease, an idea that has been strengthened by the occurrence of symptoms on exposure to the dust on Mondays or after a spell away from the mill. Other features can be made to fit this hypothesis: the period of exposure which elapses before symptoms begin might indicate the time required for sensitization to develop; difference in individual susceptibility to byssinosis might be due to inherent differences in the proneness to become sensitive; the fact that in the early stages of the illness symptoms are worse on Mondays and decrease or disappear later in the week might mean a temporary partial desensitization. There is, however, nothing conclusive or convincing in such reasoning and other explanations for the peculiarities of byssinosis are possible.

The fraction of the dust concerned in producing the disease and the mechanism of its action are still not clearly understood. Prausnitz (1936) stated that byssinotics were hypersensitive, as judged by skin tests, to a substance which could be extracted from cotton dust and concluded that allergy was a factor in aetiology. The dust was also said to contain an unidentified irritant or toxic fraction which may be different from the fraction that was considered to cause hypersensitivity. The effect of the toxic fraction on the lungs was believed to be of primary importance. In addition histamine or a similar substance has been identified in extracts of cotton dust (Maitland, Heap, and Macdonald, 1932; Macdonald and Maitland, 1934; Macdonald and Prausnitz, 1936; Haworth and Macdonald, 1937). The histamine has been shown to come from the fragments of cotton-seed, both kernels and husks, in the dust (Macdonald and Prausnitz, 1936).

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As a preliminary, the bacterial and fungal content of cotton dust has been studied. Other studies on cotton dust in relation to byssinosis, particularly the possibility of specific sensitization, will be reported in other papers.

It has long been known that large numbers of bacteria and fungi are present in cotton dust but there has been little systematic investigation of this subject, apart from the work of Prindle (1934a, 1934b) who made a survey of the organisms found in freshly collected and stored commercial raw cotton in America. The flora changed on storage; in freshly collected cotton it consisted largely of non-sporing soil bacteria and fungi of the genera *Homodendrum*, *Alternaria*, and *Fusarium*, together with smaller numbers of *Penicillium* and *Aspergillus*; after storage spore-forming bacteria and the fungi *Penicillium* and *Aspergillus* predominated.

There is no evidence that infection with the bacteria or fungi found in cotton dust is a factor in the aetiology of byssinosis. The acute illness investigated by Neal, Schneiter, and Caminita (1942), Schneiter, Neal, and Caminita (1942), and Caminita, Schneiter, Kolb, and Neal (1943) which occurred among workers exposed to dust from low grade and stained cotton was a distinct entity, not connected with byssinosis. This cotton contained large numbers of *Aerobacter cloacae*, almost to the exclusion of other bacteria. The inhalation of this organism was considered to be the cause of the illness, due to the action of its endotoxin rather than to infection or sensitization. Its abundance in this cotton was associated with particular climatic circumstances and undue exposure of the cotton to contamination from the soil. Relatively few fungi were isolated from it but they comprised the genera *Alternaria*, *Mucor*, *Rhizopus*, *Fusarium*, *Sporotrichum*, *Aspergillus*, *Cladosporium*, and *Penicillium*.

Although scattered observations have furnished some general data about the kind and numbers of bacteria and fungi in dust from cotton or from the air in cotton mills there is very little information about the possibility that biologically active substances such as toxins or allergens might have their origin in these microorganisms. Examination of some fungi showed that they were not a source of histamine (Macdonald and Prausnitz, 1936).

The scope of this paper is to examine qualitatively and quantitatively the flora of a fine fraction of dust obtained from various grades of cotton received in this country.

Source and Nature of Materials Examined

Samples of dust have been separated from "cotton fly" removed from the raw cotton by the cotton-cleaning machinery and from material

collected by the air-cleaning plant from the atmosphere in the vicinity of these machines. The varieties of cotton being processed included fine grade cottons of the Sudan type, medium grade cottons of the American type, and low grade cottons of the Indian type. The crude material was a mixture of dust and cotton fly in varying proportions depending on its origin; from the air-cleaning plant it consisted mainly of short fibres mixed with very fine dust; some flue dust from the scutcher had only a small quantity of fly; material from the Shirley cage* was made up of a large bulk of fibre with pockets of adhering and intermingled dust. These differences in the crude material were reflected in the character of the dust obtained from them, particularly in the relation of weight to volume. The crude material was teased out on a 90-mesh sieve which was passed over the upturned nozzle of a "Hoover dustette" fastened to a stand. The dust was immediately recovered from the bag and stored in glass-stoppered jars. This fine fraction of dust which passed the 90-mesh sieve is referred to throughout these investigations as "cotton dust".

The volume of dust per unit weight was estimated approximately by noting the volume of about 20 g. in a 100-ml. cylinder.

The physical character of the dust is shown in Table 1.

The moisture content of five samples was very similar, ranging from 4.0 to 6.3% and averaging less than 5%. At this low humidity any increase in the number of bacteria and moulds during storage would be unlikely to occur.

The grade of cotton being processed did not have any constant relationship to the weight per unit volume of the dust obtained from it. Dust from the air-cleaning plants was always lighter than that obtained from the cotton-cleaning machinery and its ash content was also less than that of dust from the other machines.

Microscopic Examination of Cotton Dust

Smears of watery suspensions of cotton dust were fixed by heat, defatted for 15 minutes in a bath of petroleum ether, dried, and stained or mounted appropriately. Gram-positive cocci and bacilli, and bacterial spores were present in all samples. No bacteria have been found in the lumen of fragments of fibre. Mould hyphae and

* The Shirley cage is a perforated cylinder rotating at high speed so that the cotton sucked on to its surface forms a much thinner layer than on a conventional, slow-speed cage. This permits free extraction of dust into the interior of the cage, while the cleaned cotton from its surface is enabled to go forward in a current of clean air to the next machine.

TABLE 1
SUMMARY OF SAMPLES OF COTTON WASTE EXAMINED AND PHYSICAL CHARACTERS
OF THE DUST EXTRACTED

Sample*	Machine Extracting Waste	Mix of Cotton from which Waste was Extracted	Cotton Dust (g. per 100 ml.)	Moisture (%)	Ash (%)
A	Shirley cage	Sudan	45.43	4.0	70.0
B	" "	$\frac{1}{2}$ Egyptian (Karnak) and $\frac{1}{2}$ Sudan	19.7	—	—
C	Air-cleaning plant	$\frac{1}{3}$ Menoufin, $\frac{1}{3}$ Karnak $\frac{1}{3}$ Sudan Sakel	8.36	—	—
D	Flue "dust" (opening and cleaning machinery)	$\frac{1}{2}$ Sudan Sakel, $\frac{1}{2}$ Karnak	16.36	—	—
E	Shirley cage	Sudan Sakel	31.0	5.4	62.0
F	" "	$\frac{2}{3}$ American, $\frac{1}{3}$ Brazilian, $\frac{1}{3}$ East African	27.42	4.7	55.3
G	" "	$\frac{1}{2}$ American, $\frac{1}{10}$ Uganda, $\frac{1}{2}$ Sao Paulo	32.9	4.2	63.1
H	Air-cleaning plant	American	11.17	6.3	33.5
K	Filter "bags" (opening and cleaning machinery)	$\frac{1}{2}$ American, $\frac{1}{2}$ Peruvian	9.93	—	—
L	Filter bags (opening and cleaning machinery)	$\frac{1}{2}$ Indian, $\frac{1}{2}$ Argentine, $\frac{1}{2}$ Turkish	35.76	—	—
M	Filter bags (opening and cleaning machinery)	$\frac{1}{2}$ American, $\frac{1}{2}$ Argentine, $\frac{1}{2}$ Turkish, $\frac{1}{2}$ Sudan	20.43	—	—
N	Filter bags (opening and cleaning machinery)	Changing mixture of low grade American	34.0	—	—
P	Filter bags (card-room)	Pakistan Sind, Indian Dhaller and Oomras and some Turkish	37.75	—	—

* Samples A—E were from fine grade cotton, F—K from medium grade, and L—P from low grade.

mould spores were also found; the spores were similar to those of *Aspergillus niger* and a species of *Penicillium* isolated from the dust. Large quantities of fibre and debris were seen in all samples. It was not feasible to estimate the total number of bacteria, fungi, or their spores, and it was therefore not possible to determine the ratio between viable and dead organisms; many would be dead but capable nevertheless of being a source of biologically active substances.

The Bacteria in Cotton Dust

Dust was difficult to wet especially when the cotton had been oiled in the mills. To make an even suspension 1 g. of dust was placed in a test tube and thoroughly mixed with 5 ml. of "peptone water" (1% peptone and 0.5% NaCl. in tap water) using a glass rod. When the dust appeared completely wetted a further 10 ml. of peptone water was added, the test tube closed with a rubber bung and thoroughly shaken. The whole was transferred to a glass-stoppered bottle, made up to 100 ml., and again thoroughly shaken. From this initial dilution of 1/100 w/v other dilutions were prepared as required. (Peptone water has been used as a suspending medium and a diluent in preference to saline or distilled water as it retarded the sedimentation of the suspension.)

Viable Counts.—The results of cultural studies of cotton dust are markedly affected by the techniques

and culture media employed and when assessing or comparing such results it is necessary to take this into consideration.

Preliminary observations were directed to determining the conditions which favoured the highest counts while preventing the spreading growth of some species, chiefly aerobic spore-forming bacilli, which when it occurred, rendered plates useless for counting colonies.

Pour plates were made by incorporating 1 ml. of inoculum with 15 ml. of 2% nutrient agar at 45–50°C., but they were unsuitable for counting as they were rapidly overgrown by spreaders. Sandwich plates (Fry, 1932) were therefore made from pour plates by drying them in the incubator for one hour, which fixed any organisms that were on the surface of the medium. A layer of nutrient agar was then poured over the surface and dried again for an hour which prevented spreaders growing up between the glass and the medium. Duplicate plates were made and only those having 30–300 colonies when observed with a $2\frac{1}{2} \times$ hand lens were counted. Sandwich plates gave proportional counts with different dilutions of inoculum up to about 300 bacteria per plate. Tests showed that the optimum times for incubation were 48 hours at 37°C. and 72 hours at 22°C. Results were consistent: four samples of one dust gave counts of 550, 560, 410, and 470 million organisms per g. with plates incubated at 37°C. for 48 hours. Inclusion in the medium of chemical inhibitors, 1/10,000 sodium azide (Snyder and Lichstein, 1940), 5–6% alcohol (Floyd and Dack, 1939), and 6%

sodium chloride (Lichstein and Snyder, 1941) will prevent the spreading of *Proteus*. These substances were therefore incorporated in pour plates, and although they prevented the spreading of bacteria present in the dust they were unsatisfactory because they reduced the number of colonies detectable in 72 hours when compared with sandwich plates. This was especially noticeable at 22°C. and may have been due to delayed growth, as further incubation produced an increase in the number of colonies, especially with alcohol which is volatile.

The results obtained with different methods of inhibiting spreaders are shown in Table 2.

TABLE 2

EFFECT OF CHEMICALS FOR INHIBITING "SPREADERS" ON THE VIABLE COUNT OF AEROBIC BACTERIA

Medium	Millions per g. Cultured at	
	37°C. for 48 hours	22°C. for 72 hours
Agar only	560	970
Agar +4% alcohol	104	141
Agar +6% NaCl	146	80
Agar +1/10,000 sodium azide..	76	below 3

Sandwich plates were clearly the most satisfactory of these methods for our purpose. Further tests showed that 6% agar (Hayward and Miles, 1943) prevented spreading growth and that surface inoculations, distributed over the surface to within 1 cm. of the edge ("spread plates"), gave even higher counts than sandwich plates. Six per cent. agar was, however, not suitable for use in pour plates, as the high temperature at which the agar had to be poured to ensure mixing, reduced the count; 4% agar did not prevent spreading growth.

The counts with 6% agar spread plates were not proportional to serial dilutions; if plates had more than 100 colonies, it would seem that some of the bacteria were inhibited when the plates were thickly seeded. Plates with approximately 100 colonies were therefore chosen for counting and yielded the highest viable counts of any of the methods tried.

Results.—Viable counts of the bacteria in dust from fine, medium, and low grades of cotton are shown in Table 3. A comparison of the counts obtained with sandwich plates and spread plates is also shown. In every case the plates were prepared at the same time from the same dilutions of dust. Surface inoculation consistently gave the highest viable counts; it appears that some of the bacteria did not grow in sandwich plates under microaerophilic conditions.

The relation between counts per gramme and those per millilitre was not constant due to the

varying composition of the dust. The greatest differences occurred with samples from the air-cleaning plant which were lightest (see Table 1) and had therefore the largest volume per gramme which suggests that the bacteria were associated to a greater extent with the lighter organic matter derived from the cotton plant than the heavier inorganic material.

There appeared to be broad differences in bacterial count related to the grade of cotton as determined either by sandwich or spread plates and incubated at 37°C. or 22°C. There was some area of overlapping, but fine grades had the lowest counts, and medium grades, the highest, the low grades being intermediate. The significance of this is not clear but may be related to the origin and handling of the cotton rather than to the grade of the fibre. In spread plates the viable counts ranged between 108 and 4,000 million per g.

Considering the diverse conditions under which the cotton was grown, stored, and the samples collected the results were more uniform than might have been expected.

Counts from sandwich plates incubated at 22°C. were often higher than corresponding plates incubated at 37°C.; this occurred in eight of 13 samples (Table 3). Most of the differences were within the range of experimental error and were not correlated with the grade of cotton or source of dust.

Isolation of Aerobic Bacteria.—Spread plates of 6% nutrient agar were incubated at 37°C. for 48 hours. Colonies were identified with a hand lens supplemented by stained films when necessary. The genus *Bacillus* usually predominated; the remainder of the flora was composed of several varieties of Gram-negative bacilli. Colonies of micrococci were seen in cultures from one sample of dust only, and aerobic actinomycetes from three samples* Table 4 shows the total numbers and the proportion of the two main kinds of bacteria. Every colony was taken into account. Each result represents at least two plates and usually more.

Identification of a representative sample of 21 colonies of *Bacillus* was attempted; two were *B. megatherium*, one was *B. pumilus*, the others resembled *B. pumilus*, *B. coagulans* or *B. subtilis*, differing from them in some minor respects (Bergey, 1948).

Every well separated colony of Gram-negative bacilli was subcultured; their identity is next considered.

MacConkey's agar plates were inoculated by spreading as for making counts and incubated at 37°C. for 48 hours. Some colonies of *Bacillus*

* The experience of workers at the Shirley Institute is that actinomycetes tend to appear after 72 hours of incubation (personal communication).

TABLE 3
VIABLE COUNT OF 13 SAMPLES OF COTTON DUST USING SANDWICH PLATES AND 6% AGAR
SPREAD PLATES INCUBATED AEROBICALLY

Sample*	Sandwich Plates				6% Nutrient Agar Spread Plates Incubated at 37°C. for 48 hrs.
	Incubated at 37°C. for 48 hrs.		Incubated at 22°C. for 72 hrs.		
	Millions per g.	Millions per ml.	Millions per g.	Millions per ml.	
A	65	30	73	33	—
B	240	47	263	53	418
C	152	13	133	11	206
D	80	13	77	13	156
E	66.5	21	110	34	108
F	670	184	785	215	700
G	560	184	970	319	620
G†	407	134	660	217	—
H	1,750	196	—	—	4,000
K	1,860	185	2,640	262	3,420
L	670	240	480	172	—
M	500	102	660	135	700
N	187	64	192	65	296
P	195	74	188	71	350

* Samples A—E were from fine grade cotton, F—K from medium grade, and L—P from low grade.

† After storage of the dust for 16 months in a stoppered jar in the laboratory.

grew but were disregarded. Colonies of Gram-negative bacilli were counted and every well separated colony subcultured. The viable counts of Gram-negative bacilli on this medium are shown in Table 4; comparing them with viable counts on spread 6% agar plates it will be seen that the counts on MacConkey's medium are lower, with one exception, but there is no uniformity in the proportionate reduction; it varies from 10 to 90%.

The predominant genus was *Bacterium**; a few strains were *Bact. alkaligenes*; *Achromobacter* was always present. The percentage of these genera occurring on 6% agar and on MacConkey's medium for four samples of dust is shown in Table 5.

A comparison of the two media using pure cultures indicated that MacConkey's medium inhibited some strains of *Achromobacter*. Some were completely inhibited and many others were markedly affected yielding a scanty growth of thin colonies. All strains were invariably indole-negative

and did not ferment lactose. They grew in Koser's citrate and were Voges-Proskauer positive. Methyl-red tests and gelatin liquefaction were variable.

The strains of *Bacterium* were similar irrespective of the dust from which they were isolated or the medium used. With hardly any exceptions they grew in Koser's citrate and were Voges-Proskauer positive. Only very few produced indole and between one-third and one-half were methyl-red-positive and this often coincided with a capacity to liquefy gelatin. Most produced acid and gas in glucose and sucrose in 24 hours. The results in lactose were extremely variable; only a few produced acid and gas in 24 hours, the majority taking between two and three days; varieties producing acid only or non-lactose-fermenters in five days were common. On the whole these organisms were atypical *Bact. aerogenes*. Whether isolated from 6% nutrient agar or MacConkey's agar they invariably grew on both media and were the most predominant Gram-negative organisms (see Table 5).

* As defined by Topley and Wilson (1946).

TABLE 4
BACTERIA ISOLATED FROM SURFACE CULTURES ON 6% AGAR AND ON MACCONKEY'S MEDIUM
INCUBATED AEROBICALLY AT 37°C.

Sample*	Total No. of Colonies	Aerobic Spore-formers			Gram-negative Bacilli			
		On 6% agar			On 6% agar			On MacConkey's Medium
		No. of Colonies	Millions per g.	%	No. of Colonies	Millions per g.	%	Millions per g.
A	96	69	—	71.9	27	—	28.1	67
B	289	198	286	68.5	91	131	31.5	12
C	103	44	88	42.7	59	118	57.3	102
D	74	35	74	47.3	39	82	52.7	36
E	54	41	82	75.9	13	26	24.1	21
F	444	409	645	92.1	35	55	7.9	112
G	226†	135	370	59.7	88	241	38.9	164
H	370	354	3,828	95.7	16	172	4.3	—
K	80	53	2,266	66.25	27	1,154	33.75	134
L	287	138	—	48.1	149	—	51.9	230
M	68	31	319	45.6	37	381	54.4	252
N	47	12	75	25.5	35	221	74.5	140
P	175	65	130	37.1	110	220	62.9	144

* Samples A—E were from fine grade cotton, F—K from medium grade, and L—P from low grade.
† Contained 3 micrococci.

TABLE 5
PERCENTAGE DISTRIBUTION OF GENERA OF GRAM-NEGATIVE BACTERIA GROWING AEROBICALLY AT 37°C. ON 6% NUTRIENT
AGAR AND MACCONKEY'S MEDIUM

Sample	On 6% Nutrient Agar			On MacConkey's Medium		
	<i>Bacterium</i>	<i>Achromobacter</i>	<i>Bact.alkaligenes</i>	<i>Bacterium</i>	<i>Achromobacter</i>	<i>Bact.alkaligenes</i>
B	67.8	32.2	0	72.7	27.3	0
G	77.8	22.2	0	93.75	6.25	0
K	94.7	5.3	0	94.7	5.3	0
L	74.1	18.5	7.4	72.72	22.74	4.54

Some strains of *Bacterium* formed mucoid colonies on MacConkey's agar but only slightly mucoid colonies on nutrient agar. This mucoid appearance was associated with large capsules and appeared to be derived from lactose, as nutrient agar containing 1% lactose also produced markedly mucoid colonies and large capsules (Duguid, 1948).

The general picture of the aerobic flora of the dust from these different grades of cotton was remarkably similar.

Isolation of Anaerobic Bacteria.—A 1% suspension of dust "H" was heated for 10 min. at 80°C., inoculated on plates of 6% nutrient agar containing 7% horse blood, and incubated anaerobically. One strain of *Cl. welchii* type A was isolated, four other strains of *Clostridium* (not *Cl. welchii*) were accidentally lost.

Pathogenicity of Bacteria in Cotton Dust.—Each of three samples of dust was injected intramuscularly into two guinea-pigs one hour after giving 0.2 ml. of 10% calcium chloride. Five animals died from infection due to *Cl. welchii* type A. *Cl. histolyticum* was isolated from one animal. Other anaerobes were also isolated which did not seem to correspond with any recognized species; one of them was possibly pathogenic. Aerobic bacteria were also isolated from the guinea-pigs; they were not primarily pathogenic, and their identity and characters suggested that they probably came from the dust.

The injection of suspensions of cotton dust into guinea-pigs without tissue damage, e.g. intravenously, did not result in death.

After subcutaneous injection one animal died 21 days later from a *Cl. welchii* infection. Some animals died within five days, probably from infection with a *Clostridium* which was seen in films and culture but not isolated or identified; the colonies were not like those of *Cl. welchii*. Aerobes similar to those found in cotton dust were recovered from the animals.

The incidence and identification of clostridia in cotton dust merits further study. There is no indication that they are concerned in the aetiology of byssinosis or infect those exposed to the dust.

Mycological Examination of Dust

Isolation of Fungi.—Czapek-Dox agar pH 4.2 with the addition of 3% sucrose was used for the growth and isolation of fungi, as a combination of low pH and a synthetic medium prevented the growth of some aerobic spore-formers which interfere; both malt agar pH 4.5 and Czapek-Dox agar pH 7.0 supported their growth.

Identification of Predominant Genera.—The fungi have been identified by their macroscopic appearance on spread plates and by microscopic examination after staining with lactophenol containing 0.05% cotton blue. The predominant genera were *Aspergillus*, especially *A. niger*, and *Penicillium*, but other genera have been found and the plates were often rapidly overgrown by *Mucor* which prevented the identification of slower growing fungi. The incidence of the predominant genera in a number of samples of dust is shown in Table 6.

Spores seen on microscopical examination were similar to those found in cultures of *Aspergillus* and *Penicillium*. The viable count would indicate spores rather than mycelia, as fragments of hyphae which could pass through a 90-mesh sieve would normally be too small to remain viable in the dust.

The amount of contamination of different grades of cotton by fungal spores did not run parallel to the number of bacteria, but probably too few samples have been tested to generalize on this point.

TABLE 6
VIABLE COUNTS OF FUNGI GROWING ON CZAPEK-DOX MEDIUM AND PERCENTAGE INCIDENCE OF *Aspergillus* AND *Penicillium*

Sample*	Millions per g.	Millions per ml.	Percentage of Total Fungi		
			<i>Aspergillus</i> (all varieties)	<i>A. niger</i>	<i>Penicillium</i>
A	112	50.8	55.8	42.2	1.9
B	96	18.9	—	—	—
C	31	2.6	—	—	95
D	400	61.6	100	—	—
E	60	18.6	90	90	3.3
F	18	4.9	—	—	—
G	12	3.9	42.1	21.1	21.1
H	8.5	0.95	90.7	18.6	—
K	36	3.5	44.4	27.8	27.8
L	54	19.5	92.8	92.8	3.6
M	72	14.7	75.0	41.7	4.2
N	70	23.8	71.4	60.0	8.6
P	44	16.6	82.9	65.9	4.9

* Samples A—E were from fine grade cotton, F—K from medium grade, and L—P from low grade.

Spread plates gave much higher counts than pour plates (incubation for four days at 22°C.) and were more suitable for the isolation and identification of the fungi.

Viable Counts.—Spread plates were read after incubation for two days and four days. Some colonies were small and at this stage could not be identified. Occasionally a rapidly growing species necessitated an earlier final reading before overgrowth interfered. The results with the same samples of dust as were examined bacteriologically are given in Table 6.

On the whole the fungal flora, like the bacterial flora, is similar for cotton of different grades and origins.

Summary and Conclusions

Samples of fine dust obtained from waste removed by the cleaning machinery during the processing of different grades of cotton, as well as from the atmosphere near these machines, have been examined for their physical characters and for their content of bacteria and fungi.

Dust from the air-cleaning plant was lighter than that from the cleaning machinery and its ash

content was less. There was some indication that larger numbers of bacteria were found in the lighter organic matter derived from the cotton plant than in the heavier inorganic material.

Microscopically Gram-positive cocci and bacilli, bacterial spores, fragments of mould hyphae, and mould spores were seen. Gram-negative bacteria were not easily detected though they were revealed by culture. The Gram-positive cocci were probably dead as only once were they found in culture. As it was not feasible to estimate the total number of bacteria, fungi or their spores, the ratio between viable and dead organisms could not be determined.

The technique of counting viable bacteria requires cultural conditions which prevent spreading growth while allowing the maximum number of bacteria to develop colonies. The addition to agar of 1:10,000 sodium azide, 5-6% alcohol or 6% sodium chloride inhibited spreaders but reduced the counts so that none of these chemicals was satisfactory; "sandwich plates" were better than any of the chemicals tried; surface inoculation on 6% agar plates was the best method.

The viable counts of aerobic bacteria at 37°C. ranged from 108 to 4,000 millions per g. The counts at 22°C., in most samples, would have been higher, to judge from the results obtained with sandwich plates.

Among the aerobic bacteria the genus *Bacillus* usually predominated; the remainder were Gram-negative bacilli, of which about 70% or more were *Bacterium* and the rest *Achromobacter* except for a few *Alkaligenes* in one sample.

A few aerobic actinomycetes were found in three samples and a few colonies of micrococci in one sample.

Details of the cultural and biochemical characters of the aerobic bacteria are given. They appeared not to be pathogenic for guinea-pigs.

Of the anaerobic bacteria, *Cl. welchii* (type A) was isolated once by direct plating on blood agar and from each of three samples of dust by inoculating dust and calcium chloride into guinea-pigs.

Cl. histolyticum was obtained from one sample. Several other clostridia were isolated which did not correspond with any recognized species. There is no indication that these bacteria are concerned in the aetiology of byssinosis or constitute a special risk to persons exposed to the dust.

Viable counts of fungi ranged from 85 to 400 millions per g. The predominant genera were *Aspergillus*, especially *A. niger*, and *Penicillium*.

On the whole the bacterial and fungal flora was similar for cotton of different grades and origins. The possible relation of dead and viable micro-organisms in dust to the aetiology of byssinosis has yet to be determined.

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