

Precipitin (F.L.H.) test in farmer's lung

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Farmer's lung may be defined as the disease resulting from the inhalation of the dust of mouldy hay and of other mouldy vegetable materials, with symptoms and signs attributable to a reaction in the most peripheral part of the bronchopulmonary tree. The presence, in patients suffering from farmer's lung, of precipitins against antigens in mouldy hay, reported by Pepys, Riddell, Citron, and Clayton (1961; 1962), has been confirmed by Kobayashi, Stahmann, Rankin, and Dickie (1963) and by Bishop, Melnick, and Raine (1963). Pepys, Jenkins, Festenstein, Gregory, Lacey, and Skinner (1963) have since found that the main sources of 'farmer's lung hay' (F.L.H.) antigens are thermophilic actinomycetes, the most important being *Thermopolyspora polyspora*, on which the present serological findings are based, and *Micromonospora vulgaris* (*Thermoactinomyces vulgaris*).

Earlier reports of the disease suggested that a well-defined group of symptoms and signs was typical (Campbell, 1932; Studdert, 1953; Fuller, 1953; Dickie and Rankin, 1958). It is important, however, to decide whether less typical pulmonary manifestations in a farmer exposed to mouldy hay are pathogenetically similar and should be included in the category of farmer's lung. The main features of typical acute attacks are the appearance, some hours after exposure to the dust, of crepitations together with dyspnoea, cough, and perhaps haemoptysis, malaise, chills, fever, and loss of weight. The symptoms are often more striking than the physical signs. Radiography of the lungs may show a widely distributed miliary infiltration or ground-glass appearance, which resolves within weeks or months of the attack. Reactions in the larger bronchi are not a part of farmer's lung, and a history of previous allergic asthma or bronchitis is seldom present. The pulmonary reaction in farmer's lung takes place in the peripheral part of the bronchopulmonary tree, which may be infiltrated with epithelioid cell granulomata, and is accompanied by impaired diffusion of gas and decreased compliance of the lungs, with minimal ventilatory disturbance (Dickie and Rankin, 1958, 1960; Seal, Thomas, and Griffiths, 1963; Bishop and others, 1963; Williams, 1963a, b).

In their survey of farmer's lung in the United Kingdom, Staines and Forman (1961) added to the above criteria of farmer's lung the presence of wheezing, predominantly inspiratory, and they stated that mild cases might present 'rather like dust-provoked asthmatic bronchitis', and that moderate or severe cases might start similarly. This raises the problem of distinguishing in a farmer between asthma or bronchitis and farmer's lung. A similar problem is present in farmers found to have diffuse pulmonary fibrosis, since this is a not uncommon result of repeated attacks of farmer's lung but may also develop from other causes in a patient who happens to be a farmer.

In most reports on farmer's lung the condition is described as having started with typical acute attacks, but Staines and Forman (1961) report that an insidious onset occurred equally frequently.

The serological findings reported here will be examined in relation to subjects who were or were not farmworkers, to the different modes of onset of farmer's lung, and to the presence or absence of other pulmonary disease.

MATERIALS AND METHODS

TEST EXTRACTS Freeze-dried preparations of extracts of mouldy hays in Coca's solution and of the culture medium of the following actinomycetes and fungi were dissolved at 10 mg./ml. for double-diffusion and 30 mg./ml. for immuno-electrophoretic tests (Pepys and others, 1963).

1. Extracts of specimens of the following mouldy hays were mainly used for testing: (a) mouldy hay H44, which was naturally occurring mouldy hay associated with a case of farmer's lung (Gregory and Lacey, 1963); (b) mouldy hays H1b and L8, which were prepared in the laboratory from sterile hay, the moisture content of which had been raised to 40%. This hay was inoculated with a mixed suspension of the microbial flora of H44 and was then incubated at 40° C. for two and three weeks respectively (Pepys and others, 1963); (c) tests were also made with extracts of two experimental mouldy hays which were under investigation at Rothamsted Experimental Station by Dr. P. H. Gregory and his colleagues. Mouldy hay V4 was prepared by placing chopped hay, with a moisture content of 40%, into a well-lagged barrel. The hay underwent self-heating with a gradual rise of temperature to 56° C., and extracts were prepared after

nine days. Mouldy hay V17 was prepared in a Thermo flask from chopped hay with a moisture content of 34%. This hay behaved differently from V4, showing two rapidly developing and subsiding rises of temperature to 55° C., and extracts were prepared from it after one week.

2. (a) *T. polyspora* and *M. vulgaris* were cultured on nutrient agar medium at 40° C. for four days, and extracts were prepared from the liquid which was freed from the medium by alternate freezing and thawing. (b) Preparations of the antigenic fractions of the culture filtrate extract of *T. polyspora* were made by precipitation with trichloroacetic acid. A solution of trichloroacetic acid 40% was added drop by drop to a solution of 80 mg./ml. of the freeze-dried *T. polyspora* extract to give a final concentration of 4%. After standing overnight at 4° C., the trichloroacetic acid precipitate was separated by centrifugation, and this was freeze-dried after dialysis overnight against running tap-water to remove the trichloroacetic acid.

The supernatant solution was also dialysed overnight and then concentrated twofold. Trichloroacetic acid was added once again, and the precipitate was discarded. The final supernatant solution was freeze-dried after passage through a Sephadex G 25 column. Two fractions were obtained, one in the precipitated material and the other in the supernatant. Approximately one-tenth of the original freeze-dried material was present in the precipitate, the remainder being present in the supernatant.

3. Freeze-dried extracts were prepared from the culture media of a one-month culture at 37° C. of a *Mucor* sp. on Sabouraud's glucose-peptone broth, and of a two-week culture at 27° C. of *Aspergillus fumigatus* IMJ 48337 on Czapek-Dox medium.

IMMUNOLOGICAL TESTS. Agar-gel double-diffusion and immuno-electrophoretic tests were made as described by Pepys and others (1963). The double-diffusion test was found to be more sensitive than the immuno-electrophoretic test. The latter test, however, was more discriminating, making it possible to identify specific reactions by their position in the agar-gel.

The immuno-electrophoretic test was made by a micro-method on glass slides 8 cm. square, which enabled a number of tests to be performed on the one slide. The dimensions and placing of the antigen wells and serum trough were kept constant to ensure comparability of results (Fig. 1). The antigen wells were 2 mm. in diameter and were placed midway between the serum troughs, which were 5 cm. in length, 1 mm. in diameter, and 1.2 cm. away from each other. The antigen wells were placed 1.5 cm. away from the cathodal ends of the troughs. In order to show up fine or faint precipitation arcs the immuno-electrophoretic test slides were washed overnight in 0.9% saline plus 0.1% sodium azide. The film of agar was dried between sheets of Whatman 3 MM filter paper in an oven at about 40° C. and then stained with 0.5% naphthalene black 10B in a mixture of 5 parts methanol, 4 parts water, and 1 part glacial acetic acid.

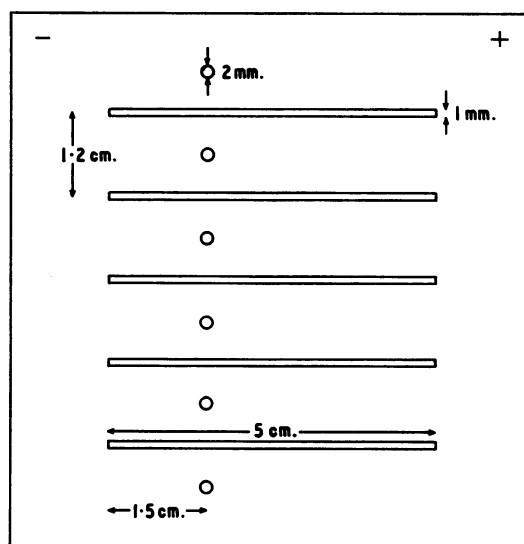


FIG. 1. Immuno-electrophoretic test pattern.

GRADING OF THE IMMUNO-ELECTROPHORETIC TEST REACTIONS The main specific precipitation arcs in tests with farmer's lung sera and extracts of mouldy hay have been described in three regions which have been labelled A, B, and C (Fig. 2). These arcs were due to antigens which are present in mouldy hay responsible for farmer's lung, and which were therefore termed 'farmer's lung hay' or F.L.H. antigens (Pepys and others, 1963). The precipitation reactions produced by these antigens in one or more of the three regions with their corresponding specific precipitins will be termed F.L.H. reactions. Since the antigens responsible for these arcs correspond with antigens present in extracts of *T. polyspora*, and since this organism is the only source of all three antigens so far isolated from mouldy hay, the arcs in the A, B, and C regions will be referred to as F.L.H. reactions (*T. polyspora* type).

An arc in the C region due to an antigen in common with *T. polyspora* was produced by extracts of *M. vulgaris*. More than one arc was frequently seen in the A, B, and C regions, and other arcs were found less frequently in contiguous regions; an example of the latter can be seen in the reaction produced by the antigens in the supernatant of the *T. polyspora* extract after trichloroacetic acid precipitation (Fig. 3). The significance of these additional arcs has not been assessed. The possibility that there are other sources of antigen in mouldy hay of importance in farmer's lung and capable of giving reactions in regions other than the A, B, and C regions has to be considered. If these are found they could be added to the range of F.L.H. antigens, together with the name of the source for purposes of identification.

The precipitation arcs in regions A, B, and C of the F.L.H. (*T. polyspora*) type have been graded in order to assess the intensity of the reaction. The presence of

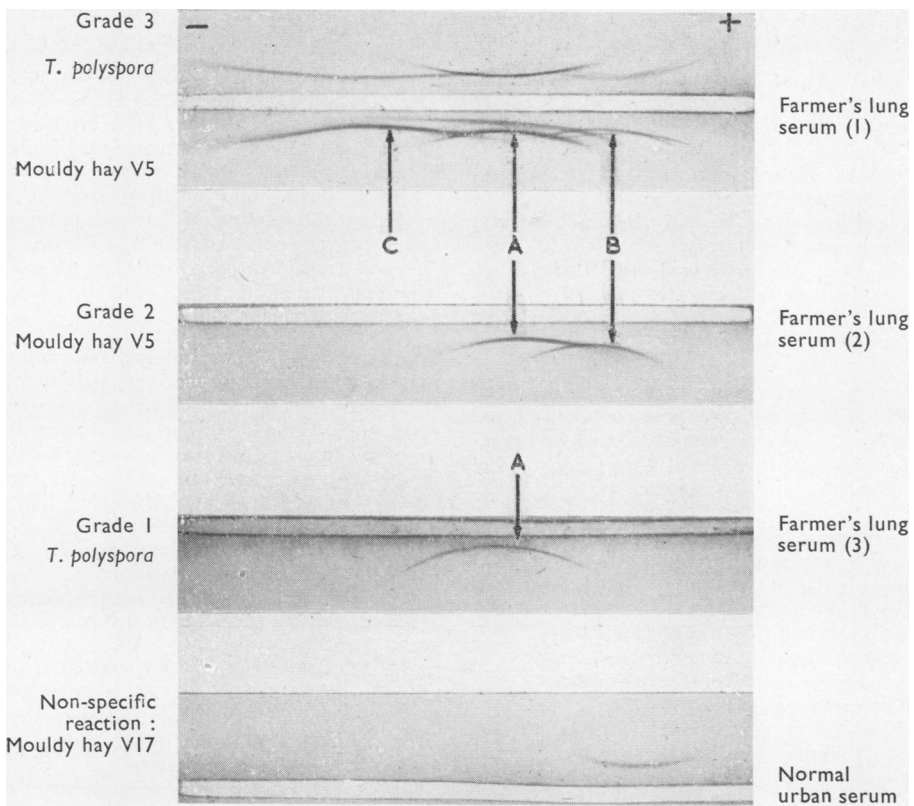


FIG. 2. Grades of F.L.H. reaction in immuno-electrophoretic test.

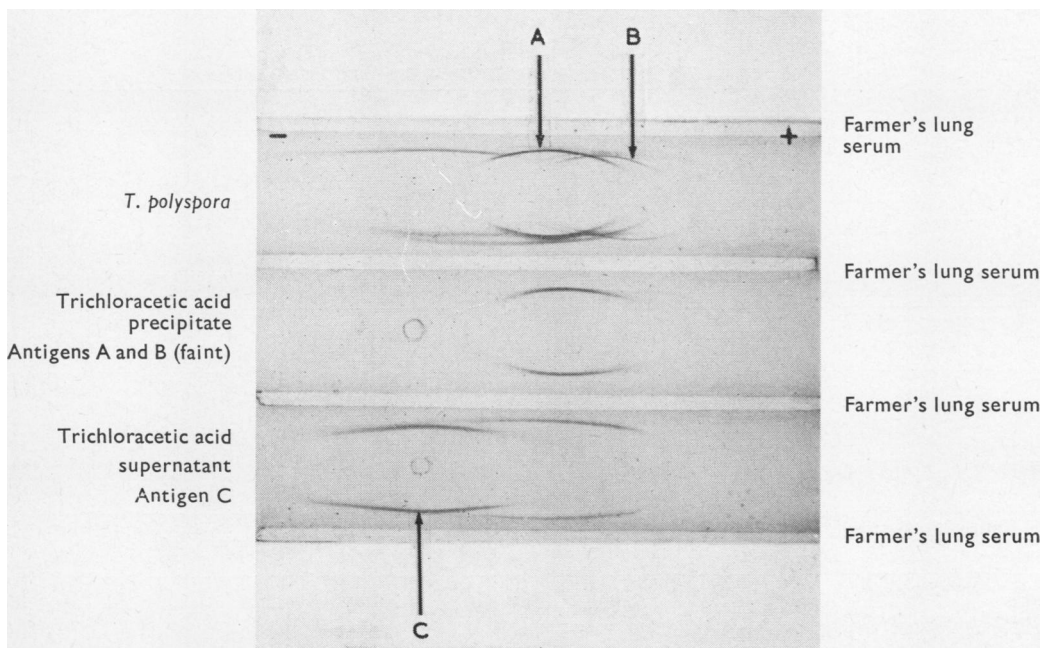


FIG. 3. *T. polyspora* trichloroacetic acid fractions.

arcs in all three regions has been termed grade 3; in two of the regions, grade 2; in one region only, grade 1; and negative reactions, grade 0.

SKIN TESTS Prick tests were performed in selected subjects with carbol-saline solutions of 10 mg./ml. of freeze-dried extracts of good hay and mouldy hay, prepared in our own laboratory, and with 10% w/v extracts of *Alternaria*, *Aspergillus fumigatus*, *Cladosporium herbarum*, dry rot, *Penicillium*, *Tricothecium*, yeasts, *Sporobolomyces*, and of mixed grass, flower, and tree pollens (Duncan-Flockhart).

INHALATION TESTS These were performed as described by Williams (1963a, b) with solutions containing 1 and 10 mg./ml. of the *T. polyspora* extract, of the two freeze-dried, trichloroacetic acid fractions of *T. polyspora*, and of extracts of mouldy hay and *M. vulgaris*.

TEST SERA Test sera were obtained from the following:—

(1) subjects not engaged in farming and not exposed to mouldy hay

(a) urban dwellers who lived in London

(b) workers exposed to other vegetable dusts

(2) farm workers exposed to mouldy hay

(a) regarded as suffering from farmer's lung

(b) with or without other lung disease

(3) a small group of farm workers exposed to and affected by other mouldy cereal dusts.

(1) *Subjects not engaged in farming* The group of sera of urban dwellers who were not exposed to mouldy hay includes sera tested in the previous study of Pepys and

others (1962) on which further tests have now been made. The total numbers were:—(1a) normal blood bank sera (60), patients with asthma (115), bronchitis (34), pulmonary aspergillosis (30), sarcoidosis (52), and interstitial fibrosis (22); (1b) sera of workers exposed to other vegetable dusts were obtained from U.K. dockers working with cereal grains (61) and cotton workers (20). Other sera were obtained from East African sisal workers (20), and tea workers (20); from West African workers on palm-nut plantations (46); and from West Indians working with bagasse (52).

(2) *Farmer's sera* Three hundred and twenty-seven sera were obtained from farmers exposed to mouldy hay. Of these, 16 were included in the previous report by Pepys and others (1963). The sera of 16 farmers exposed to other mouldy cereal dusts were also tested. Most of the sera were provided as follows:—Wales: Drs. G. O. Thomas (86), R. W. Biagi (29), J. T. Jones (20), S. T. H. Jenkins (14); Ulster: Drs. C. T. B. Adams (45), E. F. James (37); Lancaster: Dr. J. L. Russell (17); Devon: Dr. C. J. Fuller (16). Groups of less than 10 sera were obtained from other centres.

The age distribution of the total group was: 10–19 years, 3%; 20–29 years, 10%; 30–39 years, 16%; 40–49 years, 23%; 50–59 years, 31%; 60–69 years, 15%; 70 years or more, 2%.

A group of 12 sera from Icelandic patients with farmer's lung was provided by Dr. Bjornsson. These are not included in the main analysis.

(a) A total of 205 farmers were placed in the farmer's lung group. Information was sought on the clinical features listed in Table I. No questions were asked about

TABLE I
MODE OF ONSET OF CLINICAL FEATURES IN FARMER'S LUNG PATIENTS

	Tested	Groups											
		I (49%) Insidious			II (9%) Insidious and Typical			III (32%) Typical			IV (10%) Rapid Previously Insidious and or Typical		
		Tested	Positive No.	Positive %	Tested	Positive No.	Positive %	Tested	Positive No.	Positive %	Tested	Positive No.	Positive %
Total No. of patients	205				100			19			66		
Male		167	81		72	72		15	79		62	18	90
Female		38	19		28	28		4	21		4	2	10
Dyspnoea		197	96		99	99		18	95		62	18	90
Tightness		57	28		27	27		5	26		19	6	30
Wheezing		60	29		22	22		3	16		26	9	45
Cough		180	88		88	88		17	89		56	19	95
Expectoration		108	53		51	51		11	58		36	10	50
Haemoptysis		15	7		7	7		2	11		2	4	20
Malaise		96	47		43	43		9	47		31	13	65
Fever		99	48		37	37		14	74		35	13	65
No. examined during attack	172				85			19			49	19	
Wheezing		29	17		11	13		2	11		8	8	42
Crepitations		139	81		71	84		13	68		39	16	84
No. with radiographs during attack	146				68			12			52	14	
Radiograph compatible with farmer's lung		95	65		46	68		8	67		36	5	36

loss of weight, the clinicians spontaneously offering information in a small proportion of cases. Information on the presence or absence of wheezes or crepitations was available in 172 (84%) of the patients who were examined during the attacks of farmer's lung, and radiographs taken during the illness were available for 146 (71%) of the patients. Patients who had had asthma, bronchitis, and other pulmonary disease before exposure to the mouldy hay were excluded from this group unless, as in the 20 patients listed in Table II, there were additional features suggestive of farmer's lung in relation to the illness under observation. The last attack had occurred in 1963 (45%); 1962 (26%); 1961 (9%); 1960 (5%); and 1959 (10%). The remainder had their attacks from 1945 onwards, but very few had ceased being exposed to mouldy hay. The majority of the sera were examined during or shortly after the attacks, only about 10% being provided for examination two to three years or more after the last attack.

TABLE II
FEATURES SUGGESTIVE OF FARMER'S LUNG IN PATIENTS
WITH PREVIOUS LUNG DISEASE

Previous Disorder	No. of Patients	Crepitations	Malaise	Fever	Loss of Weight	Compatible Chest Radiograph
Asthma ..	4	4	4	4	1	1
Bronchitis ..	5	4	3	3	2	2
Pneumonia ..	4	2	2	1		
Undiagnosed disease ..	4	3		1		
Old tuberculosis ..	2	2				1
Silicosis ..	1		1	1		

The original descriptions of farmer's lung clearly dealt with the most striking cases, and it is these descriptions which have been followed in most of the subsequent reports. Unless it is recognized that this illness may have different modes of onset, the diagnosis is likely to be missed in many patients. The patients have been divided into four groups according to the mode of onset of the disorder (Table I). Group I, consisting of 100 patients (49%), had an insidious onset and gave a history of constant or very frequent handling of mouldy hay for weeks and sometimes months before pulmonary symptoms became severe enough for medical advice to be sought. Group II, consisting of 19 patients (9%), had an insidious onset to start with, followed later by typical acute attacks. Group III, consisting of 66 subjects (32%), gave a history of typical attacks from the beginning. In this group the usual interval between exposure and symptoms was five to six hours or more, but not infrequently fever, aches and pains, a feeling of depression, and general debility developed within one to two hours of the exposure. Group IV, consisting of 20 patients (10%), gave a history of insidious onset and subsequent typical attacks, and finally very rapid and sometimes immediate respiratory symptoms developed on exposure to the dust.

TABLE III
AGE DISTRIBUTION (%) OF FARMER'S LUNG PATIENTS

Age (yr.)	Group			
	I Insidious	II Insidious and Typical	III Typical	IV Rapid Previously Insidious and or Typical
10-19	2		2	5
20-29	12	11	9	15
30-39	19	26	17	5
40-49	21	21	27	45
50-59	34	37	26	30
60-69	11	5	18	
70	1		1	

The clinical features of the four groups of patients have been compared. Table III shows that group IV subjects (rapid onset) were slightly younger than those in the other groups. Table I shows that there were only small differences in the proportions of the patients in the groups complaining of dyspnoea, tightness of the chest, cough, expectoration of small amounts of sputum, haemoptysis, and malaise, and that there were also similar proportions with crepitations. Differences between the groups were as follows: group I (insidious onset) had the lowest proportion of patients with febrile episodes; group III (typical onset) had the highest proportion of males, suggesting that they were more heavily exposed and therefore more likely to get the typical acute attacks; and group IV (rapid onset) had the highest proportion of patients complaining of wheezing and with wheezes present, and the lowest proportion with radiographic evidence compatible with the disease, perhaps because the rapid onset of symptoms may have reduced the length of exposure and the amount of dust inhaled. Loss of weight was notified for 18% of the patients in group I, 37% in group II, 11% in group III, and 20% in group IV. It can be seen that the patients in group IV, in spite of the rapid onset of the symptoms, had malaise, fever, and crepitations with comparable frequency to the other groups, and loss of weight was also commented on as frequently.

In order to investigate the rapid bronchial reactions in group IV, skin 'prick' tests were made in 11 subjects. Only two gave positive immediate type reactions, one to an extract of good hay and the other to an extract of mouldy hay. Both patients had a history of previous allergic asthma. The negative reactions in the remainder suggest that some other mechanism is responsible for the bronchial reaction than the immediate type hypersensitivity which mediates asthma in atopic subjects.

(b) A group of 122 sera was obtained from farmers exposed to mouldy hay but not regarded as having farmer's lung. These included normal farmers in Wales and Ulster (28), who worked together with patients suffering from farmer's lung; and farmers with asthma with or without bronchitis (38), with bronchitis, with or without asthma (23), with interstitial fibrosis (15), in two of whom sarcoidosis was suspected, with sarcoidosis (9), with cardiac disease (2), with tuberculosis (1), and with undiagnosed pulmonary disease (6).

SEROLOGICAL RESULTS

(1) SUBJECTS NOT ENGAGED IN FARMING Table IV shows that in tests with extracts of mouldy hay, both by double-diffusion and by immuno-electrophoresis, against some of the sera of group 1a, that is 134 urban sera, six reacted in the double-diffusion test, but neither these nor the remaining 128 sera gave any reactions in the immuno-electrophoretic test. In the previous study by Pepys and others (1962), 51 out of 170 urban sera gave positive reactions in the double-diffusion test to extracts of mouldy hay. Immuno-electrophoretic tests were made on these 51 sera, which consisted of 16 out of 50 sarcoidosis sera, 11 out of 90 asthmatic sera, and 24 out of 30 aspergillosis sera. None of these sera gave F.L.H. reactions in the immuno-electrophoretic test. In tests with extracts of mouldy hay

TABLE IV
REACTIONS TO MOULDY HAY EXTRACTS (H44, H1b, L8)
OF SERA FROM NON-FARMING POPULATION

Clinical Condition	No. of Patients	Double-Diffusion Test	Immuno-electrophoretic Test
Normal	60	3	0
Asthma	25	1	0
Bronchitis	34	1	0
Pulmonary fibrosis	15	1	0

against some of the sera of group 1b, that is 61 dockers working with cereal grains, three gave positive reactions in the double-diffusion test. These sera were not tested by immuno-electrophoresis. Like the other sera tested above, the proportion of reactions to extracts of mouldy hay was low.

Double-diffusion tests were made with extracts of *T. polyspora* (Table V), and immuno-electrophoretic

TABLE V
REACTIONS TO *T. POLYSPORA* EXTRACT OF CONTROL SERA OF NON-FARMING POPULATION AND SUBJECTS EXPOSED TO OTHER VEGETABLE DUSTS

Clinical Condition	No. of Patients	Double-diffusion Test
<i>Urban dwellers</i>		
Normal	30	0
Asthma	30	0
Bronchitis	34	0
Sarcoidosis	10	0
Pulmonary fibrosis	20	0
<i>Workers exposed to other vegetable dust</i>		
Dock workers	20	0
Cotton workers	20	0
Sisal workers (E. Africa)	20	0
Tea workers (E. Africa)	20	0
Palm-kernel plantation workers (W. Africa)	46	0
Bagasse workers (W. Indies)	52	5

tests were made on those who gave positive reactions. No reactions were obtained with sera from group 1a, the 124 urban dwellers in the United Kingdom, or from group 1b, the 20 dockers exposed to cereal dusts and 20 cotton workers. No reactions were obtained with the sera of 20 E. African workers exposed to sisal and of 20 exposed to tea dusts, nor with the sera of 46 W. African workers on a palm-nut plantation. Of the 52 W. Indian workers exposed to bagasse, five gave positive reactions on double-diffusion but none gave reactions when tested further by immuno-electrophoresis. In tests with the extract of *T. polyspora* on 302 sera from subjects not engaged in farming, five positive reactions were obtained in bagasse workers in the double-diffusion test only. These findings confirm the absence of antibodies capable of reacting with F.L.H. antigens, shown in the test with the mouldy hay extracts in subjects not exposed to mouldy hay.

TABLE VI
PRECIPITIN REACTIONS IN THE FOUR CLINICAL GROUPS OF FARMER'S LUNG PATIENTS (205 PATIENTS)

	Total			Group I (49%) Insidious			Group II (9%) Insidious and Typical			Group III (32%) Typical			Group IV (10%) Rapid Previously Insidious and or Typical		
	Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive	
		No.	%		No.	%		No.	%		No.	%		No.	%
Double-diffusion test															
Mouldy hay	205	186	91	100	87	87	19	17	89	66	62	94	20	20	100
<i>T. polyspora</i>	194	164	85	95	72	76	18	16	89	63	58	92	18	18	100
<i>M. vulgaris</i>	193	101	52	95	46	48	18	9	50	63	35	56	17	11	65
<i>Mucor</i>	175	103	59	89	49	55	15	10	67	57	39	68	14	5	36
<i>A. fumigatus</i>	166	63	38	84	24	29	15	10	67	53	26	49	14	3	21
Immuno-electrophoretic test															
Mouldy hay	205	*182	89	100	84	84	19	17	89	66	61	92	20	20	100

* Including 3 sera which gave reactions not of the *T. polyspora* type.

FARMING POPULATION

(2a) *Farmers with farmer's lung* Table VI shows that positive reactions were obtained against extracts of mouldy hay in the double-diffusion test in 91% of the patients and in 89% in the immuno-electrophoretic test. There are no statistically significant differences that we wish to stress. The immuno-electrophoretic reactions of 87% of the sera were produced in the A, B, and C regions which have been found to be characteristic of the F.L.H. antigens of the type found in *T. polyspora*. The remaining sera regarded as giving positive immuno-electrophoretic reactions, which were not of the F.L.H. (*T. polyspora*) type, consisted of three sera which in reacting to extracts of mouldy hay gave arcs in other regions. One further serum reacted only to an extract of *Streptomyces thermoviolaceus*, another of the thermophilic actinomycetes of mouldy hay. The presence of additional sources of F.L.H. antigen other than *T. polyspora* is suggested by the reactions of this small group of four sera.

Positive reactions to extracts of *T. polyspora* were obtained with 85% of the sera in the double-diffusion test as compared with reactions of 91% of the sera to extracts of mouldy hay. The extract of the culture of *T. polyspora* on nutrient medium has been found to be less potent than when it is grown on hay, and minor antigenic differences have also been found (Jenkins, 1964). The extract of *M. vulgaris* gave reactions in 52%, *Mucor* sp. in 59%, and *A. fumigatus* in 38% of the total. In both

the double-diffusion and immuno-electrophoretic tests, positive reactions to the extracts of mouldy hay were obtained in comparable proportions of patients in all four clinical groups. This is of particular importance in group IV, the rapid onset group, showing that they were similar serologically to the other groups of patients.

The grades of F.L.H. reaction (*T. polyspora* type) in the immuno-electrophoretic test were classified according to whether arcs were produced in one or more of the regions A, B, and C, corresponding to the electrophoretic spread of the F.L.H. antigens (*T. polyspora* type) (Fig. 2). In the 87% of farmer's lung sera that gave reactions in these regions, there were 42% with grade 3 reactions, i.e., reactions in all three regions, 31% with grade 2, 14% with grade 1, and 13% with grade 0. Of the grade 2 reactions, 84% consisted of A and B arcs, with equal numbers of the remainder giving A and C or B and C arcs. Of the grade 1 reactors, there were 43% giving A arcs, 50% giving B arcs, and 7% giving C arcs.

A comparison of the clinical features in patients with the various grades of F.L.H. reaction was made in order to see whether there was any relationship between the amount of antibody and the disease (Table VII). Although the incidence of most of the clinical features listed was similar in patients in all grades, even including grade 0, definite differences were also present. There were in the strongest reactors (grade 3) 91% of males compared with 68% to 77% in the other grades. This may have

TABLE VII

GRADE OF F.L.H. REACTIONS (*T. POLYSPORA*) AND CLINICAL FEATURES IN FARMER'S LUNG PATIENTS

	Total			Grades of F.L.H. Reaction											
				3 (42%)			2 (31%)			1 (14%)			0 (13%)		
	Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive	
		No.	%		No.	%		No.	%		No.	%		No.	%
No. of patients	205	167	81	87	79	91	64	49	77	28	19	68	26	20	77
Male		38	19		8	9		15	23		9	32		6	23
Female															
Dyspnoea		197	96		85	98		60	94		28	100		24	92
Tightness		57	28		26	30		14	22		12	43		5	19
Wheezing		60	29		29	33		18	28		7	25		6	23
Cough		180	88		80	92		55	86		22	79		23	88
Expectoration		108	53		46	53		34	53		15	54		13	50
Haemoptysis		15	7		6	7		6	9		2	7		1	4
Malaise		96	47		35	40		31	48		16	57		14	54
Fever		99	48		42	48		33	52		16	57		8	31
No examined during attack ..	172	29	17	77	9	12	52	10	19	23	6	26	20	4	20
Wheezes		139	81		62	81		45	87		15	65		17	85
Crepitations															
No. of chest radiographs during attack ..	146			64			48			16			18		
Radiograph compatible with farmer's lung		95	65		37	58		33	69		14	88		11	61

TABLE VIII
GRADES OF F.L.H. REACTION AND PERCENTAGES OF POSITIVE REACTIONS TO OTHER EXTRACTS IN FARMER'S LUNG PATIENTS

Double-diffusion Test Extracts	Grade of F.L.H. Reaction												Total		
	3			2			1			0					
	Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive		Tested	Positive	
		No.	%		No.	%		No.	%		No.	%		No.	%
Mouldy hay (3)	87	87	100	64	64	100	28	28	100	26	7	27	205	186	91
<i>T. polyspora</i>	82	82	100	60	60	100	26	19	73	26	3	12	194	164	85
<i>M. vulgaris</i>	82	54	66	59	37	63	26	9	35	26	1	4	193	101	52
<i>Mucor</i> sp.	67	49	73	55	33	60	28	14	50	25	7	28	175	103	59
<i>A. fumigatus</i>	65	31	48	50	20	40	27	10	37	24	2	8	166	63	38

been due to the heavier exposure of the male to the dust. In grade 0 only 31% of patients had fever compared with 48% to 57% in the other grades, suggesting that in this grade the absence of precipitins may have been related to the intensity of the systemic reaction.

There was also a correlation between the grade of F.L.H. reaction and the incidence of reactions in double-diffusion tests to other flora of the mouldy hay (Table VIII), the proportion of precipitation reactions to extracts of *M. vulgaris* and *Mucor* sp. being greater in the higher grades. There were in the grade 3 reactors 66% and 73% respectively, as compared with 63% and 60% in grade 2, 35% and 50% in grade 1, and 4% and 28% in grade 0. These differences were statistically significant, $P > 0.001$. There were also more reactions to the extract of *A. fumigatus*, 48% in the grade 3 sera compared with 40% in grade 2, 37% in grade 1, and 8% in grade 0. These differences were statistically significant, $P > 0.01$.

Table IX shows that the higher the grade of F.L.H. reaction, *i.e.*, the more antibody present, the greater was the number of attacks. In the patients with grade 3 sera, there were 44% with three or more attacks, compared with 31% in grade 2, 17% in grade 1, and 14% in grade 0. These differences were statistically significant, $P > 0.05$. The degree of clinical sensitivity to the dust was

assessed by the length of exposure required to provoke symptoms. Table X shows that 70% and 62% respectively of the patients with grade 0 and grade 1 sera were affected only by long exposures (that is their sensitivity was low) compared with 27% and 21% in grade 2 and grade 3. On the other hand, 29% and 31% respectively of the patients with grade 2 and grade 3 sera were affected by short exposure (that is their degree of sensitivity was high) as compared with 15% and 7% respectively in the patients with grades 0 and 1 sera. The statistical significance of these differences was $P > 0.001$.

TABLE X
GRADE OF F.L.H. REACTION AND SEVERITY OF FARMER'S LUNG IN FARMER'S LUNG PATIENTS

	Grade of F.L.H. Reaction			
	0	1	2	3
No. of patients assessed	23	13	45	74
Severity of disease	(%)	(%)	(%)	(%)
Slight	34	15	11	14
Moderate	43	39	47	54
Severe	23	46	42	32
No. of patients assessed	20	13	45	71
Degree of hypersensitivity (length of exposure needed to produce attack)	(%)	(%)	(%)	(%)
Low (long exposure)	70	62	27	21
Moderate	15	31	44	48
High (short exposure)	15	7	29	31

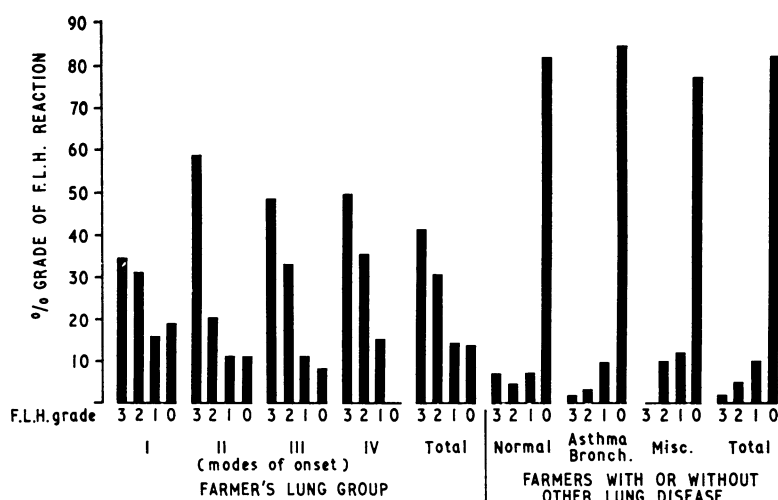
TABLE IX
GRADE OF F.L.H. REACTION AND NO. OF ATTACKS OF FARMER'S LUNG IN FARMER'S LUNG PATIENTS

Grade of F.L.H. Reaction	No. of Patients Assessed*	No. of Attacks			
		1 (%)	2 (%)	3 (%)	4 or More (%)
0	21	76	10	4	10
1	29	62	21	7	10
2	61	44	25	16	15
3	86	34	22	20	24

* Supplemented by additional patients examined after main analysis because of small numbers with information available.

Differences were also present in the severity of the attacks in patients with the various grades of serological reaction, there being more patients with slight reactions and fewer patients with severe reactions among the patients with grade 0 than in those with grades 1, 2, and 3 sera (Table X). Although this difference was not statistically significant, it is suggested, like the other findings, that the presence and amount of precipitating antibody were related to the severity of the clinical manifestations.

FIG. 4. Grade of F.L.H. reaction of sera of farmers exposed to mouldy hay.



The clinicians were asked to answer the question, Do the symptoms, signs, and duration suggest that the attacks were slight, moderate, or severe? We felt that the differences between slight and severe were readily decided, and the remainder of the patients would be regarded as having 'moderate' attacks.

These grades of severity were not known to the clinicians before the serological results, and there were no important differences in the grades of severity of disease in the different groups of onset.

The grades of F.L.H. reaction in the four clinical groups of modes of onset are shown in Figure 4. There were fewer patients with grade 3 sera and more with grade 0 sera in the group of patients whose disease started insidiously than in the other groups. The weaker serological reactions in this group may be related to the gradual mode of onset of the disease.

The group of 12 sera from Icelandic patients with farmer's lung all gave typical F.L.H. reactions: grade 3 in six; grade 2 in three; and grade 1 in three cases.

(2b) *Farmers with or without other lung disease* Table XI shows that reactions were given in 32% of the normal farmers, in 34% of the farmers with asthma or bronchitis, and in 39% of the farmers with other lung diseases in double-diffusion tests with extracts of mouldy hay. Reactions to the extract of *T. polyspora* were given in 19%, 30%, and 20% respectively. There were also low proportions of reactions to the other extracts tested.

In the immuno-electrophoretic test, F.L.H. reactions were given by 18% of normal farmers, 15% of the farmers with asthma and bronchitis, and 21% of those with other lung diseases. Figure 4 shows

TABLE XI
PRECIPITIN REACTIONS IN FARMERS WITHOUT FARMER'S LUNG

	Normal			Asthma and Bronchitis			Other Lung Diseases		
	Tested	Positive No.	Positive %	Tested	Positive No.	Positive %	Tested	Positive No.	Positive %
Double-diffusion test extracts									
Mouldy hay	28	9	32	61	21	34	33	13	39
<i>T. polyspora</i>	27	5	19	47	14	30	30	6	20
<i>M. vulgaris</i>	27	3	11	44	4	9	33	0	0
<i>Mucor</i> sp.	27	6	22	56	5	9	32	3	9
<i>A. fumigatus</i>	27	1	4	55	8	15	32	4	13
Immuno-electrophoretic test									
Mouldy hay (F.L.H. reactions)									
<i>T. polyspora</i> type	28	5	18	61	9	15	33	7	21

that small numbers gave grades 3, 2, and 1 F.L.H. reactions (*T. polyspora* type) whether or not these farmers had lung disease. In the whole group of 122 farmers not suffering from farmer's lung, F.L.H. reactions were obtained in 17%. Grade 3 reactions were obtained in three (2.5%), grade 2 in six (5%), and grade 1 in 12 (10%). By contrast, the F.L.H. reactions (*T. polyspora* type) obtained in the 87% of positive reactors in the farmer's lung group were grade 3 in 42%, grade 2 in 31%, and grade 1 in 14% (Table XII).

Three of the asthmatic patients attributed their symptoms to mouldy hay and may perhaps have been regarded as suffering from farmer's lung according to the criteria of Staines and Forman (1961). In the seven subjects with other lung diseases

TABLE XII
IMMUNO-ELECTROPHORETIC TEST—MOULDY HAY
EXTRACT

	No. of Subjects	Posi- tive	Grade of F.L.H. Reaction (<i>T. polyspora</i> type)		
			1	2	3
Normal	28	(%) 18	(%) 7	(%) 4	(%) 7
Other lung diseases	94	17	11	5	1
Farmer's lung due to:					
Mouldy hay	205	87	14	31	42
Other vegetable dusts	16	50	31	0	19

who gave positive reactions, the diagnosis of farmer's lung could not be excluded in at least two cases. One was a threshing contractor in whom the diagnosis was complicated by hypertension and left ventricular enlargement; the second patient, in whom a diagnosis of diffuse interstitial fibrosis had been made, had a long history of exposure to baled hay though without any definite attacks. Four of the positive reactors were diagnosed as having pulmonary fibrosis, in one patient possibly due to sarcoidosis, and one patient had chronic tuberculosis and asthma.

(3) PATIENTS WITH FARMER'S LUNG DUE TO OTHER VEGETABLE DUSTS Tests were performed on a group of 16 sera from patients suffering from farmer's lung due to mouldy corn (9), mouldy barley and barley straw (5), and sweating oats (2). The clinical features consisted of dyspnoea in all cases; tightness of the chest (6); wheezing (5); cough (10); malaise (5); fever (8); crepitations (7 out of 11); loss of weight (1); radiographic evidence compatible with the diagnosis (3 out of 5).

In double-diffusion tests, eight reacted to mouldy hay and to *T. polyspora*, one to *M. vulgaris*, two to *Mucor* sp., and none to *A. fumigatus*. In the immuno-electrophoretic test, eight sera (50%) gave F.L.H. reactions (*T. polyspora* type) to extracts of mouldy hay. Although the numbers are small, these results suggest that there was a lower content in the other cereal dusts of the flora found in mouldy hay.

COMPARISONS OF THE MOULDY HAY EXTRACTS

Table XIII shows that the extract of H44 gave much lower proportions of reactions with sera of grades 1, 2, and 3 than the extracts of the other two mouldy hays prepared by growing a mixed inoculum of the organisms from H44 on moistened sterile hay under favourable conditions. This finding shows the importance of using mouldy hays capable of providing potent extracts and is also relevant in that H44 was used in the previous studies (Pepys and others, 1962; Williams, 1963a, b). Its lack of potency

influenced the conclusions derived from its use, and this will be discussed again later.

Further mouldy hays were prepared by permitting the hay to become mouldy in the laboratory without any added inoculum or incubation, thus simulating the self-heating of mouldy hay under natural conditions. The mouldy hay V4 gave reactions comparable to those of other mouldy hays. V17, however, differed strikingly from the others tested in this and previous studies, giving more frequent reactions with control sera. Reactions were obtained in double-diffusion and immuno-electrophoretic

TABLE XIII
IMMUNO-ELECTROPHORETIC TEST REACTIONS IN FAR-
MER'S LUNG PATIENTS TO MOULDY HAY EXTRACTS IN
RELATION TO GRADE OF F.L.H. REACTION (133 SERA)

Grade of F.L.H. Reaction	Mouldy Hays		
	H44 (%)	Hib (%)	L8 (%)
1	38	70	65
2	40	96	98
3	70	100	100

tests as follows: in 22 normal sera, 11 and 8 respectively; in 25 sera from asthmatics, 16 and 14 respectively; in 34 sera from bronchitic patients, 15 and 9 respectively; and in 22 patients with interstitial fibrosis, 11 and only 1 respectively. The immuno-electrophoretic reactions of this extract lay well away from the F.L.H. reaction (*T. polyspora* type) towards the anode (Fig. 2). The significance of this reaction is not known, but it emphasizes the need to use the F.L.H. pattern of *T. polyspora* as a guide for the present.

REACTIONS TO *M. vulgaris*

The extract of *M. vulgaris* gave reactions in double-diffusion tests with 52% of the sera. Since this extract contains antigens which give reactions in the C region, this value corresponds well with the finding that in the immuno-electrophoretic test with extracts of mouldy hay, 48% of the sera gave reactions in the C region. Only 7% of the reactions to the mouldy hay extracts were reactions solely in the C region. As stated earlier, *M. vulgaris* and *T. polyspora* have a common antigen which produced reactions in the C region.

TRICHLORACETIC ACID FRACTIONS OF *T. polyspora* EXTRACT

The antigens precipitated from the culture filtrate extract of *T. polyspora* by trichloroacetic acid gave reactions in the A and B regions, whereas the antigens present in the supernatant gave reactions in the C region (Fig. 3). The C antigen in the

supernatant was compared directly with the antigen produced by Kobayashi and others (1963) by the extraction of mouldy hay with trichloroacetic acid solution. Both preparations gave reactions of identity in the agar-gel test and were capable of inhibiting each other's reactions.

INHALATION TESTS

Systemic and pulmonary reactions of the type encountered in farmer's lung have been reported in five affected subjects as a result of the inhalation of an extract of the culture filtrate of *T. polyspora* grown on nutrient agar (Pepys and others, 1963). Further tests have been performed with the trichloroacetic acid fractions of the *T. polyspora* extract in two additional patients and in repetition of the tests on one of the previous patients. Tests were also performed with extracts of mouldy hay and of *M. vulgaris*.

The number of controls was increased from one in the previous study to four by the testing of a further three subjects. The four controls consisted of a farmer and an agricultural inspector, both with asthma only, and two urban patients with asthma. No reactions, systemic or pulmonary, resulted in these patients from the inhalation of the highest

concentration of *T. polyspora* used, 10 mg./ml., given for the longest time, 15 minutes.

The seven patients in the farmer's lung group consisted of two patients with a typical onset and without bronchial reactions; one patient with an insidious onset but with wheezing noted from the beginning; two patients with asthma and one with bronchitis which preceded the farmer's lung; and one patient in whom bronchial reactions appeared rapidly on exposure to the dust of mouldy hay.

All these subjects gave systemic or pulmonary reactions to the *T. polyspora* extract and, where tested, to its fractions. An immediate reaction was obtained in only one, and in this case to the test with the extract of *M. vulgaris*. The systemic reactions, consisting of aches and pains and malaise, resembled the complaints of the actual disease and came on several hours after the test, being accompanied in all except one case by a rise in temperature maximal between five and seven hours. Disturbances of pulmonary function were demonstrable at the same time, pulmonary symptoms and signs only becoming obvious where strong reactions were provoked.

The inhalation tests (Table XIV) show that the A and B fraction and the C fraction of *T. polyspora*

TABLE XIV
INHALATION TESTS

Patient	Type of Onset and F.L.H. Grade of Reaction	Test Extract	Conc. (mg. ml.)	Duration of Inhalation (min.)	Rise in Temp. to ° F.	Fall In			
						F.V.C. (ml.) from to	F.E.V. (ml.) from to	Static Compliance (l. cm. H ₂ O) from to	DL _{co} from to
1	Typical Grade 3	(a) <i>T. polyspora</i>	10	10	102.5	4,250 3,250	3,300 2,800	0.181 0.094	NA
		(b) A and B fraction	10	10	99.5	3,700 3,000	2,850 2,100	0.219 0.082	NA
		(c) C fraction	10	6	No rise	3,900 3,700	3,100 2,700	0.140 0.120	NA
		(d) C fraction	10	12	100.5	3,800 3,250	3,100 2,200	0.143 0.074	NA
		(e) <i>M. vulgaris</i>	10	10	No rise	No fall	No fall	No fall	NA
2	Typical Grade 2	(a) <i>T. polyspora</i>	10	10	No rise	No fall	No fall	No fall	NA
		(b) Mouldy hay	10	7	99.5	No fall	2,790 2,300	0.146 0.102	NA
3	Insidious with wheezing Grade 3	(a) C fraction	10	10	99	4,030 3,100	3,070 2,700	No fall	No fall
		(b) A and B fraction	1	10	98.8	4,300 3,630	3,070 2,670	No fall	14.1 11.2
		(c) A and B fraction	10	10	101	3,870 3,630	2,970 2,570	No fall	No fall
		(d) <i>T. polyspora</i> grown on hay	10	10	98.8	4,070 3,730	3,000 2,600	NA	15 8
4	Asthma and farmer's lung Grade 3	1st Test (a) <i>T. polyspora</i>	10	10	98.8	No fall	No fall	NA	
		Repeat tests (a) A and B fraction	10	5	102	3,670 3,170	2,230 2,070	No fall	13.4 9.7
		(b) C fraction	10	5	99	3,600 3,370	2,270 2,070		13.2 9.4
		(c) A and B fraction	10	3	101.5	3,470 2,970	2,100 1,700	0.23 0.15	No fall
5	Asthma and farmer's lung Grade 1	(a) Mouldy hay	10	10	98.7	No change	1,600 1,400	NA	NA
		(b) <i>T. polyspora</i>	10	10	99.4	3,150 3,000	1,500 1,400	NA	NA
6	Bronchitis and farmer's lung Grade 2	(a) <i>T. polyspora</i>	10	10	No rise	No fall	1,120 1,050	0.190 0.078	NA
7	Farmer's lung and/later asthma Grade 1	(a) <i>T. polyspora</i>	10	7	No rise	2,400 1,600	1,050 750	No fall	NA
		(b) <i>T. polyspora</i>	10	15	100	2,350 1,400	900 650	0.057 0.024	NA
		(c) <i>M. vulgaris</i>	10	10					NA
						No rise, acute asthmatic reaction			

NA, not available.

were both capable of provoking reactions. In patient 2 the extract of mouldy hay provoked a more vigorous response than the *T. polyspora* extract. This may be important in selecting antigenic extracts for such tests. In general, the higher the concentration of the antigen and the longer the inhalation, the more vigorous was the reaction produced. Thus in patient 1, inhalation of the C fraction for 12 min. resulted in a temperature of 100.5° F. and a fall in static compliance, whereas the 6 min. inhalation provoked no reactions. In patient 3, the A and B fraction at 10 mg./ml. provoked a temperature of 101° F. as compared with 98.8° F. after the test with a 1 mg./ml. concentration. Patient 7 gave a vigorous reaction to *T. polyspora* extract given for 15 min. compared with a slight reaction to the 7 min. inhalation.

The pulmonary function tests showed that the changes in ventilation were not marked, except in patient 7, who will be discussed later. The D_{Lco} was measured in patients 3 and 4, both of whom showed decreases except in response to test (c) in each case. The minute volume had however risen sharply during test (c) in patient 3 from 12.4 to 17.8 l./min. and in patient 4 from 10 to 16 l./min., which probably accounts for the absence of change in D_{Lco} . Crepitations appeared in patient 1 after the inhalation of the A and B fraction (test (b)) and the C fraction (test (d)), and in patient 7 after the inhalation of *T. polyspora* extract (test (b)).

The tests show that in patients in whom farmer's lung is complicated by bronchial disease preceding or following the farmer's lung, challenge with specific antigens can provoke a febrile systemic reaction and a pulmonary reaction coming on after some hours and resembling the features regarded as characteristic of farmer's lung. Patient 7 is of interest since he began to have a rapid onset of respiratory symptoms in response to exposure to mouldy hay only after he had had attacks of farmer's lung. The inhalation of the *T. polyspora* extract provoked systemic and pulmonary symptoms like those of farmer's lung, whereas an unexpected acute asthmatic reaction followed the test with the extract of *M. vulgaris*.

More detailed testing on more typical cases is required, but our findings in this group support the suggestions that in some cases of farmer's lung there may be differences from the classical descriptions in the mode of onset and in the clinical features of the disease.

DISCUSSION

The presence of precipitins against 'farmer's lung hay' (F.L.H.) antigens derived from *T. polyspora*

and *M. vulgaris* is evidence of exposure to mouldy hay, since they are not found in subjects who have not been so exposed. The positive reactions to extracts of mouldy hay, which were obtained in the immuno-electrophoretic test with 89% of the sera of patients with farmer's lung attributed to mouldy hay, were in 87% of cases of the type produced by antigens present in *T. polyspora*; the remaining 2% gave reactions not of this type. In seeking for the explanation of the negative reactions in 11% of the farmer's lung subjects, several possibilities must be considered. Other sources of antigen may have been responsible for the disease, and this may apply particularly to the patients affected by mouldy cereal dusts other than mouldy hay, of whom only one-half gave positive reactions. Some limited evidence for this was given by the reactions obtained with three sera to other antigens in extracts of mouldy hay and with one serum to an extract of another thermophilic actinomycete, *Streptomyces thermoviolaceus*. More sensitive serological tests may also be of value, and with the identification of known sources of antigen these can now be attempted. Another possible explanation for the negative reactions in affected subjects is that the precipitins against the F.L.H. antigens may be present as a result of exposure to mouldy hay and may be playing an immuno-pathological role in some cases only or not at all. In sensitization by complex micro-organisms the reactions elicited may be mediated by more than one type of hypersensitivity for which different antigenic components may be responsible. The possibility of delayed hypersensitivity reactions of the tuberculin type as a cause of farmer's lung, with or without the participation of precipitin-mediated reactions, must be considered. Tests for delayed hypersensitivity also require pure antigens from known sources, and the recent findings may be of value in this respect as well.

Another aspect of the serological reactions that requires explanation is the appreciable group of 15% to 21% of farmers without farmer's lung whose sera gave F.L.H. reactions (*T. polyspora* type). The significance of these antibodies will need to be assessed by the follow-up of these subjects. The fact that unaffected farmers gave positive serological reactions shows that these reactions are not in themselves diagnostic of farmer's lung though they provide supporting evidence if the features of the disease are present.

The use of the pattern of arcs labelled A, B, and C, produced by the F.L.H. antigens (*T. polyspora* type) in the immuno-electrophoretic test, as a means of grading the intensity of the precipitation

reaction showed interesting correlations with other findings. In addition to the high proportion of positive reactions in farmer's lung patients, their sera also gave much higher grades of reaction than the sera of non-affected farmers. The grade 3 sera (reacting to all three F.L.H. antigens) gave higher proportions of reactions to the extracts of the other flora of mouldy hay, and the group of patients with grade 3 sera included a higher proportion of males, than the patients with the weaker grades of reaction. This suggests that the subjects with grade 3 sera were more heavily exposed to the mouldy hay, as would be expected in male farmers. The frequency and severity of attacks and the degree of sensitivity to the dust were also greater in the patients with the higher grades of serological reaction. These findings suggest that the precipitins are concerned in or run parallel with the pathogenetic mechanisms of the disease.

The immuno-electrophoretic test gave results which seem to be more significant clinically than those of the double-diffusion test. In patients with farmer's lung, similar proportions of reactions were obtained to both tests, but in the normal farmers and in those with other pulmonary diseases, almost twice as many positive reactions were obtained to extracts of mouldy hay and of *T. polyspora* in the more sensitive double-diffusion test than in the immuno-electrophoresis test. This indicates that there is less antibody in the unaffected than in the affected farmers and may account for the absence of farmer's lung reactions in them.

The sera of subjects not exposed to mouldy hay which gave weak positive reactions to extracts of mouldy hay in double-diffusion tests in the previous report by Pepys and others (1962) gave no reactions in the immuno-electrophoretic test. In this respect the reactions of the non-exposed subjects differ from those obtained in double-diffusion tests in exposed farmers with or without farmer's lung, suggesting either that different antigens were responsible or that the amount of antibody in these non-farming subjects was very low.

The addition of trichloroacetic acid to an extract of *T. polyspora* produced a deposit which contained the F.L.H. antigens A and B and a supernatant solution containing antigen C. Inhalations of antigens A and B and of antigen C both provoked systemic and pulmonary manifestations of farmer's lung in affected subjects. The antigen C in the supernatant is identical with the antigen of Kobayashi and others (1963) which was prepared by extracting mouldy hay with a trichloroacetic acid solution, evidently leaving the antigens A and B in the hay. They reported a reaction of the farmer's

lung type in a single patient who was tested by the inhalation of their extract.

Kobayashi and others (1963) claim that their antigen was more specific than the usual Coca's fluid extract of mouldy hay, because it gave reactions only in patients with farmer's lung. We have, however, shown that more than one half of our patients gave precipitin reactions with antigens A and B but not with antigen C.

The farmer's lung sera which gave grade 3 F.L.H. reactions, that is reactions to the C as well as to the A and B antigens, contained the largest variety and amount of antibodies and were obtained from patients with the most frequent and severe attacks. Positive reactions to antigens of the C type would therefore be obtained chiefly in this group of most sensitive subjects, thus giving an impression of greater specificity. Extracts containing A and B antigens as well, however, give positive reactions not only in a much larger proportion of affected subjects but also in a number of exposed but unaffected farmers.

Mouldy hays vary in their content of *T. polyspora*. A lower proportion of positive sera gave reactions to H44 than to the other mouldy hay extracts and to *T. polyspora*. In previous investigations by Pepys and others (1962) and by Williams (1963a, b), the fine dust fraction of H44 was used as a source of actinomycete antigen. This extract of the fine dust of H44 was the only one of the fungal and other extracts tested which gave any inhibition of the reactions to the additional antigens of mouldy hay when it was used to absorb the sera of patients with farmer's lung. This inhibition was, however, weak, and since the extract also failed to provoke a reaction in the inhalation test we thought that the fine dust did not contain the source of the additional antigens, although these were found in the coarse dust. We now think that the extract contained only small amounts of *T. polyspora* antigen. This assumption is supported by the fact that the inoculation of a mixed suspension of the organism of H44 into sterile hay gave under favourable conditions good growth of *T. polyspora* and an abundant supply of its antigens (Pepys and others, 1963). It was these results that led to the demonstration of *T. polyspora* as an important source of F.L.H. antigen, and it was fortuitous that H44 was used in the previous study, from which it was concluded that the antigens relevant to farmer's lung probably arose from breakdown products of the hay itself.

The potency of the antigens of *T. polyspora* demonstrated in man is also evident in cattle which have been investigated for the disease known as fog-fever, a respiratory illness which in some cases

resembles farmer's lung. Although no F.L.H. precipitins were found in the sera of animals in unaffected herds and only small numbers in other animals, almost two-thirds of the cattle suffering from fog-fever or from herds in which it was present had F.L.H. precipitins of the *T. polyspora* type in their serum (Jenkins, 1964).

Less than half of the patients with farmer's lung had typical acute attacks to start with or at some time in the course of the disease, and in the remainder it developed insidiously, particularly in farmers who were constantly exposed. The immediate or almost immediate reactions that may appear in subjects who have become very sensitive may be deceptive if the history of previous insidious or typical attacks has not been elicited. Most of the typical clinical features are present as frequently in this group as in those with the other types of onset. Clinical differences are probably due to variations in the degree of sensitivity of the subject and of the intensity of exposure.

Inhalation tests with extracts of *T. polyspora* and of mouldy hay in patients in whom farmer's lung was complicated by preceding allergic asthma or bronchitis, or by the appearance of asthmatic reactions after the farmer's lung, resulted in febrile systemic reactions and pulmonary reactions like those of farmer's lung. These responses came on some hours after exposure and support the view that a small proportion of the patients with farmer's lung may have, at the same time or as a result, bronchial manifestations as well.

The inclusion of an onset like that of 'asthmatic bronchitis' by Staines and Forman (1961) is probably responsible for the presence of 17% (16 out of 94) of patients with a past history of allergic reactions in their farmer's lung patients. Precipitin tests on the sera of their patients were made in the investigation by Pepys and others (1962). Positive reactions with the farmers' sera were obtained in double-diffusion tests with extracts of mouldy hay only with the sera of those farmers regarded as suffering from farmer's lung. This is clearly a difficult problem to resolve, but it seems that some of these patients may have been suffering from asthma or bronchitis rather than from farmer's lung, particularly since the present study has shown that precipitin reactions of the F.L.H. type may be obtained in normal farmers and in farmers with asthma and bronchitis.

In order to minimize this problem, farmers with previous asthma, bronchitis or other lung disease were excluded from the farmer's lung group unless additional supporting clinical evidence was present. The farmer's lung group included four patients with asthma, and if hay fever and rhinitis are included,

there were less than 5% of subjects in this group with a history suggestive of atopic allergic sensitivity. It is not unlikely that the grouping of patients employed here may err in the opposite direction to that of Staines and Forman (1961) by excluding patients with less familiar forms of farmer's lung, and there will almost certainly be a group in whom a final decision may not be reached.

Studies of pulmonary function may help to distinguish between patients with bronchial reactions associated with disturbances of ventilatory function and patients with farmer's lung in whom the reaction in the peripheral part of the broncho-pulmonary tree results in impaired diffusion of gases and decreased lung compliance. On the one hand, these latter features may not be present at the time of examination, and, on the other hand, Dickie and Rankin (1960) have listed a large number of disorders with similar physiological findings, including, for example, diffuse interstitial fibrosis and sarcoidosis, which give rise to difficulty in the differential diagnosis of farmer's lung.

Differences in the flora of cereal dusts and of mouldy hay are probably responsible for the lower proportion of F.L.H. reactions obtained in patients with farmer's lung due to mouldy vegetable dusts other than mouldy hay. Further investigations of these other dusts are being pursued by the same methods.

SUMMARY

Agar-gel precipitin tests were performed on the sera of 327 United Kingdom farmers, who had given a history of exposure to mouldy hay, with extracts of mouldy hay, *Thermopolyspora polyspora*, *Micromonospora vulgaris*, *Mucor* sp., and *Aspergillus fumigatus*. Positive reactions in the immuno-electrophoretic test to 'farmer's lung hay' (F.L.H.) antigens were obtained in 89% of 205 subjects regarded as having farmer's lung, and, of these, 87% were due to the F.L.H. antigens derived from *T. polyspora*. These sera gave reactions to all three of the main F.L.H. antigens (A, B, and C) in 42% of cases, to two in 31%, and to one in 14%. The wider range of the serological reaction, the greater was the frequency of reactions to the other extracts, the more frequent and severe were the attacks, the higher the degree of clinical sensitivity to the dust of mouldy hay, and the higher the proportion of males, presumably because they were more heavily exposed. F.L.H. reactions (*T. polyspora* type) were obtained with the sera of Icelandic patients with farmer's lung. In 16 patients with farmer's lung due to other vegetable dusts only 50% gave F.L.H. reactions.

In 122 farmers, who had been exposed to mouldy hay and were not suffering from farmer's lung, F.L.H. reactions were obtained in 18% of 28 apparently unaffected farmers, in 15% of 61 with asthma and bronchitis, and in 21% of 33 with other lung disease, these reactions being much weaker in intensity than in the farmer's lung patients.

In tests on the sera of subjects who had not been exposed to mouldy hay, some having been exposed to other vegetable dusts, no F.L.H. reactions were obtained against extracts of mouldy hay in tests on 304 sera, and no reactions (except in five bagasse workers) were obtained against an extract of *T. polyspora* in tests on 302 sera.

Trichloroacetic acid fractionation of *T. polyspora* extract gave a precipitate containing the A and B antigens and a supernatant containing the C antigen. Both fractions provoked systemic and pulmonary reactions in affected subjects in inhalation tests.

The mode of onset of the farmer's lung was insidious in 49% (group I), insidious and later typical in 9% (group II), typical in 32% (group III), and insidious and/or typical and later rapid in 10% (group IV). The latter group had many of the clinical and serological features of the other groups.

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