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The extracellular antigens of *Micropolyspora faeni:* their significance in farmer's lung disease

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SUMMARY

A serological analysis of the extracellular antigens of *Micropolyspora faeni* by immunodiffusion with a combination of sera revealed 29 individual antigens. A survey was made of the incidence of precipitins to the antigens in sera from patients with clinical farmer's lung disease (FLD) and other respiratory diseases. Precipitating antibody was found in 75% of farmer's lung cases and in 20% of other cases who had been exposed to the same environment. More precipitin reactions were seen in sera from severe forms of FLD than from milder forms. The distribution of precipitins to individual antigens was not significantly affected by severity of disease.

Most of the patients with precipitins to *M. faeni*, but without the symptoms of FLD, were suffering from mild or moderate symptoms of other respiratory diseases with a history of chronic onset of symptoms. The distribution of precipitins to individual antigens in this group was similar to that in clinical FLD patients but the incidence was considerably lower.

The significance of these results is discussed.

INTRODUCTION

Farmer's lung disease (FLD) is a respiratory hypersensitivity of agricultural workers, associated with the inhalation of dust from mouldy hay (Dickie & Rankin, 1958). The hypersensitivity is of the Arthus type, which is thought to be mediated by precipitating antibody, tissue damage being caused by the formation of antigen-antibody precipitates (Rose & Phills, 1967). The sera of most patients with clinical FLD have been shown to contain precipitating antibody to a thermophilic actinomycete commonly found in mouldy hay, *Micropolyspora faeni* (Pepys *et al.* 1963). A much smaller proportion of workers exposed to mouldy hay who are healthy or have other respiratory disease also show these antibodies. The organism was originally identified as *Thermopolyspora polyspora* but has recently been reclassified (Cross, Maciver & Lacey, 1968). Precipitating antibody to this organism has also been found in sera from many cattle suffering from a wide range of respiratory conditions collectively known as 'fog-fever' which have several features in common with FLD (Jenkins & Pepys, 1965). In addition, sera from farmer's lung patients often contain precipitating antibodies to *Aspergillus*

fumigatus, *Mucor* spp., *Streptomyces* spp. and other thermophilic actinomycetes, notably *Micromonospora vulgaris* (Pepys *et al.* 1963).

For some years laboratories in the Public Health Laboratory Service have aided clinicians in the diagnosis of equivocal cases of respiratory disease by testing sera against extracts of *M. faeni* by immunodiffusion techniques. Interpretation of the results of these tests is difficult as antibody cannot be detected in some cases of clinical FLD and is present in some patients thought to have other respiratory diseases. The present study was undertaken to determine if there is any qualitative difference between the precipitin contents of sera from patients with and without clinical symptoms of FLD.

MATERIALS AND METHODS

Sera

Sera from 40 patients with FLD (group A) and 54 patients with other pulmonary diseases (group B) were obtained from Public Health Laboratories throughout the country. These sera had been sent to this laboratory for serological testing. All patients had a history of exposure to mouldy farm produce. Other clinical details are summarized in Table 1.

Table 1. *Clinical details of patients*

	Group A (total no. 40)		Group B (total no. 54)	
	No.	%	No.	%
Symptoms				
Dyspnoea	33	83	33	65
Fever	12	30	16	30
Rigor	8	20	8	15
Weight loss	13	32	10	19
Severity				
Mild	8	20	14	26
Moderate	22	55	27	50
Severe	10	25	13	24
Mode of onset				
Acute	24	60	35	65
Chronic	16	40	19	35

Group A = farmer's lung disease patients.
Group B = patients with other respiratory diseases.

The two sera used for the serological analysis contained antibodies to more *M. faeni* antigens than any other available; one was from a case of FLD (H 1), the other from a cow with 'fog-fever' (B 1).

Antigen

The organism used as a source of antigen was *M. faeni*, strain 1156, which was isolated by Dr M. Lacey, Rothamsted Experimental Station, Harpenden. This was grown in continuous culture (details to be published) at 55° C. The medium used contained (per litre distilled water): casein hydrolysate (Hopkin & Williams

Ltd), 10·0 g.; dried yeast extract (Difco Ltd), 2·5 g.; sodium glycerophosphate, 2·5 g.; KCl, 0·5 g.; $MgSO_4$ (anhydrous), 0·5 g.; $Na_2HPO_4.2H_2O$, 5·34 g.; KH_2PO_4, 2·72 g. The pH was 7·0.

Culture was centrifuged and the supernatant fluid concentrated by dialysis against 40 % polyethylene glycol (Carbowax 3000, Union Carbide Ltd) in three steps, dialysing against two changes of 0·01 M phosphate buffer, pH 7·0 for 36 hr. after each concentration step. The concentrated material was finally dried from the frozen state and antigen for immunodiffusion tests reconstituted at 25 mg./ml. in 0·9 % saline.

Immunodiffusion tests

The Ouchterlony (1953) immunodiffusion technique was modified as follows. Ten ml. of 1 % Oxoid Agar No. 1 in 0·02 M phosphate buffer, pH 7·0, containing 0·1 % sodium azide, was poured into a plastic Petri dish (90 mm. diam.). Holes, 9·5 mm. diam., were cut in the gel 4·8 mm. apart in the patterns shown in Plate 1. The appropriate holes were filled with antigen and serum and then left for 40 hr. in a saturated atmosphere at room temperature. They were then washed in 0·9 % saline overnight and photographed using dark-ground illumination.

Serological analysis

Two different batches of antigen, A 19 and A 211, used at 25 and 12·5 mg./ml., were tested against two sera, H 1 and B 1, by immunodiffusion. The precipitation pattern between each antigen-antiserum pair and reactions of identity between adjacent patterns were noted; these were interpreted by established criteria (Ouchterlony, 1958).

Serum survey

Sera were tested by immunodiffusion using the arrangement shown in Plate 2. This allowed comparison of their precipitation patterns with the four standard patterns determined by the serological analysis. Precipitins were identified by noting reactions of identity with these four patterns.

RESULTS

Serological analysis

Plate 1 shows the immunodiffusion reactions between two sera and two batches of antigen. An analysis of the precipitation patterns is shown diagrammatically in Fig. 1. Line pattern components (l.p.c.) are numbered from the serum well outwards between A 19 and B 1, and the l.p.c. sequences in the reactions are shown in Table 2. Where two or more l.p.c. appear as one line in the photograph, this is indicated by brackets in Table 2. These results were reproducible provided strict adherence was made to the experimental procedure outlined.

Hereafter 'antigen 1', etc., will refer to the antigen responsible for the l.p.c. which bears that number in Fig. 1.

Small variations were seen in the antigen content of different batches of culture. In most batches antigens 18–29 were not detectable; all had slightly different sequences of l.p.c. in immunodiffusion tests. Such a difference is shown between A 19 and A 211 in Table 2.

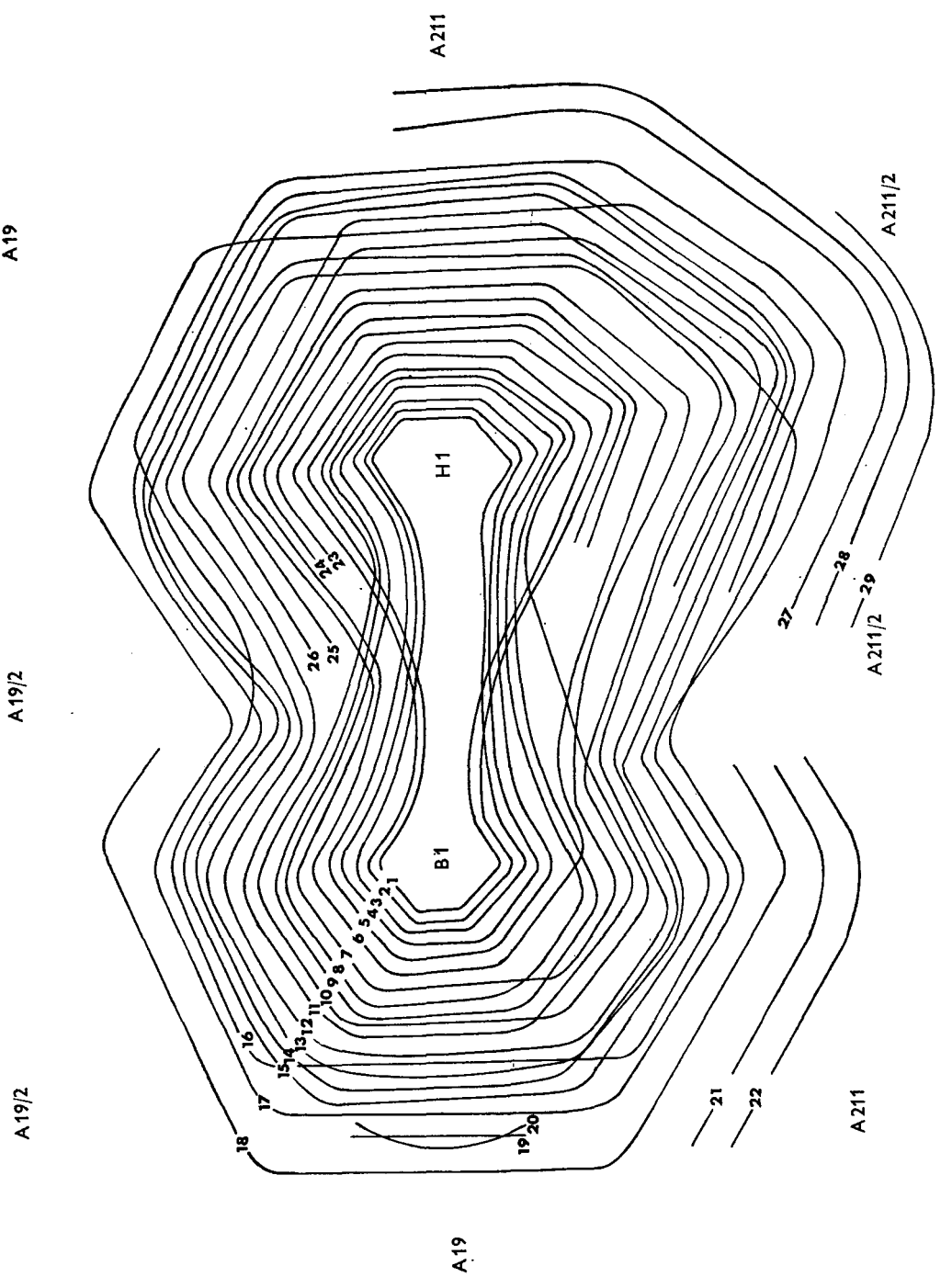

Fig. 1. Analysis and numbering system of line pattern components (l.p.c.) of the precipitin reactions in Plate 1.

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Serum survey

The incidence of precipitins to *M. faeni* antigens in patients' sera is given in Table 3. No serum, other than the two used for the serological analysis, which were not included in this survey, contained precipitins to antigens 18, 21, 22, 26, 27, 28 or 29. Precipitating antibody to *M. faeni* was detected in 75 % of sera from

Table 2. *Sequence of line pattern components in the precipitation reactions shown in Plate* 1

Reaction			
A19/B1	A19/H1	A211/B1	A211/H1
(1, 2)	(3, 4, 5)	(1, 2)	(3, 4, 5)
(3, 4, 5)	(8, 9, 10)	(3, 4, 5)	(8, 9, 10)
(6, 7)	(1, 2)	(6, 8, 9, 7, 11)	(1, 2)
(8, 9)	23	10	23
(10, 11, 12, 16)	24	13	24
	(6, 7, 25,		6
(13, 14, 15, 17)	26)	12	
	(11, 12)	14	7
(19, 20)			(11, 12)
	(13, 14, 15)	15	
18			25
	16	16	
			(17, 26, 13,
	17	18	14, 15)
		21	16
		22	27
			28

farmer's lung cases and 20 % of sera from other cases. The l.p.c. most frequently given by farmer's lung sera were: 8, 9, 10, 5, 6, 7, 13, 14 and 4, and by sera from cases of other respiratory diseases: 14, 15, 13, 8, 9, 10, 6 and 7.

Most positive sera (75 %) gave either l.p.c. 8 or 9 or 10; 57 % gave all three, together with varying numbers of other l.p.c. Of the remaining positive sera 10 % gave l.p.c. 16 and 17 only, 12·5 % gave l.p.c. 13, 14 and 15 only, 5 % gave l.p.c. 13, 14, 15, 16 and 17 only, and 2·5 % gave l.p.c. 11 and 12 only. There was no significant difference between the two groups of sera in this respect.

In Table 4 the results are shown graded by severity of disease. A considerable difference exists between the numbers of precipitins in sera from mild cases and from moderate and severe cases of both FLD and other respiratory diseases. The difference is statistically significant; $P < 0{\cdot}025$. There is no significant difference, however, between the distribution of precipitins in groups A and B in sera from patients of the same grade of severity.

Sera from patients with chronic or acute modes of onset of symptoms of FLD and other respiratory diseases show very similar distributions of precipitins. This is shown in Table 5. It appears from the results in the table that the presence of

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precipitins in sera from non-FLD patients is most frequently associated with a chronic onset of symptoms.

Table 3. *Number and percentage of sera in groups A and B reacting with each antigen*

	Group A (total no. 40)		Group B (total no. 54)		Total (total no. 94)	
Antigen	No.	%	No.	%	No.	%
1	10	25	1	2	11	12
2	11	27	1	2	12	13
3	12	30	2	4	14	15
4	19	47	2	4	21	22
5	20	50	2	4	22	23
6	20	50	4	8	24	25
7	20	50	4	8	24	25
8	23	57	6	11	29	31
9	23	57	6	11	29	31
10	23	57	6	11	29	31
11	6	15	1	2	7	7
12	6	15	1	2	7	7
13	20	50	6	11	26	28
14	20	50	7	13	27	29
15	15	37	7	13	22	23
16	14	35	3	6	17	18
17	14	35	3	6	17	18
19	1	3	—	—	1	1
20	1	3	—	—	1	1
23	14	35	2	4	16	17
24	11	27	2	4	13	14
25	4	10	1	2	5	5
Total no. positive	30	75	11	20	41	43

DISCUSSION

We have investigated the incidence of precipitating antibody to individual *M. faeni* antigens in sera from clinical farmer's lung cases and from cases of other respiratory diseases. Our purpose was to discover if there was any difference between the precipitin content of sera in the two groups.

The results show that the numbers of precipitins present in patients' sera are significantly higher in patients with moderate or severe forms of FLD than in those with milder symptoms, but that the distribution of precipitins to individual *M. faeni* antigens is similar in all grades of the disease. This was also true of the patients with other respiratory diseases. Thus there appears to be a general increase in the incidence of precipitins to all antigens of *M. faeni* with increasing severity of disease. Individual differences in this respect may well be due to variation in the antigenic composition of the strains to which patients are exposed. It is interesting to note that preliminary experiments in this laboratory suggest that antigens 1–5 correspond to the antigenic fraction 'C' of Pepys & Jenkins

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(1965), antibody to which was shown to be most frequent in the sera of patients who had suffered several attacks of FLD.

Table 4. *Precipitin reactions in sera from group A and B patients with varying grades of severity of disease*

	Group A			Group B		
Grade of disease ...	Mild	Moderate	Severe	Mild	Moderate	Severe
Total no. of sera ...	8	22	10	14	27	13
No. positive ...	6	16	8	6	4	1
No. reacting with antigen						
1	1	8	1	—	—	1
2	1	8	2	—	—	1
3	1	9	2	—	1	1
4	1	12	6	—	1	1
5	1	12	7	—	1	1
6	1	13	6	—	3	1
7	1	13	6	—	3	1
8	3	15	5	2	3	1
9	3	15	5	2	3	1
10	2	15	6	2	3	1
11	1	2	3	—	—	1
12	1	2	3	—	—	1
13	2	11	7	2	3	1
14	4	9	7	3	3	1
15	1	8	6	3	3	1
16	3	7	4	2	—	1
17	3	7	4	2	—	1
19	1	—	—	—	—	—
20	1	—	—	—	—	—
23	1	10	3	—	1	1
24	—	10	1	—	1	1
25	—	4	—	—	1	—
Average no. of l.p.c./serum	6	12	10	3	8	19

A small number of farmers' lung cases may be attributed to hypersensitivity to other organisms, notably *Micromonospora vulgaris* (Wenzel, Emanuel & Lawton, 1967). However, in many cases failure to detect antibody to *M. faeni* antigens almost certainly reflects a lack of sensitivity in immunoelectrophoresis and, to a lesser extent, in immunodiffusion tests. Jameson (1968), using a more sensitive technique, immunoosmophoresis, detected antibody in many sera from farmer's lung cases and from healthy farmers which were negative by the conventional immunodiffusion test. The numbers of precipitin lines given by these sera were fewer than those given by sera which were positive by the conventional test.

In the chronic stage FLD presents a much greater problem of diagnosis than in the acute stage. Radiological evidence of fibrosis may remain or even increase for years after an attack, without further exposure to mouldy hay, the resulting

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decrease in antibody giving rise to negative serological tests. In addition, the symptoms of FLD in this stage are similar to those of several other diseases, notably chronic 'fibroid' pulmonary tuberculosis, chronic diffuse idiopathic pulmonary fibrosis and other pneumoconioses; careful history-taking is therefore needed to establish a correct diagnosis.

Table 5. *Number of sera from acute and chronic cases in groups A and B reacting with each antigen*

No. of sera reacting with antigen	Acute cases		Chronic cases	
	Group A	Group B	Group A	Group B
1	6	—	4	1
2	7	—	4	1
3	7	—	5	2
4	11	—	8	2
5	12	—	8	2
6	12	—	8	4
7	12	—	8	4
8	14	—	9	6
9	14	—	9	6
10	14	—	9	6
11	5	—	1	1
12	5	—	1	1
13	13	1	7	5
14	13	2	7	5
15	10	—	5	5
16	10	—	4	3
17	10	—	4	3
19	—	—	1	—
20	—	—	1	—
23	7	—	7	2
24	7	—	4	2
25	3	—	1	1
Total sera	19	2	11	9

The occurrence of precipitins in healthy farmers' serum may be due to an attack of FLD in the past which has not been repeated, or to exposure to *M. faeni* to an extent that has not produced obvious symptoms. The predominance of mild and moderate symptoms shown by precipitin-positive patients with other respiratory diseases in this report suggests that in this group the occurrence of antibody may have some clinical significance. It is possible that the patients in question suffer from a mild form of FLD which has escaped diagnosis because of the presence of symptoms of other respiratory diseases.

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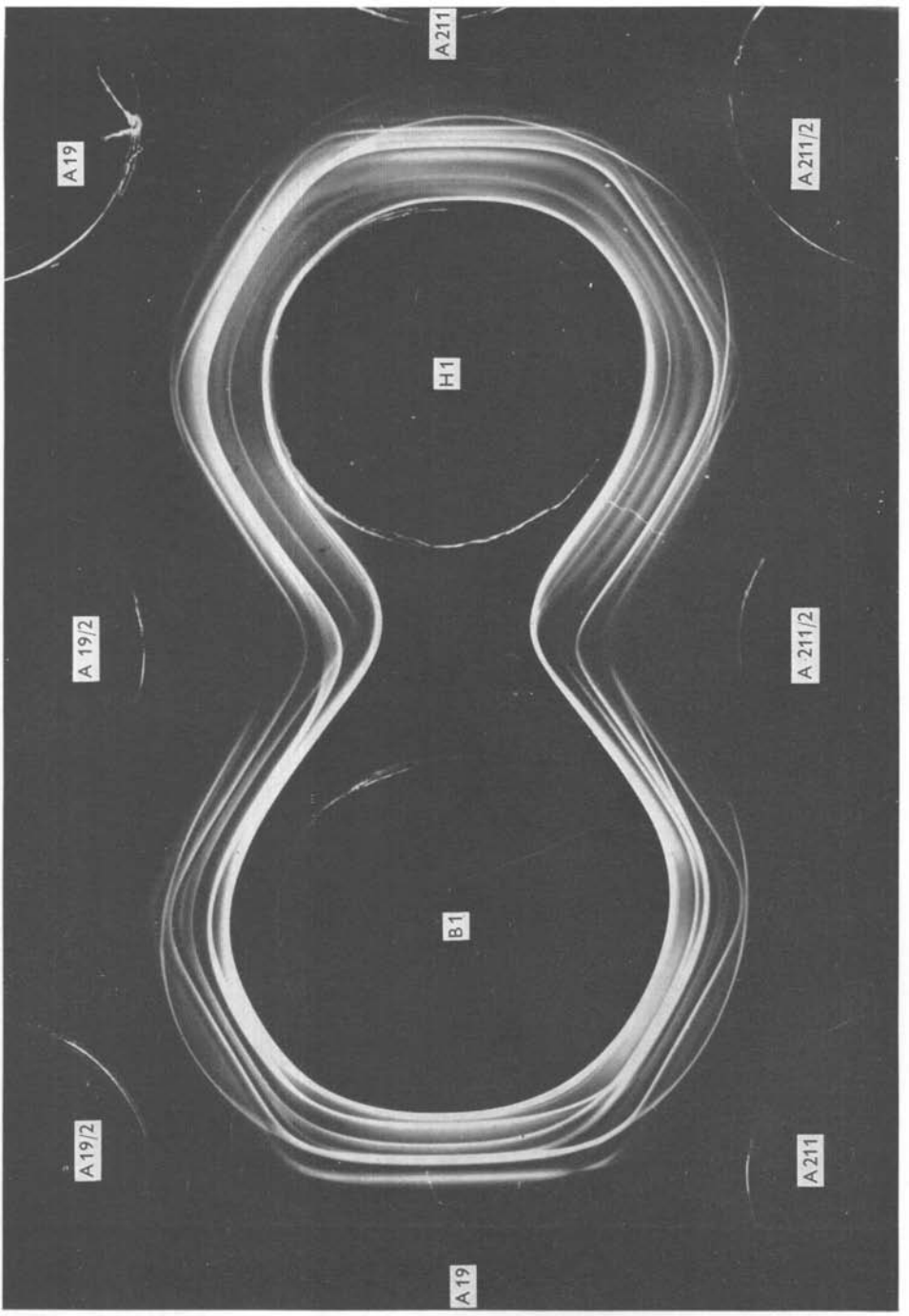

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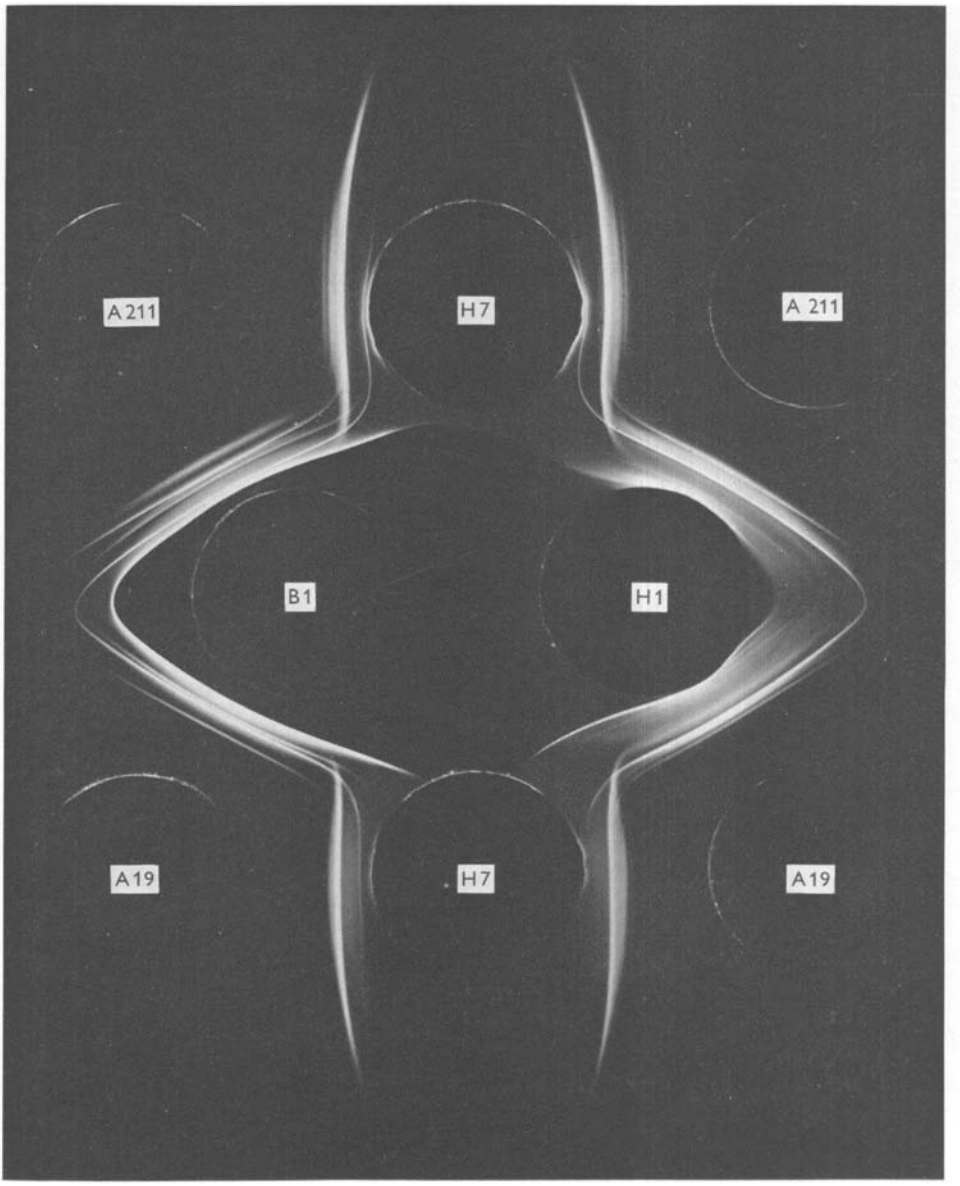

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EXPLANATION OF PLATES

Plate 1

Precipitin patterns given by two batches of antigen at 25 mg./ml. (A 19, A 211) and 12·5 mg./ml. (A 19/2, A 211/2) with a human (H 1) and a bovine (B 1) serum.

Plate 2

The arrangement of sera and antigens used in the analysis of precipitin content of human sera. The precipitation pattern between human serum (in this example H 7) and antigens A 19 and A 211 are compared with those given by four 'standard' reactions (B 1/A 19, B 1/A 211, H 1/A 19 and H 1/A 211).