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Antibody responses in patients with farmer's lung disease to antigens from *Micropolyspora faeni*

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SUMMARY

Three purified cell-wall glycoprotein antigens, *a*, *b* and *c*, sensitive to sodium periodate and resistant to pronase, were extracted from mycelium of *Micropolyspora faeni* with aqueous phenol or trichloracetic acid. Pronase-sensitive, sodium periodate-resistant protein antigens were extracted from mycelium with aqueous phenol. Immunoelectrophoresis was a critical method of assessing purity of preparation.

Antibodies to glycoprotein antigens only occurred in clinically defined cases of farmer's lung disease, whereas antibodies to protein antigens also occurred in symptomless farmers. Precipitins to *a* occurred in all cases of farmer's lung disease, and this antigen was isolated and purified.

Fractionation of pooled sera from cases of farmer's lung disease showed that precipitins were IgG globulins, whereas latex-agglutinating antibodies were IgG, IgA and especially IgM globulins. It is suggested that farmer's lung may involve a cytotoxic type II reaction, in which glycoproteins adsorbed to tissue cells react with IgG, IgA and IgM immunoglobulins in the presence of complement, causing cellular damage.

INTRODUCTION

Sera from most patients with farmer's lung disease (FLD) contain precipitating antibodies to the thermophilic actinomycete *Micropolyspora faeni* and less frequently to *Thermoactinomyces vulgaris* (Pepys *et al.* 1963). However, about 20% of farmers exposed to mouldy hay, but without overt clinical FLD, also have precipitating antibodies to *M. faeni* (Pepys & Jenkins, 1965). Fletcher, Rondle & Murray (1970) showed that precipitating antibodies to two antigens of *M. faeni* appeared more frequently than others. These antigens were later shown to be cell wall components, extractable from *M. faeni* mycelium by trichloracetic acid (TCA), and containing arabinose, galactose and glucosamine (Fletcher & Rondle, 1973). Kobayashi, Stahmann, Rankin & Dickie (1963) found that a similar TCA extract of mouldy hay gave precipitin reactions with sera of all patients with symptoms and 17 of 29 symptomless farmers, some of whom had completely recovered, and no reaction with 38 controls. Moreover, Barbee, Dickin & Rankin (1965) found that aerosol inhalation of a TCA extract of mouldy hay produced symptoms in six FLD patients identical with those of acute FLD. Pepys & Jenkins (1965),

however, showed that both the TCA extract and a TCA precipitate of *M. faeni* culture filtrate provoked systemic and pulmonary reactions in affected subjects on inhalation.

The present study was undertaken to identify and purify glycoprotein and protein antigens of *M. faeni*, and to measure the rate of incidence of serum antibodies to such purified antigens in farmers with clinical FLD and suitable controls. Both gel diffusion and latex agglutination methods were used and an attempt was made to identify the class of reactive antibody.

MATERIALS AND METHODS

Cultures

The three strains of *M. faeni* examined were 1156 (Fletcher *et al.* 1970), and 5280 and 9355, isolated from the sputa of patients with FLD by Dr B. Moore, Public Health Laboratory, Exeter.

The liquid growth medium was that described by Fletcher *et al.* (1970). One volume of a 5-day culture was added to each 100 vol. of medium, and grown for 48 hr. at 50° C. with vigorous stirring. Determination of the microbial nitrogen present by the micro-kjeldahl method showed that the logarithmic phase of growth ended at 48 hr. (Fletcher, 1971). Cells were harvested at 20,000 *g* in an MSE continuous-action rotor, washed three times in 0·02 M phosphate-buffered saline, pH 7·2 (PBS), and stored at −30° C. until used.

Preparation of M. faeni *antigens by physical methods*

Culture supernatant (CS) antigens

Antigens from the supernatant after centrifugation of culture medium used for growth of *M. faeni* were prepared as described by Fletcher *et al.* (1970).

Mycelial (MU) antigen

Washed mycelium was disrupted by ultrasonic treatment as described by Fletcher & Rondle (1973), except that sonication was for 20 min., when examination by microscopy showed that no intact mycelium was present.

Cell walls

Cell walls were prepared from the deposit of the preparation of MU antigen according to Fletcher & Rondle (1973).

Preparation of M. faeni *antigens by chemical extraction*

Phenol

Mycelium or cell walls, suspended in water, were shaken vigorously with an equal volume of aqueous phenol (90 %, w/w) for 2 hr. at 4° C. (Westphal, Luderitz & Bister, 1952; Gierer & Schramm, 1956). After separation the aqueous and phenolic layers were dialysed against water to remove phenol. The opalescent aqueous phase was lyophilized (extract PA). The insoluble material which separated

from the phenolic phase was also lyophilized and extracted for 30 min. at 37° C. with PBS, centrifuged at 10,000 *g* for 1 min. and the supernatant used in serological tests (extract PP).

Trichloracetic acid

Washed mycelium (20 mg./ml.) in PBS was stirred in 5% (w/w) TCA at 4° C. for 24 hr. The insoluble residue, deposited at 10,000 *g*, was re-extracted in the same way. After dialysis against PBS, ethanol was added to the extract to a final concentration of 90% (v/v), held for 24 hr. at 4° C. and centrifuged at 10,000 *g* for 20 min. The deposit was resuspended and dialysed against distilled water and lyophilized (extract TE). The supernatant from the ethanol precipitation was further precipitated with 3 vol. of acetone containing a trace of sodium acetate for 24 hr. at 4° C., centrifuged at 10,000 *g* for 20 min., the deposit dialysed against distilled water and lyophilized (extract TEA).

Alternatively, some crude TCA extracts of *M. faeni* were precipitated directly with 3 vol. of acetone and a trace of sodium acetate, the deposits from centrifugation dialysed against distilled water and lyophilized (extract TA).

Antisera

For antigenic analysis, strongly reacting sera from 25 patients with clinically defined FLD were pooled and lyophilized in small volumes. This serum, H2, was identical in gel diffusion tests with the serum H1 used by Fletcher *et al.* (1970).

For a survey of the incidence of antibodies to selected antigens, sera received in this laboratory for routine serological testing were used. Each serum was initially tested for precipitating antibodies to *M. faeni* MU antigens.

Group A (76 patients) had clinically defined FLD and precipitins to *M. faeni*. Group B (66 patients) had pulmonary diseases other than FLD and precipitins to *M. faeni*. Group C (75 subjects) were healthy urban dwellers with no history of exposure to mouldy farm produce. Each group was tested in gel diffusion with *M. faeni* PA, TA and PP antigens, and in latex agglutination tests with *M. faeni* MU and TA antigens.

Serological tests

Gel diffusion

The method was that of Fletcher *et al.* (1970), except that 1·2% Difco Noble agar was used. MU and PP antigens were tested at 25 mg./ml. and other extracts at 2 mg./ml. In the survey of human sera, these and 1/10 dilutions were used.

Immunoelectrophoresis

The method of Pepys & Jenkins (1965) was used. MU and PP antigens were tested at 40 mg./ml. and other extracts at 5 mg./ml.

Latex agglutination

Latex (Difco polystyrene, 0·81 μm. particle size) was sensitized as follows: 1 volume of antigen (10 mg./ml. in PBS) was added to 4 vol. of latex solution and

mixed gently for 2 min. This mixture was diluted with 95 vol. of PBS and shaken for 2 hr. at room temperature. Such sensitized latex suspensions remained active for at least 3 months stored at 4° C. Control sensitized latex was prepared similarly, but excluding antigen.

Doubling dilutions of sera in PBS containing 0·2% (w/v) bovine serum albumin were prepared in 0·4 ml. volumes in perspex WHO pattern plates containing eight rows of ten cups. The dilutions ranged from 1/8 to 1/2048. Two 0·02 ml. drops of sensitized latex suspension were added to each dilution. The plates were held for 2 hr. at 37° C. and 48 hr. at room temperature. The degree of agglutination was recorded on a scale from 4+ to negative, and the end-point taken as 2+ or greater to help exclude non-specific agglutination. In all tests the last cup of each row of dilutions contained buffer and sensitized latex particles only, and each serum was titrated against control sensitized latex. Known positive (H2) and negative human sera were always included.

For absorption of latex agglutinating (LA) antibody, 0·15 ml. of H2 serum was held for 72 hr. at 4° C. with 1·5 mg. of antigen, and the precipitate was removed by centrifugation. Control serum was held in the same way with PBS.

Treatments of antigens

Pronase

Five volumes of MU or PP antigens at 35 mg./ml. or other extracts at 3 mg./ml. were held with 1 volume of pronase (Sigma Chemical Company, St Louis, Missouri, U.S.A.) at 10 mg./ml. for 6 hr. at 37° C. A second volume of pronase was added and the mixture further held for 18 hr. at 37° C. Control antigens were held with PBS under the same conditions.

Sodium periodate

Five volumes of MU or PP antigens at 30 mg./ml. or other extracts at 3 mg./ml. were held with 1 volume of 0·02 M sodium periodate for 24 hr. at room temperature (21° C.) in the dark. Control suspensions were similarly held with PBS.

Preparation of antibody fractions

Mercaptoethanol

H2 serum was held with an equal volume of 0·2 M 2-mercaptoethanol as described by Strannegård & Yurchision (1969).

Absorption with specific antisera

One volume of H2 serum was held with 1 volume each of all paired combinations of goat anti-human IgG, IgA, or IgM globulins (Nordic Pharmaceuticals and Diagnostics, Tillburg, Holland) for 48 hr. at 4° C. and the precipitates removed by centrifugation.

Fractionation of serum

Crude gamma-globulin was separated from 2 ml. of H2 serum with 50% saturated ammonium sulphate, and the precipitate resuspended in 2 ml. of 0·1 M

Table 1. *Properties of* Micropolyspora faeni *antigens*

	MU antigen			
	a	*b*	*c*	13 antigens
CS antigen	*a*	−	−	16 antigens
Periodate sensitivity	+	+	+	−
Pronase sensitivity	−	−	−	+
Phenol extract				
PA	+	+	+	−
PP	−	−	−	+
TCA-acetone extract	+	+	+	−
TCA-acetone ethanol extract	+	−	−	−
I.E. region	C	C	C	A, B

+, Presence or sensitivity; −, absence or resistance, of antigens.
PA, PP, Aqueous or phenol phases respectively after phenol extraction.

Tris-HCl buffer, pH 7·5. Samples of 1 ml. were applied to Bio-Gel A-5m (Bio-Rad Laboratories, Richmond, Calif., U.S.A.), equilibrated in the same buffer packed into a column (45 × 2·5 cm.). Elution was performed using the same buffer, and 3 ml. fractions collected and the absorption at 280 nm. recorded in an Optica CF 4 Spectrophotometer. Each fraction was tested in gel diffusion with goat anti-human IgG, IgA and IgM globulins, and those containing each antibody were pooled and concentrated to 1 ml. by dialysis against polyethylene glycol.

RESULTS

Extraction of antigens

Gel diffusion

In gel diffusion tests with H2 serum, two groups of antigens in strain 1156 MU and CS preparations were distinguished by their sensitivity to sodium periodate and pronase respectively, and their presence in chemical extracts. The results are summarized in Table 1. MU contained three antigens designated *a*, *b* and *c*, each sensitive to sodium periodate, with *a* closest to the antiserum well, whereas only *a* was present in CS antigen (Pl. 1, fig. 1). Of a total of 17 pronase-sensitive antigens detected in both preparations, 12 were present in both MU and CS antigens, 4 only in CS antigen and 1 only in MU antigen. A similar distribution of sensitive antigens was observed in strains 5280 and 9535.

Separation of sodium periodate and pronase sensitive antigens was achieved with aqueous phenol extraction of mycelium or cell walls. Gel diffusion tests with H2 serum showed that antigens *a*, *b* and *c* passed into the aqueous phase (PA extract, Pl. 1, fig. 2), gave a positive result with Molisch reagent, remained sensitive to sodium periodate and resistant to pronase. The 13 pronase-sensitive antigens were recovered from the phenol phase, remained sensitive to pronase and resistant to sodium periodate. Similar PA and PP extracts prepared from *M. faeni* strains 5280 and 9535 gave reactions of complete identity with those of strain 1156.

Extraction of *M. faeni* 1156, 5280 and 9535 mycelium or cell walls with TCA

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Table 2. *Absorption of latex agglutinating (LA) antibody from H 2 serum (titre 1024) by extracts of* Micropolyspora faeni

(Sera titrated against latex coated with MU antigen at 10 mg./ml.)

H2 serum absorbed with	Factor by which titre decreased
Nil (control)	1
PBS	0
MU antigen	⩾ 128
CS antigen	32
MU + periodate	4
MU + pronase	64
PP extract	2
PA extract	32
TA extract	32
TE extract	32
TEA extract	16

followed by acetone precipitation (TA extract) yielded the three sodium periodate-sensitive antigens which remained sensitive to sodium periodate and resistant to pronase, and which all gave reactions of complete identity with the PA extract in gel diffusion tests with HS serum (Pl. 1, fig. 2). These antigens were also precipitated with 90% (v/v) ethanol (TE extract, Pl. 1, fig. 2), but further precipitation of the ethanol supernatant with acetone yielded only antigen *a* (TEA extract, Pl. 1, fig. 2).

Serial dilutions of the TA or TEA extracts showed that in gel diffusion tests with H2 serum, antigen *c* reacted at concentrations of 400 μg./ml. or above, *b* at 100 μg. per ml. or above, but *a* reacted even at 2 μg./ml.

Immunoelectrophoresis

The immunoelectrophoretic patterns of PA, TA, TE, TEA and PP extracts with H 2 serum are shown in Pl. 1, figs. 3 and 4. Both the PA and TA extracts gave three arcs of precipitation adjacent to the antiserum well (the 'C' region of Pepys & Jenkins, 1965). The TE extract gave in addition to three 'C' region arcs, several arcs towards the anode (A and B regions of Pepys & Jenkins, 1965), as did the supernatant of the TA (TA-SN) precipitation at high concentrations. Crude TCA extracts before ethanol or acetone precipitation also gave 'A', 'B' and 'C' region arcs. After treatment of the TE extract with pronase, these 'A' and 'B' region arcs disappeared. As in gel diffusion, the TEA extract gave only one line of precipitation, in the 'C' region. The pronase-sensitive antigens in the PP extract gave arcs in only the A and B regions.

Latex agglutination

The results of absorbing LA antibodies are shown in Table 2. LA antibody was fully absorbed with MU antigen and mostly with CS antigen. The absorbing ability of MU antigen was significantly decreased after treatment with sodium periodate, but little altered after pronase treatment. The significant role of sodium

Table 3. *Agglutination, by H 2 serum, of latex coated with extracts of* Micropolyspora faeni

Latex coated with	Titre H 2 serum
H_2O control	< 8
MU extract	1024
PP extract	< 8
PA extract	64
TA extract	512
TE extract	256
TEA extract	128

Table 4. *Agglutination of latex coated with* Micropolyspora faeni *MU antigen, and precipitin activity against* M. faeni *MU or TA antigens, by fractions of H 2 serum*

H 2 absorbed with	Titre of latex agglutinating antibody	Precipitins against	
		MU	TA
PBS control	1024	+	+
mercaptoethanol	< 8	±	±
IgA + IgM (= IgG)	256	+	+
IgG + IgM (= IgA)	512	−	−
IgG + IgA (= IgM)	1024	−	−
H 2 serum fractions			
(IgG)	128	+	+
(IgA)	64	−	−
(IgM)	1024	−	−

periodate antigens was confirmed by the strong absorption of LA antibody with PA, TA, TE and TEA extracts, and not PP extract.

The results of coating latex with PP, PA, TA, TE and TEA extracts are shown in Table 3. PP extracts were not active. However, extracts containing the sodium periodate-sensitive antigens effectively coated latex, with TA extract being the most active.

Activity of antibody fractions

H 2 serum treated with 2-mercaptoethanol was completely inactive in LA tests although its precipitating activity was reduced in gel diffusion with MU and TA antigens (Table 4).

After absorption of H 2 serum with goat antiserum to IgG, IgA and IgM globulins, only the IgG fraction possessed precipitin activity whereas the IgG, IgA and IgM all possessed significant LA activity (Table 4).

Fractionation of H 2 serum on Bio-Gel A-5m is shown in Fig. 1. Again, only IgG possessed precipitating activity, although all antibody fractions contained LA activity (Table 4).

Survey of human sera

The incidence of precipitins and LA antibodies in group A and B sera is given in Table 5. No antibodies were detected in any test in group C sera. The average LA titre of group C sera was 1/8, and none was above 1/16. A positive LA titre, therefore, was taken as 1/32 or higher.

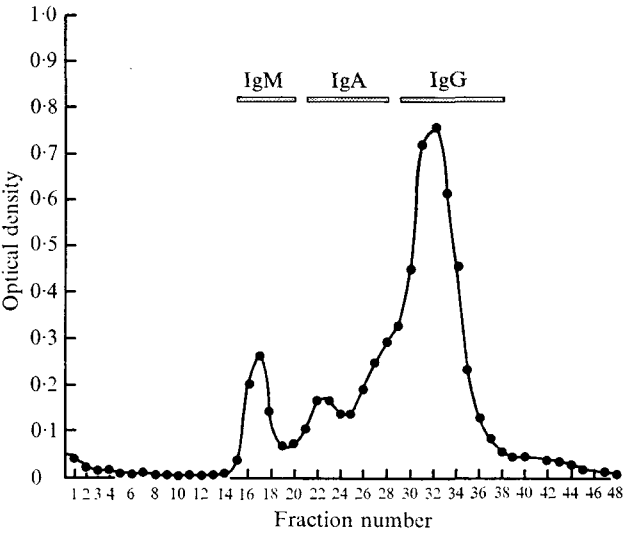

Fig. 1. Fractionation of H 2 serum on Bio-Gel A-5m.

Table 5. *Number of sera in Groups A and B reacting in precipitin and latex agglutination (LA) tests with* Micropolyspora faeni *antigens*

	Group A	Group B
Total number ...	76	66
Sera with precipitins to		
PP antigens	73	62
PA antigens	74	3
TA antigens	74	3
a antigen	74	0
b antigen	36	2
c antigen	6	1
Sera with LA antibodies to		
MU antigens	76	2
TA antigens	76	2

Almost all group A (73/76) and group B (62/66) sera with precipitins to *M. faeni* MU antigen contained precipitins to pronase sensitive (PP) antigens. However, precipitins to *M. faeni* sodium-periodate-sensitive (PA and TA) antigens, occurred in 74/76 sera from group A and in only 3/66 group B sera. Of these 74 positive group A sera, 74 (100%) were positive to *a*, 36 (49%) were positive to *b*, and 6 (8%) were positive to *c*; of the 3 positive group B sera, 2 were positive to *b* and 1 was positive to *c*.

Similarly LA antibodies to MU and TA antigens occurred in all group A sera and only the 2 group B sera with precipitins to *b*.

DISCUSSION

The results obtained by chemical extraction of *M. faeni* mycelium and treatment of sonicated mycelial antigen, (MU) antigen, with sodium periodate and

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pronase, show that this thermophilic actinomycete possesses two chemically distinct groups of antigens. The three individual antigens in the first group are sodium periodate-sensitive and pronase-resistant, pass into the aqueous phase of phenol extraction, and are soluble in TCA. Their predominantly carbohydrate nature and the insensitivity to pronase of the small amounts of bound protein identifies them as glycoproteins. Antigens in the second group are sodium periodate-resistant, pronase-sensitive proteins, and are soluble in phenol. The three glycoprotein antigens are derived from the cell wall. Only one (*a*) is released into the culture medium during the period of growth used, although extending the period of incubation may well release other glycoproteins into the growth medium through autolysis. Both groups of antigens appear to be identical in each of the three strains of *M. faeni* examined.

It appears that glycoproteins prepared by phenol or TCA extraction followed by precipitation with acetone do not migrate towards the anode during immunoelectrophoresis, but remain adjacent to the antigen well, i.e. in the 'C' region as described by Pepys & Jenkins (1965). Other workers have found that cruder TCA extracts of *M. faeni* mycelium or mouldy hay (Pepys & Jenkins, 1965; Fletcher & Rondle, 1973) or ethanol precipitates of such extracts (Kobayashi *et al.* 1963; LaBerge & Stahmann, 1966*a*, *b*) give reactions not only in the 'C' region, but also in the 'A' and 'B' regions as well. This study has shown that arcs reacting in the 'A' and 'B' regions in such TCA extracts are formed by small amounts of pronase-sensitive proteins. It appears therefore essential that in any critical study the purity of extracted glycoproteins should be confirmed by immunoelectrophoresis when arcs should be formed only in the 'C' region.

In gel diffusion of the three glycoprotein antigens detected, *a* appears to be the most reactive. A combination of ethanol and acetone precipitations separated some of *a* present in the crude TCA extract from the other antigens. Preliminary studies on the inhibition of glycoprotein antigen *a* in quantitative precipitation have shown that arabinose and glucosamine are involved in the precipitation with human immune serum.

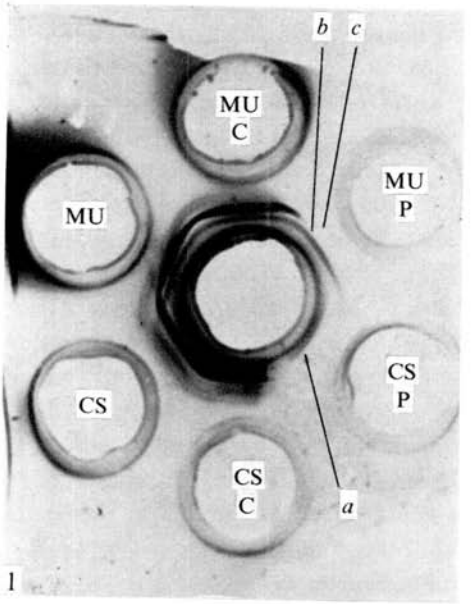
Gel diffusion tests have previously shown that sera from some subjects without clinical FLD but with exposure to mouldy hay contain precipitins to *M. faeni* antigens (Pepys & Jenkins, 1965; Fletcher *et al.* 1970). The results of this survey of human sera using purified antigens have show that precipitins to the three glycoproteins occurred only in sera from patients with FLD, whereas precipitins to protein antigens were present and presumably occurred as a result of exposure to mouldy hay, both in FLD cases and in patients with other respiratory illnesses. This extends the findings of Fletcher *et al.* (1970) that antibodies to glycoproteins occurred more frequently than antibodies to other types of antigen in sera from patients with FLD and rarely in sera from patients without FLD; however, the antigen used in this earlier study was sonicated mycelial supernatant (MU) and not purified glycoproteins. Of interest in this respect is the finding by Pepys & Jenkins (1965) that less than 50 % of their patients with FLD contained antibodies precipitating in the 'C' region; their antigen, however, appeared to contain only one arc in the 'C' region.

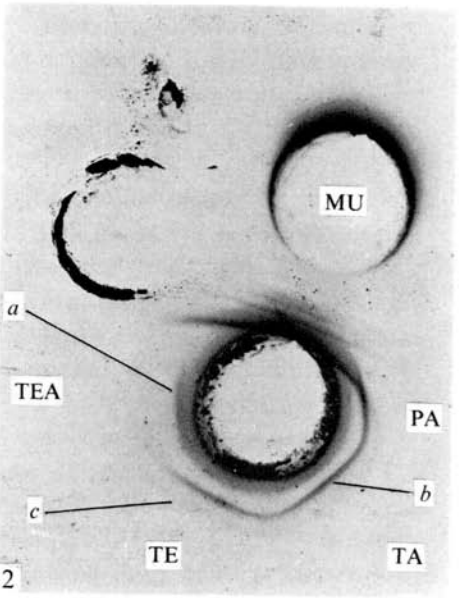
The occurrence of antibodies to glycoproteins only in cases of FLD was also confirmed by using latex coated with purified glycoproteins or whole MU antigen. In the latter case, absorption studies showed that only antibodies to glycoproteins appear to agglutinate the latex particles. Latex with *M. faeni* glycoproteins, therefore, appears to be a more specific test than gel diffusion for the detection of antibodies only involved in FLD.

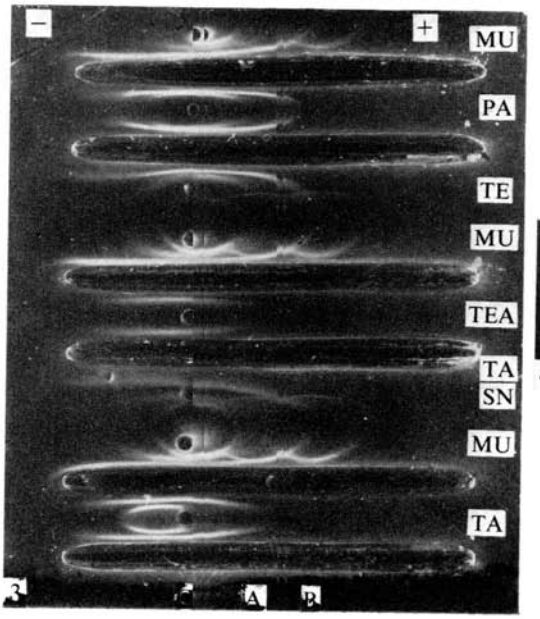
Isolation of the three major classes of immunoglobulins of human serum has shown that precipitins to *M. faeni* antigens are IgG globulins and confirms the suggestion made by Pike (1967) that IgG are effective precipitins. In contrast, LA antibodies were found mostly in the IgM globulins, although IgG and IgA were also active. Thus, the LA test estimates the three classes of antibodies, and the findings are consistent with Pike's suggestion that smaller amounts of IgM than IgG are required for agglutination. The higher LA titres of sera from patients in the acute phase of FLD may, therefore, represent a higher concentration of IgM than IgG or IgA globulins in these patients. It is suggested that lower LA titres in the subacute or chronic cases may represent a fall in the concentration of IgM globulins and their replacement by IgG.

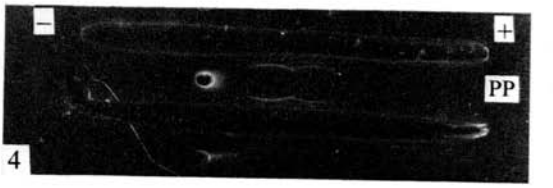
The immunological processes involved in the pathogenesis of FLD have yet to be fully elucidated. Pepys & Jenkins (1965) and Pepys (1969) have suggested the role of precipitins in an Arthus type III reaction. However, Wenzel, Emanuel & Gray (1971) studied the walls of bronchioles in patients with acute FLD stained with fluorescein-labelled IgG, IgA and IgM globulins, and showed that the three globulins were found in plasma cells and histiocytes. In addition, the C 3 component of complement was fixed in the histiocytes, suggesting that the pathogenesis of FLD may involve a cytotoxic type II reaction, at least in the acute phase. It was suggested that the absorption of antigen evidently renders tissue cells susceptible to cytotoxic antibody and complement. The present survey of human sera has shown that precipitating and agglutinating antibodies (i.e. IgG, IgA and IgM globulins) to glycoproteins occur only in those patients with FLD. It is possible, therefore, that *M. faeni* glycoproteins fix onto tissue cells and bring about a cytotoxic type II reaction in the presence of specific antibody. It is noteworthy that bacterial polysaccharides have a tendency to become fixed onto red cells (Coombs & Gell, 1968).

Until further work has revealed whether either or both type II and type III, or indeed the delayed type IV, reactions are involved in FLD, it would seem advisable that all three classes of immunoglobulins should be measured in the serological diagnosis of FLD. It is possible that some patients with FLD but without detectable precipitins (Pepys & Jenkins, 1965), and therefore without IgG immunoglobulins, may possess IgA or IgM globulins active in the type II reaction and only detectable by agglutination tests. Such studies are planned.









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REFERENCES

BARBEE, R. A., DICKIE, H. A. & RANKIN, J. (1965). Pathogenicity of specific glycopeptide antigen in Farmer's Lung. *Proceedings of the Society for Experimental Biology and Medicine* **118**, 546.

COOMBS, R. R. A. & GELL, P. G. H. (1968). *Clinical Aspects of Immunology* (ed. P. G. H. Gell and R. R. A. Coombs), p. 27. Oxford: Blackwell.

FLETCHER, S. M., RONDLE, C. J. M. & MURRAY, I. G. (1970). The extracellular antigens of *Micropolyspora faeni*: their significance in farmer's lung disease. *Journal of Hygiene* **68**, 41.

FLETCHER, S. M. (1971). Antigens of *Micropolyspora faeni* and their relevance in farmer's lung disease. Ph.D. Thesis, University of London.

FLETCHER, S. M. & RONDLE, C. J. M. (1973). *Micropolyspora faeni* and farmer's lung disease. *Journal of Hygiene* **71**, 185.

GIERER, A. & SCHRAM, G. (1956). Die Infektiosität der Nucleinsaure aus Tabakmosaikvirus. *Zeitschrift für Naturforschung* **11***b*, 138.

KOBAYASHI, M., STAHMANN, M. A., RANKIN, J. & DICKIE, H.A. (1963). Antigens in mouldy hay as the cause of Farmer's Lung. *Proceedings of the Society for Experimental Biology and Medicine* **113**, 427.

LABERGE, D. E. & STAHMANN, M. A. (1966*a*). Antigens from mouldy hay involved in Farmer's Lung. *Proceedings of the Society for Experimental Biology and Medicine* **121**, 458.

LABERGE, D. E. & STAHMANN, M. A. (1966*b*). Antigens from *Thermopolyspora polyspora* involved in Farmer's Lung. *Proceedings of the Society for Experimental Biology and Medicine* **121**, 463.

PEPYS, J. & JENKINS, P. A. (1965). Precipitin (F.L.H.) test in Farmer's Lung. *Thorax* **20**, 21.

PEPYS, J., JENKINS, P. A., FESTENSTEIN, G. N., GREGORY, P. H., LACEY, M. E. & SKINNER, F. A. (1963). Farmer's Lung. Thermophilic actinomycetes as a source of 'farmer's lung hay' antigens. *Lancet* ii, 607.

PEPYS, J. (1969). Hypersensitivity diseases of the lungs due to fungi and organic dusts. *Monographs in Allergy*, no. 4. Basel: Karger.

PIKE, R. M. (1967). Antibody heterogeneity and serological reactions. *Bacteriological Reviews* **31**, 157.

STRANNEGÅRD, O. & YURCHISION, A. (1969). Formation of agglutinating and reaginic antibodies in rabbits following oral administration of soluble and particulate antigens. *International Archives of Allergy* **35**, 579.

WENZEL, F. J., EMANUEL, D. A. & GRAY, B. S. (1971). Immunofluorescent studies in patients with Farmer's Lung. *Journal of Allergy and Clinical Immunology* **48**, 224.

WESTPHAL, O., LUDERITZ, O. & BISTER, F. (1952). Über die Extraction von Bakterien mit Phenol/Wasser. *Zeitschrift für Naturforschung* **7***b*, 148.

EXPLANATION OF PLATE

Fig. 1. Precipitin reactions given by MU and CS preparations and after treatment with pronase (MU.P and CS.P), and controls (MU.C and CS.C), revealing *a*, *b* and *c* antigens.

Fig. 2. Precipitin reactions given by MU preparation compared with PA, TA, TE and TEA extracts, showing, *a*, *b* and *c* antigens, and isolated *a* antigen.

Fig. 3. Immunoelectrophoresis of MU, PA, TA, TE, TEA and TA.SN extracts. Letters refer to C, A and B regions in text.

Fig. 4. Immunoelectrophoresis of PP extract, showing arcs in 'A' and 'B' regions.