

Candida albicans precipitins in respiratory disease in man

J. Pepys, M.B. (W'srand), M.R.C.P., F.R.C.P.E.,
Jennifer A. Faux, B.Sc., Joan L. Longbottom, M.Sc., Ph.D.,
D. S. McCarthy, M.D., M.R.C.P., and
F. E. Hargreave, M.R.C.P., Brompton, London, England

Precipitins against mannan and less frequently against protein antigens of C. albicans Group A are common in asthma and especially in asthmatic patients with pulmonary eosinophilia. The mannan A precipitins are associated with Arthus, Type III, skin test reactions to mannan A. Inhalation tests, particularly with the culture filtrate, containing both mannan A and protein antigens, provoked systemic reactions coming on after 4 to 8 hours and immediate and late asthmatic reactions. Precipitins to C. albicans are present in most of the patients with asthma and pulmonary eosinophilia in whom precipitins to A. fumigatus, the commonest cause in the United Kingdom, are absent. Precipitin-mediated reactions to C. albicans may be clinically relevant in these cases. The mannan antigens are potently antigenic, a single skin test resulting in the appearance of precipitins in all subjects, in an increase in the size of the Type III skin test reactions, and in the number of patients giving such reactions.

C*andida albicans* presents a challenging problem in man. On the one hand, it causes deep-seated or superficial infections in patients with other diseases or in patients under treatment with antibiotic or corticosteroid drugs. On the other hand, it is often regarded as a commensal or contaminant because of the frequency with which it is cultured from the sputum, excreta, or body surfaces. There is a very extensive literature ^{1, 2} showing, even in apparently healthy subjects, a high incidence of immunological responses and allergic sensitivity to extracts, usually crude, of *C. albicans*, though it is not clear whether these are of pathogenetic importance.

Precise understanding of these immunological responses depends upon the use of purified antigens such as the polysaccharide, cell-wall mannans described

From the Department of Clinical Immunology, Department of Medicine, Institute of Diseases of the Chest, Brompton, London, S.W.3.

These investigations were made with the support of the British Research Council.

Received for publication Dec. 4, 1967.

by Summers, Grollman, and Hasenclever (1964).³ This is all the more necessary in precipitin tests, for example, where widely differing results, attributable to both the test materials and the test methods, have been reported. Some workers have found precipitins only in heavily infected subjects,⁴⁻⁶ whereas others⁷ using very sensitive methods have found precipitins against the mannan antigens in all normal subjects, though more strongly in heavily infected subjects. The present report shows that tests with mannan antigens provide evidence to suggest mainly that the precipitating antibody is capable of mediating hypersensitive reactions and also that there is some association, requiring further confirmation, between the precipitins and respiratory disease.

MATERIALS AND METHODS

Antigens tested

1. Mannans from *C. albicans* Group A (B 311) and B (792) prepared from cell-wall extracts by precipitation as copper complexes.^{3, 22} The mannan A had a N₂ content of 1.1 per cent and the B of 1.8 per cent.

2. Mannans from *C. albicans* Group A (A 207/5/3) prepared from aerated cultures on glucose (2 per cent)–peptone (1 per cent) broth by the same methods as antigens 1. These were shown here to be antigenically identical to the mannan A (B 311) and to be similar chemically, containing predominantly mannose with traces of glucose and a N₂ content of 0.91 per cent.

3. A dialyzed and freeze-dried formamide extract⁸ of the cells of *C. albicans* Group A (A 207/5/3). This contained considerably more glucose than antigens 1 and 2 and had a N₂ content of 5.21 per cent.

4. A β -naphthol extract⁴ of cells of an unknown strain of *C. Albicans* prepared in 1960 (before Groups A and B had been discovered). This contained polysaccharides shown here to be antigenically identical to mannan B (792).

5. A Seitz-filtered, dialyzed, and freeze-dried culture filtrate prepared in 1960 by culturing an unknown strain of *C. albicans* for 3½ months in glucose (2 per cent)–peptone dialysate (1 per cent) broth. This contained mannose, glucose, and 3.5 per cent N₂, the polysaccharides being shown here to be antigenically identical to Group A. The culture filtrate, which contained protein as well as mannan antigens, is termed “filtrate, Sabouraud-dialyzed peptone,” or Fsd extract.

Serological tests

Precipitin tests were made by double-diffusion and immunoelectrophoresis in agar gel as for the study of *Aspergillus* antigens.⁹ The double-diffusion tests were made in a 3.5 mm. thick layer of 1.5 per cent Ionagar II (Oxoid) prepared in equal parts of isotonic solutions of citric acid phosphate buffer, pH 7, and saline, with the addition of 0.1 per cent sodium azide. The central serum well was 12.5 mm. in diameter, and the 6 peripheral wells were 4 mm. in diameter and lay 6 mm. from the center. Precipitation arcs appeared after 1 day up to 1 week, and final results were recorded from plates stained for protein. Negative sera were retested after threefold concentration. In quantitative precipitin tests with the mannan A in one patient giving a strong reaction, approximately

0.25 mg. per milliliter of precipitating antibody was found. In another subject, initially giving a negative precipitin test and no measurable amount of antibody, repetition of the precipitin test 1, 2, and 3 weeks after skin testing gave a positive precipitation reaction, and 0.18 to 0.21 mg. per milliliter of precipitating antibody was found. This suggests that approximately this amount of precipitating antibody is relevant to the skin and other reactions elicited by tests with the extracts at 10 mg. per milliliter. Where positive precipitin reactions were obtained only after threefold concentration of the serum, it would seem that the undiluted serum contained about one third of the amount of precipitin in the above 2 patients.

Sera were absorbed for inhibition tests by adding the test extract to the serum to give a concentration of 10 mg. per milliliter, followed by centrifugation after standing for 2 to 4 hours at room temperature.

Types of precipitation reaction

Fuzzy precipitation arcs of the H type associated with polysaccharide-type antigens were given by the heat-stable mannan antigens. Lower concentrations of mannan A gave well-defined arcs, up to 2 to 3 with certain sera (Fig. 1). The Fsd (culture filtrate) gave, with some sera, an inner H type arc giving reactions of identity with mannan A and 1 to 2 well-defined R type arcs, the latter being due to heat-labile antigens regarded as probably of protein nature (Fig. 3). As has been found before with other fungal polysaccharide-type antigens,¹⁰ periodic acid oxidation of the mannan A resulted in complete loss of precipitinogenic activity, attributable to disruption of the polysaccharide. In gel electrophoresis of the mannan A there was only polysaccharide staining, corresponding with the position of the H type precipitation arc, while elec-

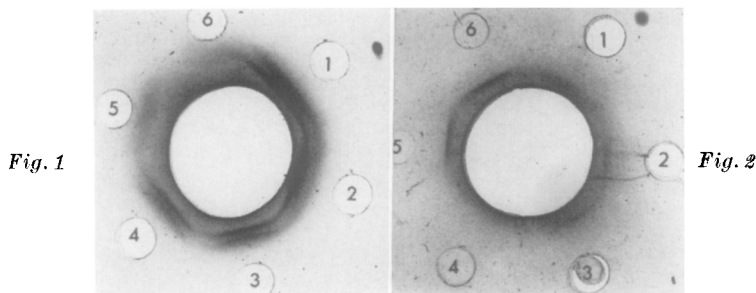


Fig. 1

Double-diffusion test against serum of Patient R. W. (No. 4) with mannan from *C. albicans* Group A. Test concentrations starting from black spot: (1) 1 mg. per milliliter; (2) 0.5 mg. per milliliter; (3) 0.2 mg. per milliliter; (4) 0.1 mg. per milliliter; (5) 0.05 mg. per milliliter; and (6) 0.01 mg. per milliliter. Note presence of 2 to 3 precipitation arcs.

Fig. 2

Double-diffusion test with culture filtrate (Fsd) and mannans of *C. albicans* Group A. Test concentrations from black spot: culture filtrate (Fsd)—(1) 30 mg. per milliliter; (2) 20 mg. per milliliter; and (3) 10 mg. per milliliter; and mannan—(4) 10 mg. per milliliter; (5) 1 mg. per milliliter; and (6) 0.5 mg. per milliliter. Note weak reactions to mannan A component of culture filtrate plus doubtful reaction to mannan A at 10 mg. per milliliter and strong reactions at 1 and 0.5 mg. per milliliter.

trophoresis of the Fsd showed an additional protein-staining area corresponding with the R type reactions.

Immunoelectrophoresis tests of 6 positive sera showed that the reactions to the mannan A occurred in the region of the immunoglobulins. IgG antibodies separated¹¹ from 2 sera gave precipitation reactions in one case to mannan A and B and in the other to the protein antigens of Fsd as well (Fig. 3).

In preliminary comparisons of mannan A (B 311) and Fsd on 18 sera, 8 reacted to mannan A at 0.5 and 1 mg. per milliliter, and, it should be noted, only 3 of these reacted at 10 mg. per milliliter. All 8 sera reacted to the Fsd at 30, 20, and 10 mg. per milliliter. Absorption of the sera by Fsd inhibited the mannan A reaction, whereas mannan A absorption inhibited only the polysaccharide reaction of the Fsd but not the reaction to the protein antigen. In

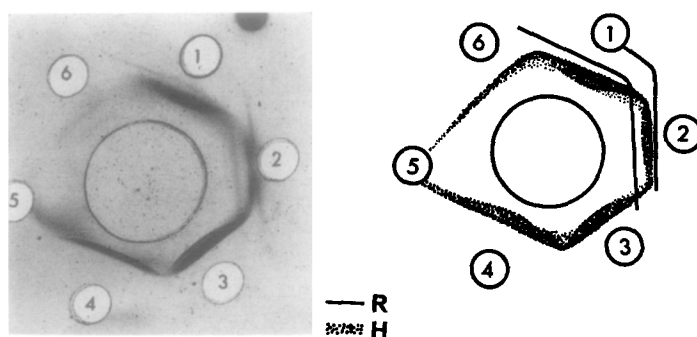


Fig. 3

Double-diffusion test with culture filtrates and mannans of *C. albicans* Groups A and B against concentrated IgG (60 mg. per milliliter) of Patient K. S. (No. 2). Tests from black spot: (1) culture filtrate (Fsd) of Group A (20 mg. per milliliter) giving well-defined R type precipitation arcs to protein antigens and fuzzy H type to mannans. (2) Protein fraction (ammonium sulfate 100 per cent precipitation) of Fsd of Group A at 20 mg. per milliliter, giving more intense multiple R type arcs to protein antigens and weak H reaction to trace of mannan. (3) Mannan A (1 mg. per milliliter). Note stronger reaction than to mannan A (10 mg. per milliliter). (4) Mannan A (10 mg. per milliliter). Note no R type arcs to (3) and (4), i.e., absence of protein antigens. (5) Culture filtrate of Group B (20 mg. per milliliter)—doubtful reactions. (6) Mannan B (1 mg. per milliliter). Weak H type reaction.

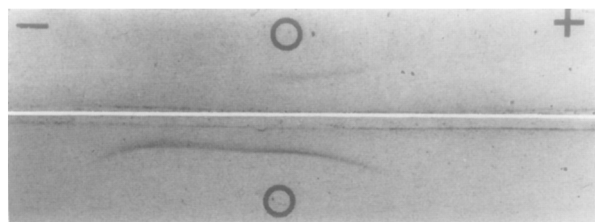


Fig. 4

Immunoelectrophoresis test of mannan A and mannan B at 1 mg. per milliliter against serum of Patient K. S. (No. 2) in the trough. Note precipitation arcs to fast and slow-moving components of mannan A (below). Mannan B (above) reacting only like the fast-moving component of the mannan A.

a further 28 sera with mannan A precipitins, precipitins against the protein antigens of Fsd were present in 8. The Fsd gave weaker polysaccharide reactions than the mannan A at comparable concentrations, and consequently more emphasis was placed upon the frequency of the latter. Only one of all the sera tested had precipitins to the protein antigen only.

The Group A strain of *C. albicans* is cultured more frequently in man than Group B.^{12, 13} Mannan B gave reactions in only 7 out of 25 sera with mannan A precipitins and was shown here to contain some of the antigens of mannan A (Fig. 4), as reported in experimental animals.³ Only 3 of all the sera tested gave reactions to mannan B only.

The formamide extract of Group A (A 207/5/3) tested at 1 and 10 mg. per milliliter gave reactions of identity with, and was inhibited by absorption with, mannan A. The β -naphthol extract of what was probably a Group B strain was similarly shown to contain antigens identical to mannan B (792).

Skin tests

Prick and intracutaneous tests were made with solutions of mannan A and B and with Fsd at 10 mg. per milliliter, lower concentrations being too weak. A carbol-saline extract of *C. albicans* (Bencard) gave far fewer and weaker reactions.

The types of reaction are as defined by Gell and Coombs.²¹ Immediate Type I weal and flare reactions were elicited by prick tests with all of the antigens. In certain patients a dual skin test reaction was elicited, particularly by intracutaneous tests. This dual reaction consisted of an immediate Type I reaction, followed after its resolution by a soft, edematous reaction, 20 to 80 mm. or more in diameter, which came on 3 to 4 hours after the test, was maximal at 7 to 8 hours, and was resolving or resolved within 24 hours (Fig. 5). This



Fig. 5

Type III, Arthus reaction to intracutaneous test (0.01 to 0.02 ml.) with mannan of *C. albicans* Group A (10 mg. per milliliter) in Patient C. P. (No. 3). Note large soft swelling with minimal central induration 5 hours after the test.

second reaction is regarded as a Type III, Arthus, precipitin-mediated reaction. Delayed, tuberculin type, Type IV reactions appearing after 24 to 48 hours were elicited in almost all subjects by the culture filtrate, Fsd, which contained protein as well as mannan antigen. In about one half of the subjects the Type III reactions to the mannan were followed after 24 to 48 hours by small, indurated, slightly edematous reactions which gave recurrent flare-ups for 1 week or more. These latter reactions were indistinguishable from Type IV reactions, and all showed powdery desquamation of the pigmented areas.

RESULTS

Precipitins to mannan A (Table I)

In patients with asthma, defined as reversible airways obstruction together with evidence of extrinsic sensitivity and/or blood and sputum eosinophilia, precipitin reactions were obtained more frequently than in nonasthmatic subjects. More patients (72.2 per cent) with asthma complicated by pulmonary eosinophilia had precipitins than those with only asthma (55 per cent). There were only slightly more patients with precipitins in subjects giving Type I prick test reactions to Fsd, and there was no difference in their frequency in patients regarded as having extrinsic or intrinsic asthma. Precipitins were, however, not uncommon in apparently healthy subjects (22.7 per cent) and in nonasthmatic patients with various lung diseases (18.7 per cent).

Asthma and pulmonary eosinophilia

The complication of asthma by transitory infiltrations of the collapse-consolidation type seen on x-ray of the lungs, together with eosinophilia of the blood or the sputum, is quite common in the United Kingdom and is referred to as one of the forms of asthma and pulmonary eosinophilia. The group of 36 patients with asthma and pulmonary eosinophilia (Table I) includes 11 who were specially tested because they had no precipitins to *Aspergillus fumigatus*. *A. fumigatus* precipitins have been found in over 90 per cent of patients with this disease in the United Kingdom.¹⁴ *C. albicans* mannan A precipitins were present in 9 of the 11 patients who had no *Aspergillus* precipitins. In 18 who had precipitins against both polysaccharide and protein antigens of *A. fumigatus*,

Table I. *Precipitins to mannan A (1 mg. per milliliter)*

Diagnosis	No. tested	Serum		
		Unconcentrated (No. pos./% pos.)	Concentrated (No. pos./% pos.)	Total (No. pos./% pos.)
Asthma	40	10/25	12/30	22/55
Asthma and pulmonary eosinophilia	36	12/33.3	14/38.8	26/72.2
Miscellaneous lung diseases	16	1/6.2	2/12.5	3/18.7
Healthy subjects	22	1/4.5	4/18.2	5/22.7

C. albicans mannan A precipitins were also present in 17, whereas in 7 who had precipitins only against the protein antigens of *A. fumigatus*, none had *C. albicans* mannan A precipitins.

Skin tests: Prick tests, immediate Type I reactions

Three preparations of *C. albicans*, all tested at 10 mg. per milliliter, showed the following positive reactions: to the culture filtrate (Fsd) in 17 out of 18 patients, the average diameter of the weals being 3.4 mm.; to the mannan A in 15 out of 18, the average diameter of the weals being 2.2 mm.; and to the mannan B in 12 out of 16 tested, the average diameter being 0.8 mm. The greater strength and frequency of the reactions to the Fsd are attributable to its content of both protein and mannan antigens.

The following reactions to the Fsd extract were obtained. In the 40 asthmatic patients (Table I), there were slightly more positive Type I reactions in atopic subjects with extrinsic sensitivity (16 out of 21) than in patients with late onset, "intrinsic" asthma without evidence of extrinsic sensitivity (13 out of 19). A slightly lower proportion of the patients with asthma and pulmonary eosinophilia (11 out of 17) gave positive reactions. Fewer positive reactions were obtained in the patients with miscellaneous respiratory diseases (3 out of 6 tested), and there were no reactions in 9 healthy subjects.

Intracutaneous tests

Type III, Arthus, skin test reactions and precipitins. Intracutaneous injections of 0.01 to 0.02 ml. of both mannan A and Fsd were given to 30 subjects. Dual reactions were given by 13 to the mannan A and by 11 to the Fsd. Two further groups of 10 each were tested with either mannan A or Fsd, and 3 in each group gave dual reactions. Thus, out of the 40 tested with each antigen, dual reactions were given to mannan A in 16 and to Fsd in 14 subjects.

In the total group of 50 subjects, mannan A precipitins were present in 22; 17 of these gave dual reactions to either antigen or to both, and 13 gave Type I prick test reactions. Of these, 12 gave precipitin reactions with unconcentrated serum and 5 with serum after concentration. In the 28 without precipitins, 4 gave dual reactions and 3 gave prick test reactions. In the total of 21 subjects who gave dual reactions, there were 7 out of 15 with asthma, 7 out of 11 with asthma and pulmonary eosinophilia, 1 out of 7 with tuberculosis, 6 out of 10 with miscellaneous lung diseases, and none out of 7 healthy subjects.

Effects of intracutaneous tests on precipitins and repetition of the skin tests. In 6 patients (fibrosing alveolitis, 2; asthma, 1; asthma and pulmonary eosinophilia, 1; bronchiectasis, 1) it was found, on repetition of the skin tests with both mannan A and Fsd, that after an interval of 8 to 14 days the number giving dual reactions increased from 2 to 6, and the number with mannan precipitins increased from 1 to 5.

Skin tests were repeated after 1 week in 21 subjects who were first tested with both mannan A and Fsd (3), or only with mannan A (10), or Fsd (8). Table II shows that a first test with mannan A by itself, like the other tests,

Table II. *Effect of initial skin tests on subsequent dual reactions to the mannan A*

First test with	No. tested	Total giving dual reactions	
		First test	Retested after 1 week
Mannan A and Fsd	3	0	2
Mannan A	10	2	5
Fsd	8	1	2

Table III. *Effect of intracutaneous tests on the precipitins and repetition of the skin tests with both Fsd and mannan A*

Groups tested	No.	Total giving dual reactions		Total No. with precipitins			
		Initially	1 Week	Initially	4 Days	1 Week	2 to 3 Weeks
Healthy subjects	7	0	4	1	1	2	6/6
Tuberculosis	7	1	2	1	2	3	6/6
Miscellaneous lung disease	7	2	3	2	2	3	5/5

Table IV. *Inhalation tests with Fsd (culture filtrate) and mannan antigens*

Diagnosis	1. P. P. (F, 38 yrs.) Asthma and pulmonary eosinophilia and pleurisy	2 K. S. (M, 27 yrs.) Asthma and pulmonary eosinophilia and bronchiectasis
Sputum culture for <i>Candida albicans</i>	Negative	Positive
Skin test (Fsd) Prick (immediate) Intracutaneous (dual)	Positive Positive	Positive Positive
Precipitins against mannan A against Fsd (protein)	Positive (unconc. serum) Positive (unconc. serum)	Positive (conc. serum) Positive (unconc. serum)
Inhalation test (duration/volume) Fsd 10 mg./ml. Mannan A 10 mg./ml.	5 min./1.5 ml. 5 min./1.5 ml.	7 min./2.5 ml.
	First test	Second test
Pulmonary reaction	Immediate asthma followed by late asthmatic reaction after 8½ hrs.	Nil Immediate asthma On repetition of test 6 days later, only late asthmatic reac- tion after 6 hrs.
Systemic reaction (malaise, chills) hours after test	8	Nil 4
Peak fever (°F.) /hours after test	100.4/9½	Nil 99.7/6 101/24 103/32
W.B.C./24 hrs.	10,000-17,500	6,200-10,000 5,000-20,000
Blood eosinophilia (mm. ³ /24-48 hrs.)	400-580	250-400 430-1,100

N. A. = Not available.

resulted in an increase in the number of subjects giving dual reactions on retesting. Fewer and weaker reactions were given by the Fsd, attributable to its lesser content of mannan A.

The 21 subjects could be divided into 3 clinical groups (Table III). This showed that there was an increase in dual skin test reactions on retesting healthy subjects as well as subjects with lung diseases. The numbers with precipitins also showed an increase, 1 previously negative reactor giving positive mannan A reactions after 4 days, 3 further positive reactions appearing after 1 week, and all the sera tested having precipitins by 2 to 3 weeks.

Inhalation tests (Table IV)

These were performed as in farmer's lung¹⁵ and in bird fancier's lung and related diseases.¹⁶ All the asthmatic reactions were reversible by isoprenaline inhalation. No reactions were obtained to control tests with carbol-saline solution in any of the subjects, or in 4 subjects tested with the Fsd at 1 mg. per milliliter, or in 2 tested with mannan A at 1 mg. per milliliter. All 10 were tested with Fsd at 10 mg. per milliliter.

<i>3 C. P. (M, 54 yrs.) Pleural eosinophilia and old tuberculosis</i>		<i>4 E. W. (M, 14 yrs.) Asthma and pulmonary eosinophilia</i>
Positive		Plugs with yeast cells
Negative		Positive
Positive		Negative
Positive (unconc. serum)		Positive (conc. serum)
Positive (unconc. serum)		Positive (conc. serum)
10 min./2.5 ml. 5 min./1.5 ml.		10 min./2 ml.
<i>First test</i>	<i>Second test</i>	
Dyspnea and wheeze at 4 hrs.; cough and crepitant rales at 24 hrs.	Immediate asthma On repetition of test 1 day later, only late asthmatic reaction after 4 hrs.	Late asthmatic reaction after 5½ hrs.
6	Nil	8
103/9	Nil	99.8/8
6,000-13,300	No change	10,000-27,000
280-580	230-380	600-1,800

Table IV. *Cont'd*

<i>Diagnosis</i>	<i>5. V. A. (F, 45 yrs.) Late onset asthma</i>	<i>6. G. H. S. (M, 58 yrs.) Aspergilloma</i>
Sputum culture for <i>Candida albicans</i>	Negative	Negative
Skin test (Fsd)		
Prick (immediate)	Negative	Positive
Intracutaneous (dual)	Negative	Positive
Precipitins		
against mannan A	Negative	Positive (unconc. serum)
against Fsd (protein)	Negative	Positive (unconc. serum)
Inhalation test (duration/volume)		
Fsd 10 mg./ml.	10 min./1.8 ml.	10 min./3.5 ml.
Mannan A 10 mg./ml.		
Pulmonary reaction	Immediate asthma, followed by late asthmatic reaction after 15¼ hrs.	Nil
Systemic reaction (malaise, chills) hours after test	7½	Nil
Peak fever (°F.) /hours after test	99/24	100.8/10
W.B.C./24 hrs.	4,500-9,000	17,500-20,000
Blood eosinophilia (mm. ³ /24-48 hrs.)	470-770	1,040-1,340

N. A. = Not available.

Systemic reactions, fever and malaise, appeared after 4 to 8 hours in 6 patients, accompanied by an increase at 24 hours in the white blood count and eosinophil counts. In 5 of these 6 patients and in 1 who had no systemic reaction (No. 7, P. S.), bronchial reactions were also produced. In 3 of these patients immediate asthmatic reactions were produced followed in 2 cases by a further asthmatic reaction after about 8 and 16 hours, respectively. In the third patient (2, K. S.), repetition of the test after 6 days was followed by a late asthmatic reaction coming on after 6 hours without a preceding immediate reaction. In 1 patient (7, P. S.) only a late asthmatic reaction appeared after 15 hours. In 3 patients tests were made with the mannan A antigen. No reactions were provoked in Patient P. P. (No. 1), who reacted to the Fsd test, nor in J. B. (No. 9), who gave no reactions to either test. In the third patient, C. P. (No. 3), the mannan provoked an immediate asthmatic reaction, and, on repetition of the test 1 day later, a late reaction appeared without a preceding immediate reaction. None of the patients tested with the mannan A gave a systemic reaction, suggesting that the protein antigen in the Fsd may have been more important in this respect.

Of 3 patients giving negative precipitin reactions (5, V. A.; 8, M. E.; and

7. P. S. (F, 49 yrs.) <i>Late onset asthma</i>	8. M. E. (M, 43 yrs.) <i>Bronchiectasis</i>	9. J. B. (F, 23 yrs.) <i>Asthma and pulmonary eosinophilia</i>		10. A. S. (M, 57 yrs.) <i>Histocytosis-X</i>
Negative	Positive	Positive		N. A.
Positive Positive	Positive Positive	Positive Negative		Negative N. A.
Positive (unconc. serum) Negative	Negative Negative	Positive (conc. serum) Negative		Negative Negative
6 min./1.5 ml.	10 min./2.2 ml.	10 min./2.5 ml. 5 min./2 ml.		7 min./1.75 ml.
Late asthmatic reaction after 15 hrs.	Nil	<i>First test</i> Nil	<i>Second test</i> Nil	Nil
Nil	Nil	Nil	Nil	Nil
Nil	Nil	Nil	Nil	Nil
8,600-6,800	Nil	No change	7,800-17,600	No change
400-480	Nil	No change	No change	N. A.

10, A. S.), 1 (5, V. A.) gave bronchial and systemic reactions to the inhalation tests.

These tests show that inhalation of the antigens of *C. albicans* may be followed by both systemic and pulmonary reactions. The immediate and late asthmatic reactions and what seems to have been an alveolar reaction in Subject C. P. (No. 3) appear to be due to the tests, though no conclusions can be drawn as yet regarding their relevance to clinical disease.

DISCUSSION

These investigations have shown the presence of precipitins against the antigens of *C. albicans* in agar gel double-diffusion tests of unconcentrated serum and serum concentrated threefold, with the pattern and size of test wells employed here. Precipitins were found against both polysaccharide and protein antigens of *C. albicans* Group A. They were present, more frequently against the polysaccharide, cell-wall mannans, in many patients with uncomplicated asthma and with asthma and pulmonary eosinophilia, as well as in smaller numbers of patients with other respiratory diseases and healthy subjects. The amounts of precipitating antibody shown by these tests appear to

be related to skin and inhalation test reactions regarded as Arthus, Type III reactions. These reactions were of the same type as those described in patients with pulmonary aspergillosis,⁹ bird fancier's lung,¹⁶ and related diseases,¹⁴ in which precipitins are present against the appropriate antigens and in which by immunofluorescence tests on the dual skin test reactions Pepys and colleagues¹⁹ have shown the presence of β 1C component of complement and of immunoglobulins compatible with an Arthus reaction.

The production of Arthus, Type III reactions to the mannan of *C. albicans* Group A in 17 out of 22 subjects with precipitins, as against 4 out of 28 without precipitins, shows the close association of the precipitins to these reactions. The appearance, at 24 to 48 hours, at the sites of the Arthus reactions of recurrent indurated reactions indistinguishable from Type IV reactions may be due to the peptide content of the mannan or perhaps to some as yet unrecognized reaction to polysaccharide antigens.

The systemic reactions, coming on after some hours, to the inhalation tests were like those attributed to precipitin-mediated reactions in farmer's lung, bird fancier's lung, and related diseases. The rapidly developing asthmatic reactions can be attributed to Type I allergy, whereas the more slowly developing asthma and the appearance of systemic manifestations after about 4 to 8 hours would correspond with the speed of appearance of precipitin-mediated Arthus, Type III, reactions. Similar responses have been reported by Pepys²⁰ in patients with bird fancier's lung, the presence of precipitins being associated with a late asthmatic reaction to inhalation tests. These findings show that the precipitins against the antigens of *C. albicans* should be regarded as of possible pathogenic significance, because they can mediate Arthus, Type III reactions which are capable of damaging tissues.

There is some limited evidence in the patients with asthma and pulmonary eosinophilia to suggest that reactions to the *C. albicans* antigens are of clinical importance. The majority of patients with asthma and pulmonary eosinophilia in the United Kingdom have evidence of Type I and Type III allergy to *A. fumigatus*. In 11 patients without such evidence, precipitins against *C. albicans* mannan A were present in 9. The problem is complicated by the presence of mannan A precipitins in those patients with precipitins against both polysaccharide and protein antigens of *A. fumigatus*, but not in those with precipitins against the protein antigens only. It might have been expected, under the circumstances, that the mannan A and the *A. fumigatus* polysaccharide would show cross-reactions. Their failure to do so may be due to chemical differences, the reacting sugar in the gluco-galacto-mannan peptide of *A. fumigatus* being galactose,¹⁰ which is not present in the mannan A of *C. albicans*. The presence together, in some cases, of precipitins against polysaccharides from both the fungi may be due to infection with both of them or perhaps reflect a heightened sensitivity of some subjects to polysaccharide antigens in general.

The frequency with which precipitins have been found in this study and in that of Chew and Theus⁷ contrasts strikingly with the reports⁴⁻⁶ that precipitins were found only in subjects with deep-seated infection with *C. albicans* in response to tests with a variety of extracts, all of which were shown in this study and by Chew and Theus⁷ to contain mannan antigens. The differ-

ences in the frequency of reactions may be due to the use of too strong concentrations of mannan, which reduces the number of visible precipitation reactions either by causing them to take place in the central serum well or because the reactions of the H type given by the mannans are readily soluble in excess antigen. The number of reactions of weak sera would be reduced in this way. In patients with deep-seated or extensive infection, larger amounts of precipitin are present, capable of giving reactions with high concentrations of antigen. This is a probable explanation of the reports of positive reactions only in such subjects.

There are also differences between our findings and those of Chew and Theus,⁷ who found precipitins in the sera of all normal subjects when these were concentrated fifteenfold and tested in Preer tubes. They, however, obtained fewer precipitation reactions in double-diffusion tests against unconcentrated sera than we did. They used a test pattern in which the serum and antigen wells were of the same size, whereas we used a much larger serum than antigen well, thus increasing the amount of antibody available for the reaction. Furthermore, they do not state what concentration of mannan was used; if this were high, it would contribute to an excess of antigen, thereby decreasing the number of reactions. As stated earlier, the level of precipitins demonstrated in our tests (about 0.18 mg. per milliliter) is of pathogenetic importance, since there was a good correlation between the precipitin reactions and the production of Type III skin and inhalation test reactions.

The mannans of *C. albicans* are also of unusual potency. Skin test with them stimulated in all subjects, healthy or otherwise, the appearance of or increase of precipitins in the tests employed here. In view of the universal presence of precipitins reported by Chew and Theus,⁷ it seems that both the precipitins which appeared in 4 to 7 days and those which appeared within 2 to 3 weeks were due to a secondary response. The Type III component of the dual skin test reactions was also increased in strength and frequency as a result of the initial skin tests. Similar findings have been reported with histoplasmin antigens.¹⁷

These findings may reflect a process by which liberation of antigen, due to growth of the fungus or infection with it, could stimulate both increasing amounts of precipitating antibody and the production of Arthus reactions more frequently and of greater severity. Such a mechanism could be responsible for the changes reported in established cases of *Candida* infection of the lung, including lung abscess, pulmonary candidoma, chronic granulomatous and fibrotic changes, and bronchiectasis.¹ Reactions of the same type could also affect other organs in which *Candida* infections are common.

Local and constitutional reactions to the injection of extracts of *C. albicans* in asthma have been reported frequently. Indeed, one of the strongest precipitin reactions observed by us was obtained with the serum of a patient having such injections. The implications of the increase in precipitins following injections of extracts of *C. albicans* for treatment purposes must be examined. The production of Arthus reactions on skin testing and of systemic reactions on inhalation testing show that these precipitins are of pathogenetic importance. The possibility that they may be responsible for unrecognized reactions oc-

curing elsewhere in the body cannot be excluded. Indeed, the effects of the skin tests themselves create difficulties in the further study of these antigens. Nevertheless there is a report¹⁸ that injection treatment led to a decrease in immediate and delayed bronchial reactions to inhalation tests. The methods and extracts employed were different from ours, and comparisons are not possible.

Increased understanding of the allergic reactions to *C. albicans* and their correlation with disease should help to determine their role, even though their assessment is made more difficult by the reactions of the same nature which can be elicited in healthy subjects with precipitins. This problem is common to other "opportunistic" pathogens which, because of their ubiquitous distribution, have often been considered to have no pathological importance.

We wish to thank Drs. H. F. Hasenclever and W. O. Mitchell for providing mannans for testing, Dr. I. G. Murray for providing cultures, and the clinicians of the Brompton Hospital and elsewhere for their generous cooperation.

REFERENCES

1. Winner, H. I., and Hurley, R.: *Candida albicans*, London, 1964, J. & A. Churchill, Inc.
2. Winner, H. I., and Hurley, R.: Symposium on *Candida* Infections, Edinburgh, 1966, E. & S. Livingstone, Ltd.
3. Summers, D. F., Grollman, A. P., and Hasenclever, H. F.: Polysaccharide Antigens of *Candida* Cell Wall, *J. Immunol.* **92**: 491, 1964.
4. Elinov, N. P., and Zaikina, N. A.: The Precipitin Test in the Serological Diagnosis of Moniliasis, *Zh. Mikrobiol. Moscou* **30**: 40, 1959.
5. Stallybrass, F. C.: *Candida* Precipitins, *J. Path. & Bact.* **87**: 89, 1964 a.
6. Taschdjian, C. L., Kozinn, P. J., and Caroline, L.: Immune Studies in Candidiasis. III. Precipitating Antibodies in Systemic Candidiasis, *Sabouraudia* **3**: 312, 1964.
7. Chew, W. H., and Theus, T. L.: *Candida* Precipitins, *J. Immunol.* **98**: 220, 1967.
8. Fuller, A. T.: Formamide Method for Extraction of Polysaccharides From Haemolytic Streptococci, *Brit. J. Exper. Path.* **19**: 130, 1938.
9. Longbottom, J. L., and Pepys, J.: Pulmonary Aspergillosis. Diagnostic and Immunological Significance of Antigens and C-substance in *Aspergillus fumigatus*, *J. Path. & Bact.* **88**: 141, 1964.
10. Longbottom, J. L.: University of London Ph.D. Thesis, 1964.
11. Levy, H. B., and Sober, H. A.: A Simple Chromatographic Method for Preparation of γ -globulin, *Proc. Soc. Exper. Biol. & Med.* **103**: 250, 1960.
12. Hasenclever, H. F., and Mitchell, W. O.: Antigenic Studies of *Candida*. IV. The Relationship of the Antigenic Groups of *Candida albicans* to Their Isolation From Various Clinical Specimens, *Sabouraudia* **2**: 201, 1963.
13. Stallybrass, F. C.: The Incidence of the Serological Groups of *Candida albicans* in Southern England, *J. Hyg.* **62**: 395, 1964 b.
14. Pepys, J.: Pulmonary Hypersensitivity Disease Due to Inhaled Organic Antigens, *Postgrad. M. J.* **42**: 698, 1966.
15. Williams, J. V.: Pulmonary Function Studies in Patients With Farmer's Lung, *Thorax* **18**: 182, 1963.
16. Hargreave, F. E., Pepys, J., Longbottom, J. L., and Wraith, D. G.: Bird Breeder's (Fancier's) Lung, *Lancet* **1**: 445, 1966.
17. Campbell, C. C.: Problems Associated With Antigenic Analysis of *Histoplasma capsulatum* and Other Mycotic Agents, *Am. Rev. Resp. Dis.* **92**: 113, 1965.
18. Itkin, I. H., and Dennis, M.: Bronchial Hypersensitivity to *Candida albicans*, *J. ALLERGY* **37**: 187, 1966.
19. Pepys, J., Turner-Warwick, Margaret, Dawson, Patricia L., and Hinson, K. F. W.: Arthus (Type III) Skin Test Reactions in Man: Clinical and Immunopathological Features. Proceedings of Sixth International Congress of International Association of Allergology, 1967. To be published.
20. Pepys, J.: Hypersensitivity to Inhaled Organic Antigens, *J. Roy. Coll. Phys.* **2**: 42, 1967.
21. Gell, P. G. H., and Coombs, R. R. A.: *Clinical Aspects of Immunology*, Oxford, 1964, Blackwell Scientific Publications.
22. Peat, S., Whelan, W. J., and Edwards, T. E.: Polysaccharides of Baker's Yeast. Part IV. Mannan, *J. Chem. Soc.*: 29, 1961.