parasites in tissue cultures also offers advantages over previously available procedures in that all of the equipment required is readily available in any laboratory. This relatively simple method can be utilized by laboratories which may have to suspend toxoplasma tests temporarily since after one passage, organisms can be returned to the refrigerator for an additional period of storage.

Summary. *Toxoplasma gondii* cultivated in roller tube cultures of Hep-2 cells produce large numbers of free, extracellular parasites for the performance of dye tests. The successful storage of toxoplasma in refrigerated tissue culture preparations for up to 120 days and in the frozen state for as long as 360 days is described.

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Pathogenicity of Specific Glycopeptide Antigen in Farmers Lung.* (29901)

ROBERT A. BARBEE,** HELEN A. DICKIE AND JOHN RANKIN
(Introduced by O. O. Meyer)
Department of Medicine and Cardiopulmonary Research Laboratory, University of Wisconsin, Madison

We have previously described(1,2) the clinical, physiological and pathological features of Farmers Lung. Characteristically, the typical clinical syndrome consists of chills, fever, cough and shortness of breath 4 to 8 hours after exposure to moldy hay or forage and subsequent development of a diffuse granulomatous interstitial pneumonitis. Recently Kobayashi(3) and Pepys(4) have demonstrated that the serum of patients with this disease reacts with antigens extracted from moldy hay to form specific precipitin lines in agar gel immunodiffusion. This observation, however, does not prove a causal relationship between exposure to moldy hay and subsequent development of pulmonary disease.

To establish that such a cause and effect relationship does exist, a provocative challenge test utilizing these antigens was employed in a group of patients with Farmers Lung and a comparable control group. The clinical response was observed, and pulmonary function studies were performed on both groups following this aerosol inhalation challenge.

Materials and methods. Antigens were extracted from a sample of moldy hay which was known to have caused the typical clinical syndrome in one of our patients in the following manner. After defatting with acetone, the sample was extracted with 8% trichloroacetic acid (TCA). Precipitable fractions were obtained by treating the TCA soluble

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** Postdoctoral Fellow, Nat. Inst. Allergy & Infect. Dis., U.S.P.H.S.

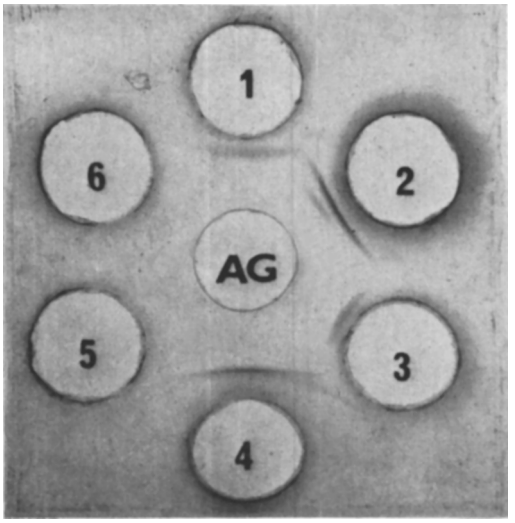


FIG. 1. Immunodiffusion reaction in agar gel stained with amido black. The center well contains Farmers Lung antigen (AG) and the numbered outer wells contain the sera of the six patients whose data are included in the present study. 1 to 4 show precipitin reactions, 5 and 6 do not.

extract with graded concentrations of ethyl alcohol. Those fractions which precipitated at alcohol concentrations above 50% were found to be the most active in demonstrating precipitating antibody and, thus, were used in the provocative challenge. These alcohol precipitates were lyophilized, diluted in sterile saline and Seitz filtered prior to aerosolization.

Modified Ouchterlony immunodiffusion and immunoelectrophoresis were used to demonstrate serum precipitins. An example of the reaction obtained with this method is shown in Fig. 1.

Aerosol challenge was administered by means of a standard DeVilbiss No. 40 nebulizer charged with a saline solution of the antigen extract. After trial, a single challenge with 100 mg was used. In one patient, a second challenge with 100 mg was given 8 hours after the first (see Table I).

Following challenge, temperature recordings were made every one to two hours for at least 14 hours, or until it had returned to normal. Total and differential white blood counts were determined before and at 5 hours following challenge in most subjects, and also at 24 hours in the patient group.

Diffusing capacity for CO was measured using the single breath method modified after Ogilvie, Forster *et al*(6). Maximum inspiratory and expiratory flow rates were determined on a special servospirometer, and arterial gas studies were performed according to the techniques of Van Slyke and Neill.

Results. Six patients with Farmers Lung and 10 control subjects were challenged with this moldy hay antigen by aerosol inhalation. The immunologic status of the patients receiving the antigen challenge, as judged by double diffusion in agar gel, was as follows: 4 had definite precipitins; one was negative; and one was either weakly positive or negative on different occasions (Fig. 1). None of the 10 controls showed precipitin reactions.

Clinical. With minor variations the observed clinical symptoms were qualitatively identical to those which the patient noted during a naturally occurring episode of the disease. In most cases this means chills, fever, productive cough and shortness of breath. The degree to which these symptoms appeared was related to the individual sensitivity to moldy hay. For example, Patient No. 2, who reacts with high fever and malaise after minimal exposure to moldy hay in the barn, developed a fever of over 103°F during the post-challenge period (Fig. 2). On the other hand, as has been the case in his naturally occurring episodes, Patient No. 3 showed little febrile response but experienced more cough and shortness of breath.

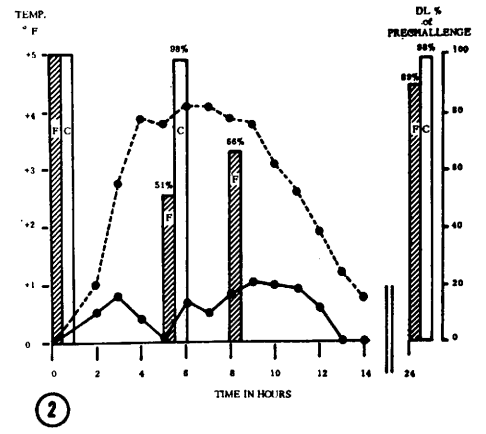
Temperature. The average temperature response of the patient and control groups is shown in Fig. 3. Patient temperature responses during the 14-hour post-challenge period averaged 2.70° above the pre-challenge day temperature. That this antigen is not completely innocuous in the non-sensitized control subject is shown by the .91°F temperature rise seen in this group during the same 14-hour post-challenge period. The significance of this and possible explanations will be discussed later.

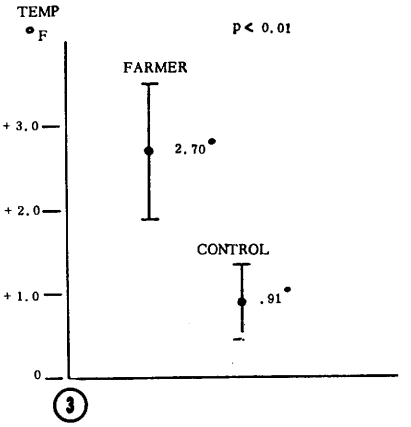
White blood count. Changes in WBC are noted in Fig. 4. Both experimental groups showed a marked rise in total WBC which was limited primarily to the granulocytic series. This leucocytosis occurred in the con-

trol subjects despite only minimal temperature rise and in the presence of only mild malaise. The total WBC in the patient group was slightly higher at 5 hours than in the controls, and of some interest was the almost universal decrease in total lymphocytes in the patients.

Diffusing capacity. Decrease in pulmonary diffusing capacity for CO(D_L) is the major physiologic alteration encountered (Fig. 2, Patient 2). Five hours following aerosol inhalation D_L was 51% of the pre-challenge level, rising to 66% at 8 hours, and approaching the previous level by 24 hours. On the other hand, in the controls the temperature response was minimal, and no detectable changes in D_L were apparent.†

Although some variation is seen in the patient group, the overall pattern is quite consistent. Three of the four patients whose serum contained precipitins showed significant decreases in diffusing capacity and/or arterial oxygen saturation during the acute febrile episode approximately 5 hours following challenge. As a group, the 4 patients with demonstrable antibodies against Farmers Lung antigen showed a 24.4% drop in D_L 5 hours following challenge, falling from 23.8 cc/min/mmHg to 18.0 cc/min/mmHg. By 24 hours the D_L for these 4 patients had returned virtually to the pre-challenge level. Patients whose serum reacted weakly or not at all showed essentially no change in any of the parameters of lung function meas-





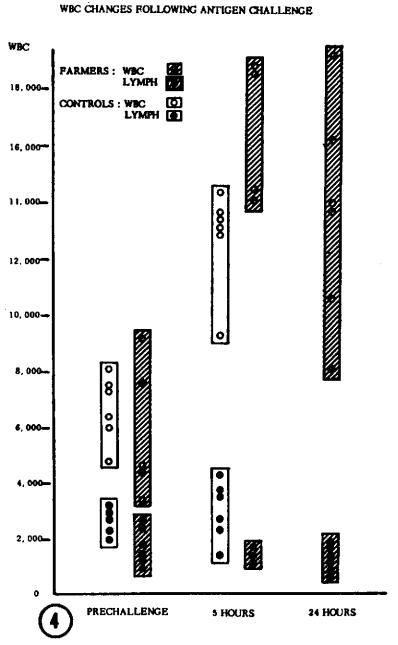


FIG. 2. Temperature response in degrees F of one of the patients compared with that of a control subject. Patient response seen on stippled line; control response on solid line. Changes in single breath CO diffusing capacity represented by vertical bars as a percentage of pre-challenge value. Patient response (F) is seen on striped bars and control (C) on open bars.

FIG. 3. Temperature responses in degrees F are shown for the Farmers and the controls during the 14-hr period following aerosol inhalation of 100 mg of Farmers Lung antigen. Average response is shown by the circles and the extremes in each group by the ends of the bars.

FIG. 4. An elevation in total WBC is seen in both groups following antigen inhalation. Of possible significance immunologically is the decreased total lymphocyte count noted in the subjects with Farmers Lung.

† Individual values are shown in Table 1. The patient numbers correspond to those used in the immunodiffusion plate (Fig. 1).

TABLE I.

Patient	Precipitins	Antigen dose (mg)	Maximum inspiratory flow rate (liters/min)* 0 hr	+5 hr	+8 hr	+24 hr	Maximum expiratory flow rate (liters/min) 0 hr	+5 hr	+8 hr	+24 hr	Diffusing capacity for CO† (cc/min/mmHg) 0 hr	+5 hr	+8 hr	+24 hr	Arterial oxygen saturation (%) 0 hr	+5 hr	+24 hr
1	+	100	400	450		480	430	470		460	26.4	24.5		22.6	93.8	91.3	95.0
2	+	100	340	220		310	460	350		430	23.9	12.1	15.8	21.3	90.7	82.0	90.0
3	+	100	340	300	300	320	410	380	300	420	19.7	14.9	16.7	23.1	92.6	92.9	92.2
4	+	100	440	140	240	350	270	270	250	290	25.3	20.3	26.5	24.2	92.0	85.6	92.3
Avg			380	280	270	360	390	370	270	400	23.8	18.0	19.7	22.8	92.3	88.0	92.4
5	±	100	270	240		300	390	350		410	21.3	21.9		20.7	93.1		
6	—	200	460	400		380	430	450		440	20.1	22.2		18.0	94.1		95.4
Control																	
1	—	100	360			460	470			440	43.0	41.5		38.8			
2	—	100	410			460	440			420	32.9	34.6		38.8			
3	—	100	520			610	590			570	44.6	43.5		45.7			
4	—	100	440			460	470			460	37.0	36.3		36.2			
5	—	100	330			350	410			390	26.6	25.6		22.8			
6	—	100	470			360	540			520	37.1	40.7		36.0			
7	—	100									30.1	33.1		32.7			
8	—	100									30.3			28.7			
9	—	100									35.3	30.6		34.0			
10	—	100									42.4			40.8			
Avg			420			450	490			470	35.9	35.7		34.9			

* Maximal inspiratory flow rates and maximal expiratory flow rates were measured on special servospirometer. Average normal values lie between 450 and 600 liters/min.

† Pulmonary diffusing capacity for carbon monoxide was measured by a modification of the breath-holding technique of Ogilvie, Forster, Blakemore, and Morton(6). Predicted $D_{L_{CO}}$ = surface area × 18.85 − 6.8.

ured. In this regard, these latter 2 patients reacted in the same manner as 9 of the 10 control subjects. Control No. 9, in whom D_L decreased from 35.3 to 30.6, was the only one of the 10 control subjects to show any change in diffusing capacity. During this same post-challenge period the arterial oxygen saturation of the 4 serologically positive patients fell from 92.3% to 88%. By 24 hours this change had also disappeared.

Flow rates. Two of the four patients with precipitins showed significant changes in either maximum inspiratory or expiratory flow rates. Because these tests are dependent upon maximal effort on the part of the subjects, one must be cautious in interpreting a fall in flow rate which occurs during a time when the subject is acutely ill with fever and malaise.

Discussion. The purpose of this study was to demonstrate that antigens which are extracted from moldy hay and which are known to produce precipitin reactions in agar gel immunodiffusion are also capable of producing lung disease. Clinically and physiologically, the febrile episodes resulting from inhalation of these antigens were qualitatively identical to the naturally occurring episodes of Farmers Lung. In those patients, the severity of the response appeared possibly to be related to the level of circulating antibody. Neither Patients No. 5 nor 6 had been exposed to moldy hay for several years, and one might expect that their reactivity to one mild exposure would not produce dramatic changes, either clinical or physiological. In each case the febrile response of these 2 subjects was greater than the controls but less than that seen in the first 4 patients, all of whom had acute episodes of disease within the past 2 years.

In its present state, the antigens used in this study are not completely innocuous even in presumably non-sensitized controls. The mild temperature elevation and rise in WBC indicates the presence of a contaminant which produces some systemic reaction in all subjects. It is tempting to attribute this reaction to the presence of contaminating endotoxin. No direct evidence for this is available, however.

Despite the above systemic febrile reaction in controls, no detectable change in diffusion was present 5 hours following challenge. This contrasts with the drop in D_L and arterial oxygen saturation which occurred transiently in the patient group. Small qualitative and quantitative differences in total WBC and lymphocyte response were also noted. These physiologic and hematologic changes, coupled with the reproduction of clinical symptoms of Farmers Lung, appear to demonstrate an etiologic relationship between the antigen employed and the production of Farmers Lung.

Summary. Six patients with Farmers Lung and 10 controls were challenged with an antigen extracted from moldy hay by aerosol inhalation. Precipitin reactions in agar gel immunodiffusion were strongly positive in 4 patients, weakly reactive in one, and not present in one. Clinical, hematologic and physiologic data are presented on the 6 patients challenged in an identical manner and on the 10 control subjects. Responses to this challenge in the patient group were qualitatively identical to the reaction each patient experienced when exposed to moldy hay in the barn or silo. These moldy hay antigens are, thus, concluded to be the etiologic agents responsible for the disease, Farmers Lung.

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