

Farmer's lung

Part I An immunological study of some antigenic components of mouldy foodstuffs

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The essential feature of farmer's lung is that it is respiratory distress occurring six to nine hours after the inhalation of the dust of mouldy vegetable material (Studdert, 1953; Dickie and Rankin, 1958). It is unlike asthma in that the patient has difficulty in inspiration rather than in expiration (Duncan, 1945; Fuller, 1953; Frank, 1958). The lungs of affected subjects have an interstitial fibrosis, are infiltrated by histiocytes and lymphocytes, and contain small granulomata and tubercles (Fuller, 1958; Frank, 1958; Dickie and Rankin, 1958; Baldus and Peter, 1960).

Although for some time the condition has been thought to be allergic in origin, the first real evidence of its allergic nature was only found recently by Pepys, Riddell, Citron, and Clayton (1961), who demonstrated the presence of circulating precipitating antibodies to extracts of mouldy hay and some moulds in the sera of these patients.

Little work has been done to investigate the aetiology of this condition by exposing experimental animals to the mouldy dusts. Zettergren (1950) found that rabbits exposed to the sterile dust of mouldy hay seeded with *Candida albicans* developed small granulomata and tubercles in the lungs resembling those described in farmer's lung in man, but he observed no clinical effects similar to the disease. In another study (Parish, 1961), similar granulomata and tubercles were found in the lungs of rabbits and guinea-pigs previously sensitized by the inhalation of native mouldy hay dust or dust treated to make it sterile and then seeded with cultures of *C. albicans*, *Aspergillus fumigatus*, or *Mycobacterium phlei*. There was no delayed respiratory distress when the dust of native mouldy hay, or sterile dust seeded with cultures of these microorganisms was inhaled by animals not previously exposed to them under experimental conditions and whose sera contained no precipi-

tating antibodies to the antigens of the dust or microorganisms. However, respiratory distress delayed five and a half to nine hours followed the inhalation of mouldy hay dust in 14 of 19 rabbits, which had precipitating antibodies in their sera to extracts of mouldy hay, out of a group of 118 animals which had been long exposed to mouldy vegetable material. This delayed respiratory distress, accompanied by the formation of tubercles in the lungs, was considered to be similar to that found in farmer's lung.

This report describes experiments carried out to investigate the antigen or antigens in mouldy foodstuffs that stimulate the formation of the precipitating antibodies and that may be responsible for the condition. The presence of a common antigen or antigens in mouldy hay or cereals, not present in the clean material or the moulds tested, was demonstrated by the Schultz-Dale technique and could also be detected by double diffusion gel plate precipitin tests. The precipitating antibody in the sera of rabbits long exposed to mouldy vegetable material was shown to be a gamma globulin by immunoelectrophoresis in agar gel.

MATERIALS AND METHODS

EXTRACTS OF MOULDY HAY, CEREALS, AND FUNGI Four different samples of mouldy hay, designated MH1, MH2, MH3, and MH4, and two samples of clean hay, designated CH1 and CH2, were used in this study.

Aqueous extracts of the hay samples were prepared by wetting each sample for about six hours in tap water at room temperature, removing each sample to a stone floor, pounding it until crushed, and then placing the material back into the same water for four days at 4°C. It was then twisted until most of the water was expressed and the extract was allowed to stand for four hours; this was decanted and the supernatant filtered through a coarse filter (Whatman No. 1). The filtrate was concentrated by suspending it in

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dialysis tubing in a cold air current at 4°C. for 24 hours. It was then dialysed against running tap water, filtered through a Seitz E.K. filter, and freeze-dried to a powder. This powder was reconstituted in either saline or Dale's solution in a concentration of 15 mg./ml. and dialysed against the same medium before use. Extracts used for neutralization of sera containing antibody were not reconstituted but were used in the powdered state, as described under 'gel plate precipitin tests' on p. 85.

Suspensions of fungi were frozen and thawed three times, homogenized, centrifuged at 3,500 r.p.m. for one hour, Seitz E.K. filtered, dialysed, and freeze-dried. An extract was also made of uninoculated Sabouraud's medium incubated with, and treated as, the inoculated plates. Only early growth of each of the fungal cultures was extracted since extracts of older cultures were sometimes toxic to the ileum in the Schultz-Dale test. Extracts of older cultures of *A. fumigatus* could destroy the response of the ileum to histamine.

EXPERIMENTAL PRODUCTION OF MOULDY HAY A sample of clean hay was divided into three portions. Most of the bale was left in a room under normal conditions of storage. The second portion was wetted with tap water, tied into a bundle with string, and sealed in a polythene bag. This was incubated for four weeks at 37°C. The third portion was inoculated with a suspension of all organisms obtained from a saline extract of mouldy hay and grown on a number of different solid media for 12 days at room temperature and at 37°C. This portion was then tied with string, sealed in a polythene bag, and incubated with the second portion at 37°C. for four weeks. At the end of four weeks the three portions were extracted and freeze-dried.

EXPERIMENTAL PRODUCTION OF MOULDY BARLEY STRAW Aqueous extracts were prepared of barley straw which had been stored in a barn for nine months. A further sample was soaked in water, compressed in a tight bundle under 2 cwt. for 48 hours, sealed in a polythene bag, and incubated at 39°C. for eight weeks. Aqueous extracts were then prepared as before.

SENSITIZATION OF GUINEA-PIGS The guinea-pigs used in the Schultz-Dale tests were sensitized by two intraperitoneal injections at a two-day interval. Each injection of 1 ml. contained either 15 mg. of mouldy hay extract or 10 mg. of fungal extract. The guinea-pigs were killed and bled 21 to 32 days later.

THE SCHULTZ-DALE TECHNIQUE A smooth muscle preparation, strips of either intestine or uterus of actively sensitized guinea-pigs, will contract in the presence of the specific antigen in the Schultz-Dale bath. By this method it is possible to detect very small amounts of soluble antigen, which are too small to be detected by other immunological techniques. Once the smooth muscle preparation has contracted after the addition of antigen, it becomes desensitized to

that antigen if sufficient is added, but it will contract again when challenged with other antigens to which the animal has been sensitized. Thus it is possible to test for the antigenic similarity of different extracts by finding out which of them will completely desensitize the tissue preparation. It is also possible to detect the presence of certain antigens, such as those of microorganisms, in a complex material, such as mouldy hay extract, originally used to sensitize the animal.

The apparatus (Fig. 1) resembled that described by Coulson (1953). The glass coil used for warming the salt solutions before they entered the test chamber, and the test chamber itself, were enclosed in a water-bath maintained at 37°C. The lower end of the $\frac{1}{2}$ -in. strip of ileum or uterus suspended vertically in the test chamber was fixed to a hook on the tube which supplied air at the rate of one bubble per second. The free upper end of the tissue was attached by a thread to a light aluminium pointer, which marked a trace on a smoked drum to record contractions. The chamber normally contained 3.5 ml. of the salt solution. The balanced salt solution used was that of Dale and Laidlaw (1912) with the low calcium level and without the dextrose. Atropine was added, using 1 ml. of 1:10,000 atropine sulphate to 1 litre of salt solution.

Terminal ileum was used in preference to a strip of uterus since it produced larger and quicker contractions, though the sensitivity of the two tissues was the same. Before challenge with any antigen, the ileum was shown to give a good contraction to 1:10 million histamine acid phosphate in Dale's solution, and, when a number of challenges were made with different antigens on the same strip of ileum, a good contraction was obtained with the histamine after each challenge before proceeding to the next. Histamine was added at one-minute intervals, except after challenge with an antigen when the smooth muscle preparation took slightly longer to relax after a contraction, and the next addition of histamine was made as soon as the pointer had returned to the base line. The bath was washed out completely three times with the salt solution after every addition of antigen or histamine. Each challenge consisted of 0.5 ml. of the histamine solution or antigen containing the soluble extracts described.

The following precautions were taken to prevent non-specific contractions. (1) The tissues were always gently handled with clean instruments and washed free of all contents when removed from the body. (2) The temperature of the coil and test chamber was maintained evenly by the thermostatic heater of the water-bath. All solutions to be added to the test chamber were first placed in a rack over the water-bath and were therefore at the same temperature when tested on the tissue. (3) The level of the water-bath was kept up to the tip of the test chamber so that no variation in temperature within the chamber would cause convection currents. (4) Silk thread was used between the upper free end of the strips of tissue and the pointer, so that air bubbles could not be trapped.

on its smooth surface and cause vibration. (5) The test solutions were added dropwise over the edge of the test chamber to prevent mechanical disturbance to the muscle preparation.

All the antigen-containing extracts were first tested on ileum from normal unsensitized animals to determine whether they were free from smooth muscle contracting substances that would produce non-specific contractions when the extracts were added to the tissues of specifically sensitized animals.

GEL PLATE PRECIPITIN TESTS Tests were carried out using rabbit sera containing precipitins resulting from

natural exposure to mouldy cereals taken before the animals were challenged. In the neutralization tests, 3 mg. of the powdered extracts was added to 0.4 ml. of serum and left at 4°C. overnight before being centrifuged and added to the gel plates.

IMMUNOELECTROPHORESIS A modification of the microtechnique of Grabar (1960) was employed, in which five sera could be tested simultaneously on a glass slide 8 centimetres square. One hundred and twenty volts were passed through the 1.5% agar gel in barbital buffer, pH 8.6, I=0.06, for one hour. Lateral troughs 4 mm. each side of the reservoir

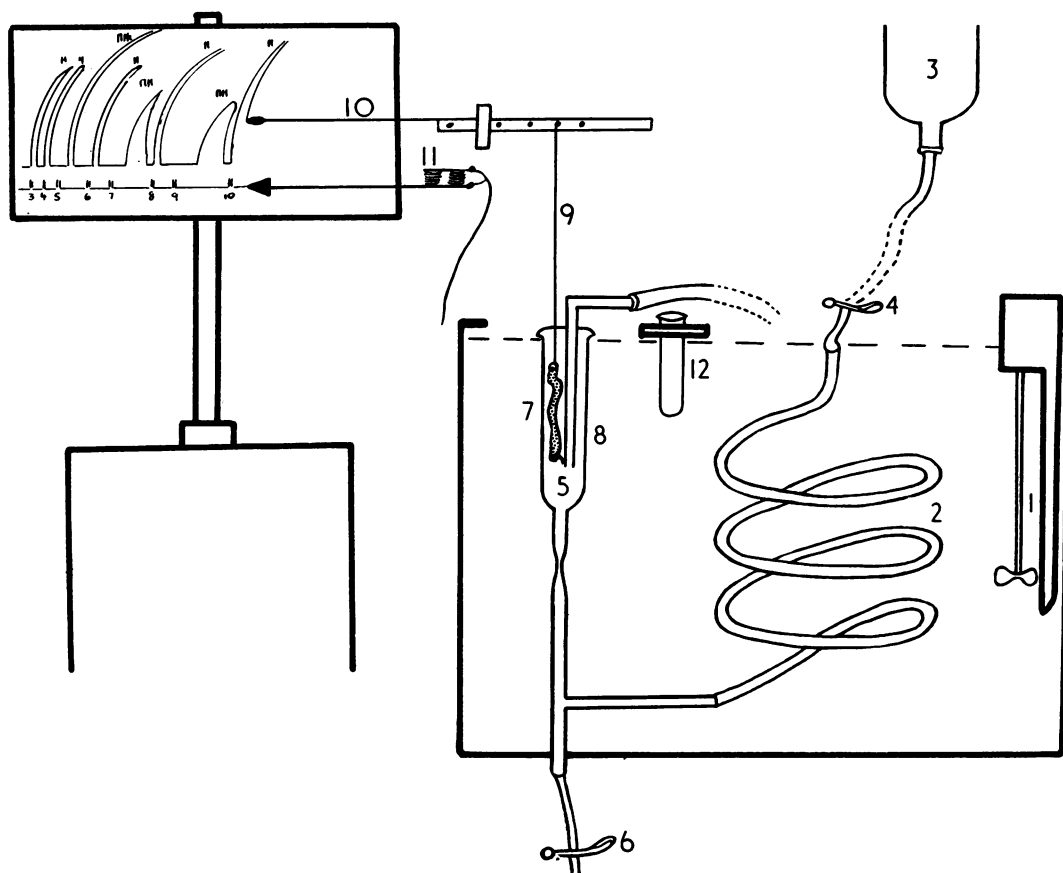


FIG. 1. Diagram of the Schultz-Dale apparatus. The water-bath maintained at an even temperature by the heater and propeller (1) contains a glass coil (2) which allows the balanced salt solution to reach the temperature of the bath. The salt solution passes from the raised reservoir (3) by gravity, and flow is controlled by a spring clip (4); it passes into the test chamber (5) and may be flushed out by releasing the spring clip (6) below the water-bath. In the test chamber a strip of ileum or uterus (7) is fixed at the lower end to a hook on the end of a tube (8) supplying air from a pump. The upper end of the tissue is attached by a thread (9) to a light aluminium pointer (10) on a gimbals. This records the contractions on the smoked drum revolving at 8 mm./minute. An electric push-button marker (11) records the moment at which extracts or histamine are added to the chamber. Tubes containing extracts or histamine to be added to the test chamber are warmed to bath temperature in the rack (12) next to the chamber.

contained mouldy hay extract on one side and goat anti-rabbit serum on the other to facilitate identification of the component containing precipitating antibody. Precipitation was allowed to develop for 12 to 24 hours and the slide was then stained with Amido-Schwarz.

RESULTS

SCHULTZ-DALE TESTS The following results were obtained.

Antigens of mouldy hay extracts It was found that fresh saline extracts of clean or mouldy hay contained a factor causing an immediate contraction of ileum or uterus from normal guinea-pigs that resembled the contraction caused by histamine. This factor was found to be resistant to boiling for 10 minutes and could be removed by dialysis against tap water or physiological saline. The dialysed, freeze-dried extracts reconstituted in saline or Dale's solution contained no such activity when tested on strips of ileum from normal guinea-pigs.

Strips of ileum obtained from sensitized guinea-pigs produced a powerful contraction when tested with the specific antigen. After a strip had recovered from such a contraction and been demonstrated to be capable of contracting on the addition of 1:10 million histamine, it failed to contract on the further addition of the same antigen, or in rare instances produced a feeble contraction if it was not completely desensitized. In these instances there was no reaction after a third exposure to the antigen.

When a number of antigen extracts obtained from different sources were added in turn to the test chamber, the strip of ileum having been shown to be sensitive to histamine between each challenge, it was possible to demonstrate the presence or absence of antigens in the samples which were common to those present in the extract originally used to sensitize the animal. Table I shows the results of testing different strips of ileum with four different samples of mouldy hay, two samples of clean hay, and extracts of *C. albicans*, *A. fumigatus*, and *Penicillium notatum*. The ileum was shown to give a contraction to 1:10 million histamine between each challenge by antigen.

The order of addition of these antigen extracts was selected so that each extract was used at least once as the first challenge on a fresh strip of ileum from the sensitized guinea-pig. Each extract of mouldy hay was also used once as the second challenge on a strip of ileum. The strip of ileum A provided evidence that, after it had been desensitized by addition of the antigen to which the

TABLE I

THE RESULTS OF CHALLENGING STRIPS OF ILEUM OBTAINED FROM A GUINEA-PIG, SENSITIZED TO AN EXTRACT OF MH1, WITH EXTRACTS OF MOULDY HAY, CLEAN HAY, AND FUNGI BY THE SCHULTZ-DALE TECHNIQUE

Strip of Ileum	Numerical Order in which Extracts of Antigens were Added to Each Strip of Ileum, and Result of Test				
A	MH1 +	MH1 -	MH2 -	MH3 -	MH4 -
B	MH2 +	MH3 ±	MH4 +	MH1 -	
C	MH3 +	MH2 ±	MH4 +	MH1 -	
D	MH4 +	MH3 -	MH2 -	MH1 -	
E	MH4 +	MH1 -			
F	MH1 +	MH4 -			
G	CH1 +	MH1 +			
H	CH2 +	MH1 +			
I	<i>A. fumigatus</i> -	<i>C. albicans</i> -	<i>P. notatum</i> ±	MH1 +	MH1 -
J	<i>C. albicans</i> -	<i>A. fumigatus</i> -	<i>P. notatum</i> -	MH1 +	MH1 -

MH1, MH2, MH3, and MH4 = extracts of four samples of mouldy hay

CH1 and CH2 = extracts of two samples of clean hay

+, contractions; ±, weak contractions; -, no contractions of ileum

animal had been sensitized (*i.e.*, MH1), the other extracts caused no contraction, thus providing a further control test as they did not cause contraction of the ileum of a normal, unsensitized guinea-pig. The extract MH1 was the last to be added to the strips of ileum B, C, D, and E, and demonstrated that the previous extracts contained all the antigens necessary to desensitize these strips of ileum. Strips of ileum E and F gave evidence of the antigenic similarity of MH1 and MH4.

Similar experiments were carried out using the ileum of other guinea-pigs which had been sensitized to each of the other three samples of mouldy hay, *i.e.*, MH2, MH3, and MH4. These tests carried out on animals sensitized to *each* of these extracts confirmed the following findings.

1 Extracts of mouldy hay would actively sensitize guinea-pigs.

2 A guinea-pig sensitized to mouldy hay was also sensitized to extracts of clean hay, but there were other antigens present in mouldy hay not present in clean hay; therefore clean hay would not completely desensitize a guinea-pig.

3 Samples of MH1 and MH4 cross-reacted completely, but samples of MH2 and MH3 each contained an antigen or antigens not found in the other three samples.

4 Extracts of MH2 contained an antigen apparently derived from *Candida* species, and extracts of MH3 contained an antigen apparently derived from *Penicillium* species. These antigens were distinct from those referred to in 3.

5 Different extracts of each sample prepared at different times did not contain identical antigenic components, or animals did not respond in the same way to the same injection. Thus tissues of

some guinea-pigs sensitized to mouldy hay, e.g. MH1 (Table I), contracted weakly to extracts of *Aspergillus* or *Penicillium*, though this response was unreliable even with different strips of ileum from the same animal.

Antigens of extracts of mouldy cereals Segments of the ileum of a guinea-pig sensitized to an extract of MH4 were challenged by extracts of normal and mouldy crushed oats and bran. In the results presented in Table II, it can be seen that the

TABLE II

THE RESULTS OF CHALLENGING STRIPS OF ILEUM OBTAINED FROM A GUINEA-PIG, SENSITIZED TO AN EXTRACT OF MH4, WITH EXTRACTS OF CLEAN AND MOULDY CRUSHED OATS AND BRAN, AND CLEAN HAY

Strip Numerical Order in which Extracts of Antigen were Added to of Each Strip of Ileum, and Result of Test

A	MH4	+	CH1	-	O	-	MO	-	B	-	MB	-
B	CH1	+	MH4	+	O	-	MO	-	B	-	MB	-
C	O	±	CH1	+	MH4	+						
D	O	±	MO	+	MH4	+						
E	B	-	MB	-	MH4	+						
F	MB	-	B	-	MO	+	MH4	+				

O=crushed oats; MO=mouldy crushed oats; B=bran; MB=mouldy bran. Other symbols as in Table I

mouldy oats had an antigen or antigens in common with the mouldy hay, but there was not complete identity between the two, since mouldy oats would not desensitize the ileum. Mouldy bran, on the other hand, in spite of its fungal content, did not cross-react with mouldy hay. However, only this one extract was tested.

Antigens of extracts of artificial mouldy hay Extracts were prepared from the clean hay, which had been treated in an attempt to render it 'mouldy' under laboratory conditions, to discover whether it developed antigens found in native mouldy hay. These extracts were tested on strips of ileum taken from a guinea-pig sensitized to an extract of native mouldy hay, MH4.

It was found that the sample of clean hay incubated in a wet state had developed a new antigen or antigens not present in the original clean sample (see Table III). A further antigen or antigens were present in the third sample of clean hay, which had been incubated in the wet state together with microorganisms obtained from mouldy hay, but the antigen or antigens may have been those of the microorganisms added rather than new antigens produced in the hay. This third sample did not contain all the antigens found in native mouldy hay.

Antigens of extracts of artificial mouldy straw Extracts of barley straw incubated at 39°C. for eight weeks were found to have antigenic similarity

TABLE III

THE RESULTS OF CHALLENGING STRIPS OF ILEUM OBTAINED FROM A GUINEA-PIG SENSITIZED TO AN EXTRACT OF MH4, WITH EXTRACTS OF CLEAN HAY AND CLEAN HAY INOCULATED WITH THE MICRO-FLORA OF MOULDY HAY

Strip Numerical Order in which Extracts of Antigens were Added to of Each Strip of Ileum, and Result of Test

A	CH	+	CHinc.	+	CHinc.M	+	MH4	+
B	CHinc.	+	CH	-	CHinc.M	+	MH4	+
C	CHinc.M	+	MH4	+				
D	MH4	+	CHinc.M	-				

CH=extract of clean hay. CHinc.=extract of the same clean hay which had been wetted and incubated. CHinc.M=extract of the same clean hay which had been wetted and incubated with moulds from mouldy hay. Other symbols as in Table I

to mouldy hay but not complete identity, as the sensitized ileum was still able to contract weakly on challenge by mouldy hay extract after it had reacted to the mouldy straw antigen complex (Table IV).

TABLE IV

THE RESULTS OF CHALLENGING STRIPS OF ILEUM, OBTAINED FROM A GUINEA-PIG SENSITIZED TO AN EXTRACT OF MH4, WITH EXTRACTS OF BARLEY STRAW AND BARLEY STRAW WETTED AND INCUBATED FOR EIGHT WEEKS

Strip Numerical Order in which Extracts of Antigens were Added to Each Strip of Ileum, and Result of Test

A	MH4	+	BSinc.	-	BS	-
B	BSinc.	+	MH4	±	MH4	-
C	BS	±	BSinc.	±	MH4	±

BS=extract of barley straw. BSinc.=extract of barley straw which had been wetted, compressed and incubated. Other symbols as in Table I

GEL PLATE PRECIPITIN TESTS Sera which produced one or two precipitation lines to extracts of mouldy hay, and which had been obtained from nine rabbits whose sensitization had resulted from long exposure to mouldy foodstuffs, were examined by gel plate precipitin tests to detect the presence of any antibody to the bacteria or fungi.

These sera contained no precipitins to extracts of *A. fumigatus*, *P. notatum*, *C. albicans*, *Cladosporium herbarum*, or *Bacillus subtilis*, and extracts of these organisms failed to neutralize the precipitins to mouldy hay.

Extracts in powdered form of clean hay added to the sera were able partially or completely to remove the second fine line in those sera producing a double band, though they would not form a precipitate band when tested against the sera; they did not affect the stronger line.

Extracts in powdered form of mouldy hay were able to neutralize the sera, which then had no precipitating antibody to extracts of the three different samples of mouldy hay tested.

The sera of the guinea-pigs that had been sensitized to mouldy hay or fungal extracts by injection for the Schultz-Dale tests, which were

obtained at the time that the animals were killed, were also tested in the gel plates. None of these sera had precipitins to mouldy hay or fungi.

IMMUNOELECTROPHORESIS The sera of four rabbits containing a single precipitation line to mouldy hay extract in the gel plate tests were fractionated by electrophoresis in agar gel and then tested against goat anti-rabbit serum and extracts of mouldy hay in alternate troughs. All four sera developed a single precipitation line to mouldy hay antigen, and, by comparison with the goat anti-rabbit serum precipitation pattern, the precipitins were solely in the γ globulin fraction.

DISCUSSION

There is no doubt that there are antigens present in mouldy hay that are not present in clean hay, though extracts of different samples of mouldy hay may not contain identical antigenic components. This difference in antigenic content is demonstrated in this study by the failure of two of the four different samples of mouldy hay completely to desensitize the strips of ileum obtained from guinea-pigs actively sensitized to each of the extracts of mouldy hay and tested by the Schultz-Dale technique. Moreover, extracts prepared at different times from one sample may vary antigenically, or, alternatively, animals may vary in their response to the same complex material. A similar difference in antigenic composition of extracts of mouldy hay was found by Pepys *et al.* (1961) in precipitation tests using sera from patients with farmer's lung.

Despite the variability of the extracts, the precipitins produced by rabbits after long exposure to mouldy foodstuffs reacted to some component in all four samples of mouldy hay tested, and the evidence obtained to date suggests that there may be a common factor in mouldy vegetable material capable of sensitizing man or animals.

Experimental animals sensitized by exposure to mouldy foodstuffs had respiratory distress five and a half to nine hours after inhaling mouldy hay dust but not after inhaling *C. albicans*, *A. fumigatus*, *P. notatum* or forage acari (Parish, 1961). These animals were not desensitized by subcutaneous injection of the fungi or of extracts of clean hay, but for a period of seven days they were desensitized after injection of mouldy hay extract. Further details of these experiments are to be published.

The sera of these sensitized rabbits obtained before challenge had precipitins to mouldy hay,

which could be neutralized by a powdered extract of mouldy hay, and the immunoelectrophoretic pattern of the sera revealed that they were precipitating γ globulin antibodies. The precipitins of these sera were not neutralized by extracts of *A. fumigatus*, *P. notatum*, *C. albicans*, *Cl. herbarum* or *B. subtilis*, but a powdered extract of clean hay was able partially or completely to remove the minor component of those sera able to produce two precipitation bands to mouldy hay extract.

These observations closely resemble those described in the condition of farmer's lung. When subjects with farmer's lung inhale extracts of mouldy hay under experimental conditions, after a delay of some hours most of them experience dyspnoea identical with that found in the acute disease (Williams, 1961). This is not produced by extracts of *Aspergillus*, *Penicillium*, *Mucor*, or *C. albicans*, or by grass pollens.

The sera from patients with farmer's lung contain precipitins to mouldy hay extract (Pepys *et al.*, 1961) and, though some of these sera also contain precipitins to some of the fungi isolated from mouldy hay, inhibition tests with extracts of these fungi could not remove all the components, so that certain mouldy hay antigens remained.

The tests carried out using the Schultz-Dale technique also provided evidence that there were antigens in mouldy hay which were not present in clean hay or in the moulds tested. It is not possible to determine the number of sensitizing antigens in mouldy hay by this technique, as can be counted in the precipitating systems in the gel plate test, but the Schultz-Dale technique has the advantage of being extremely sensitive and capable of detecting the small amounts of antigen that could not be found by precipitation techniques.

Thus it was possible to demonstrate a common antigen or antigens in mouldy hay, mouldy crushed oats, artificially inoculated incubated hay, and incubated wet barley straw nine months old. These antigens did not appear to be present in the clean materials. The experimentally produced mouldy hay was heavily inoculated with a wide range of organisms from mouldy hay, but the barley straw, which produced the best cross-reaction with guinea-pig tissue sensitized to mouldy hay, was only soaked in tap water and compressed before incubation. It is possible that temperatures of 37°C. and 39°C. are insufficiently high to mimic the overheating of wet stored hay.

It still remains to be determined whether the same antigen from mouldy straw or cereals can be extracted in sufficient amount to react with sera from subjects with farmer's lung containing

precipitins to mouldy hay. But, on clinical evidence, the condition of farmer's lung may occur in susceptible subjects after exposure to mouldy grain or straw (Fawcitt, 1938; Törnell, 1946; Fuller, 1953; Dickie and Rankin, 1958).

It still has to be demonstrated whether the precipitating antibodies found in the sera of patients with farmer's lung are responsible for the delayed dyspnoea characteristic of the condition. If precipitating antibody does mediate the respiratory distress, then the antigen of mouldy hay causing the strong precipitation line in gel plates may be responsible for the disease. However, it is also possible that the precipitating antibodies are evidence only of sensitization of a susceptible subject and may be a concomitant process and not the cause of the condition.

The nature of the antigen or antigen complex remains unknown, but it does not appear to be a part of any of the microorganisms tested, and it could be some degradation product of vegetable material.

SUMMARY

Patients affected by the condition known as farmer's lung develop delayed respiratory distress after inhaling mouldy hay dust, and precipitating antibodies to extracts of mouldy hay have been found in their sera. In this study the sera of rabbits, which had produced precipitating antibody to extracts of mouldy hay as a result of long exposure to mouldy vegetable material and which developed delayed respiratory distress following the inhalation of mouldy hay dust, were examined in gel plate precipitin tests. This enabled a comparison to be made with the results reported in similar tests on sera obtained from patients with farmer's lung.

The antibody in the sera of rabbits only formed a precipitate against extracts of mouldy hay. This antibody, which was found to be a γ globulin, could be neutralized by powdered extracts of mouldy hay but not by extracts of *Candida*

albicans, *Aspergillus fumigatus*, *Penicillium notatum*, *Cladosporium herbarum*, or *Bacillus subtilis*. Extracts of clean hay did not neutralize the major precipitating antibody but could partially or completely remove a weaker antibody contained in some sera. These results resemble those reported in investigations of the sera of subjects with farmer's lung.

The Schultz-Dale technique was used to test extracts of vegetable material for similarity of antigen content. Some cross-reaction was found between antigens of clean hay, straw, and cereals, and those contained in extracts of mouldy hay. Mouldy hay was found to contain antigens not present in extracts of other vegetable material tested. Greater similarity, but not complete identity, was found between the antigens of mouldy oats and experimentally produced mouldy hay and straw and extracts of native mouldy hay.

The antigen or antigens responsible for the sensitization of man or animals have not been identified.

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