

Extrinsic allergic alveolitis due to rat serum proteins

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Summary

In a research assistant with recurrent episodes of extrinsic allergic alveolitis on exposure to rats, typical systemic and pulmonary reactions on inhalation and positive reaction on prick testing were elicited only by tests with rat serum; precipitins were present against rat serum and rat pelt, but not rat fur, and were also present against rat urine, which may contain large amounts of serum protein and which may have been a main source of antigenic exposure.

Introduction

The production of extrinsic allergic alveolitis by the inhalation of a wide range of inhaled organic dusts is well established. These include thermophilic actinomycetes and fungal spores, and with particular relevance to the present report, heterologous serum proteins, of avian origin causing bird fancier's lung and of bovine and porcine origin, causing pituitary snuff taker's lung (Pepys, 1969). All manner of such dusts have to be regarded as potential causes and the present report describes a case due to the inhalation of rat serum proteins in a laboratory worker working with rats.

Case history

A 23-year-old female research assistant developed episodes of breathlessness, fever, weight loss, persistent cough and malaise in November 1972 after working intensively with rats. She had worked with them in the preceding year as a biology student. Her symptoms improved without treatment when off work. From November 1972 to April 1973 there were ten further such episodes, and those requiring hospital admission are outlined together with the relevant investigations in Table 1.

The symptoms usually appeared about 6–8 hr after arrival at work and became increasingly severe after exposure on successive days. At the time of attacks basal crepitations and rales in both lung fields were heard. Improvement always followed when she was away from her working environment. The relationship of the appearance

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Table 1. Chronological record of patient's symptoms and relevant investigations

Date	Symptoms	Radiology	Clinical findings	Pulmonary function	Laboratory findings	Exposure	Illustration
30.11.72	Breathlessness, fever, cough, weight loss 6.25 kg				Positive precipitin test to rat serum proteins	Operating on and caring for rats for the previous 2 months	
11.12.72	Improvement in fever. Still cough and breathlessness	Bilateral micronodular shadowing				Stopped work	Fig. 1
22.12.72	Marked improvement in breathlessness and cough. No fever. Some weight gain	Marked clearing in radiographic shadowing				Stopped work	Fig. 2
27.2.73	Recurrence of breathlessness, fever and cough	Return of radiographic bilateral mottling	Basal crepitant rales	FEV ₁ 2200 ml FVC 2650 ml DL _{co} 17.5 ml CO/min/mmHg	Hb 13.1 g% WBC 10,000 mm ³ No eosinophilia	Return to work and caring for rats again	Fig. 3
4.3.73	Improvement in symptoms, no cough, weight increasing	No change	No auscultatory findings	FEV ₁ 2200 ml FVC 2600 ml DL _{co} 18 CO/min/mmHg	Hb 13.0 g% WBC 6000 mm ³ No eosinophilia	Stopped work	
30.4.73	Recurrence of symptoms after 3 days work between 24.4.73-27.4.73	Definite worsening in radiographic shadowing	Basal crepitant rales	FEV ₁ 3300 ml FVC 4000 ml DL _{co} 20.0 CO/min/mmHg	Hb 11.9 g% WBC 11,000 mm ³ ESR 20 mm/hr	Return to work. Same job.	Fig. 4

Table 1. (cont.)

3.5.73	Improvement in symptoms although fever still persists	No change	No auscultatory findings	No change	Hb 12.3 g% WBC 8100 mm ³ ESR 29 mm/hr	Stopped work
8.5.73	Further improvement in symptoms. No fever	No change	No change	DL _{co} 17.5 CO/min/mmHg		Stopped work
9.5.73 15.5.73	Hospital—Investigations with bronchial aerosol challenge					
5.6.73	No symptoms		Nil	DL _{co} 21.6		No further work exposure
25.6.73	No symptoms		Nil	DL _{co} 20.9		
2.7.73	Slight temperature for 3 days, no cough no breathlessness	No change	Nil	DL _{co} 20.2	Hb 12.3 g% WBC 9600 mm ³ ESR 37 mm/hr	Inadvertent exposure to rats for 8 hr
29.11.73	No further symptoms, regained all weight loss, no cough, no breathlessness	Definite improvement in radiographic shadowing	Nil	DL _{co} 28.2	Precipitins to rat serum still present	No further exposure Fig. 5

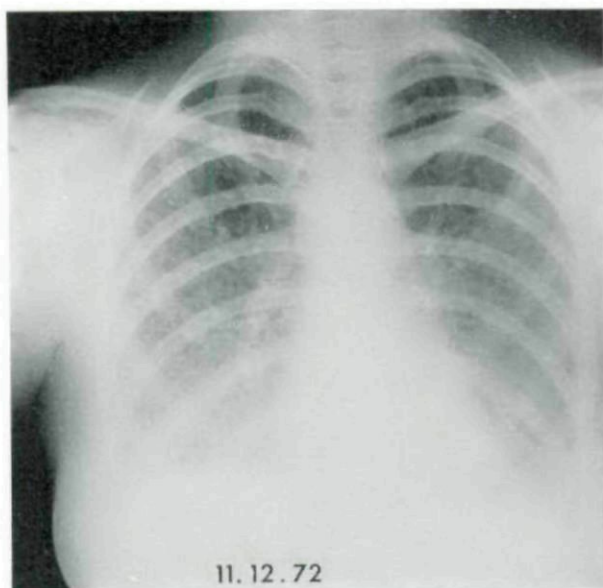


Fig. 1. (11.12.72) Bilateral micronodular shadowing during acute episode.

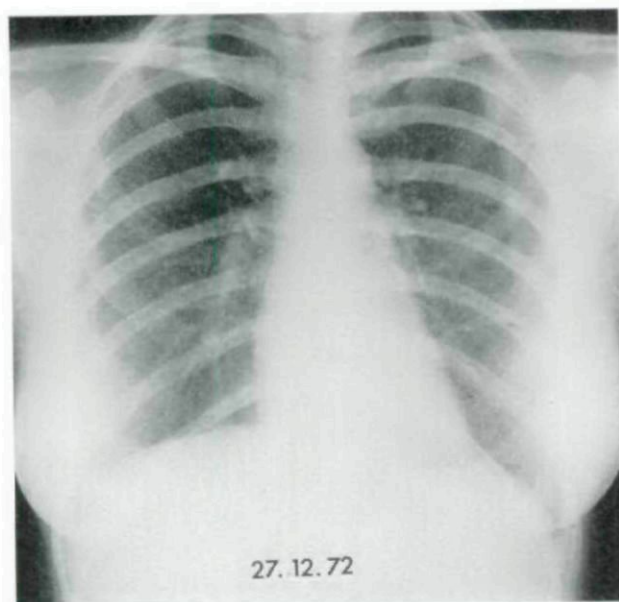


Fig. 2. (27.12.72) Marked clearing within 11 days after cessation of exposure to rats.

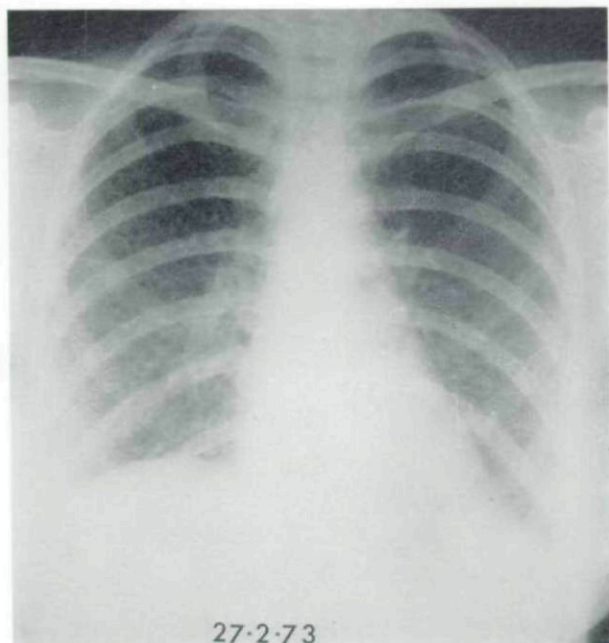


Fig. 3. (27.2.73) Recurrence of bilateral mottled shadows on return to work and re-exposure to rats.

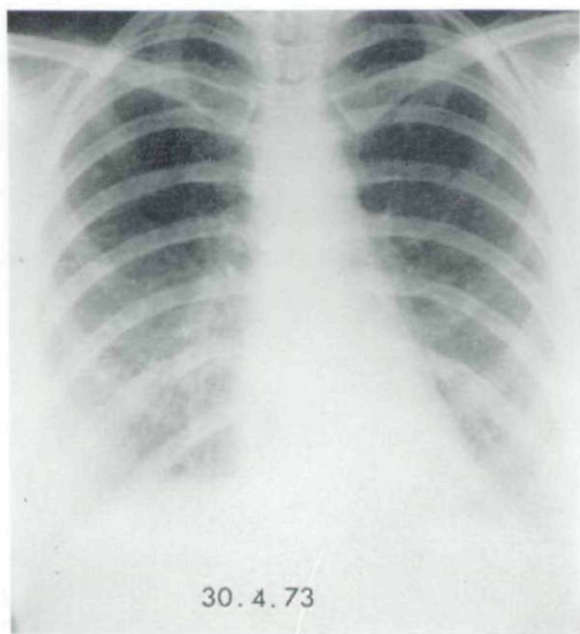


Fig. 4. (30.4.73) Further recurrence on re-exposure to rats, after a period of improvement on cessation of work.

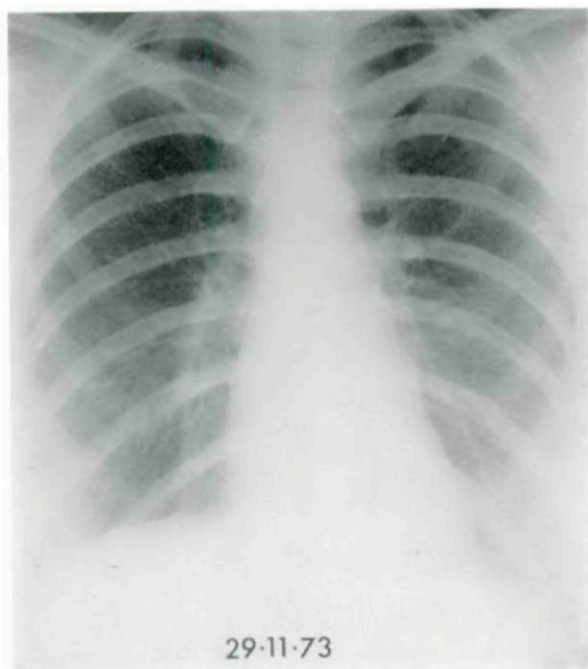


Fig. 5. (29.11.73) Improvement in radiographic appearances after cessation of exposure.

of micronodular shadowing on chest radiographs to work exposure is shown in Table 1 (Figs 1–4). There was no hilar node enlargement at any time.

Occupational history

During the course of the day the patient had to handle, anaesthetize and shave rats. In addition the cages were carefully cleaned and various antibiotic powders were scattered on the floors to avoid wound infections. The clipping process often caused animals to bleed and the mixture of dried blood-stained fur was swept away, giving rise to dust in the air. This was repeated daily. Rat cake was the main diet. Large elderly rats, which were used in the experiments, are known to lose large amounts of serum proteins in their urine (Table 2).

Investigations

Skin tests

Prick tests were made with the following routine common allergen extracts (Beechams): *Alternaria*, *Aspergillus fumigatus* and *A. terreus*, *Cladosporium herbarum*, dry rot, *Sporobolomyces roseus*, yeast, *Candida albicans*, grass, shrub and tree pollens, milk, egg, wheat, fish, mixed nuts, cat, dog, feathers, horse, house dust, *Dermatophagoides farinae* and *D. pteronyssinus* and nettle pollen. Prick tests were also made with carbol-saline extracts of *Sitophilus granarius*, wheat, barley and maize and a range of dilutions of rat serum. A Kveim test with K12 lot 5 was made.

Serology

Agar gel double diffusion tests were performed for precipitins to freeze dried wheat

extract (60 mg/ml), barley extract (60 mg/ml), maize extract (60 mg/ml), extracts of *Sitophilus granarius* (1 mg/ml, 10 mg/ml), and rat serum (Ouchterlony, 1953). Rat serum, rat urine and rat fur extract (5% w/v) were examined by immunoelectrophoresis for their reactions with the patient's serum to rabbit antiserum against rat-serum proteins (Grabar, 1960).

Inhalation tests

These were made (1) using aerosols of the suspected allergens and (2) by simulated occupational type exposure. Aerosols of the allergens were produced by passing oxygen at 8 l/min through a Wright's nebulizer, and the patient inhaled them from a re-breathing bag, the same apparatus being used for each test. The concentration of extract tested was determined by prick testing, the initial aerosol test being made with the concentration giving a weal not larger than 3 mm diam. A control inhalation test was made with Coca's fluid in the first instance. No more than one test was performed on any one day and usually only three tests in 1 week. The following extracts were used: freeze dried extract of weevil infested grain 1 mg/ml and 10 mg/ml; rat fur extract 5% w/v; sterilized rat serum 1/100 and 1/10 dilution. The duration of exposure was from 2 to 30 min (Table 3).

Occupational type exposure was made in an enclosed chamber for periods shown in Table 3. Two tests were performed, one where the patient clipped the hair of six Portland Wistar rats with which she had worked previously and another where she was exposed to the dust cloud from a mixture of the ampicillin powder plus lactose (Pepys, Pickering & Loudon, 1972; Davies, Hendrick & Pepys, 1974).

Pulmonary function tests

Respiratory function tests were measured by FEV₁, FVC and VC using a wet spirometer and Vitalograph. Total lung volumes were measured using a whole body phethysmograph. The carbon monoxide gas transfer (DL_{CO}) was measured using the single breath and steady state technique.

Blood tests

Measurements were made of (a) haemoglobin, (b) total white blood cell counts, (c) wet eosinophil counts on repeated occasions. In addition, tests for viral antibody and for Brucella agglutinins were made.

Other tests

Serum proteins and estimation of α , β and γ -globulin by paper electrophoresis, and measurement of individual globulins. Serial, frequent, temperature recordings were made.

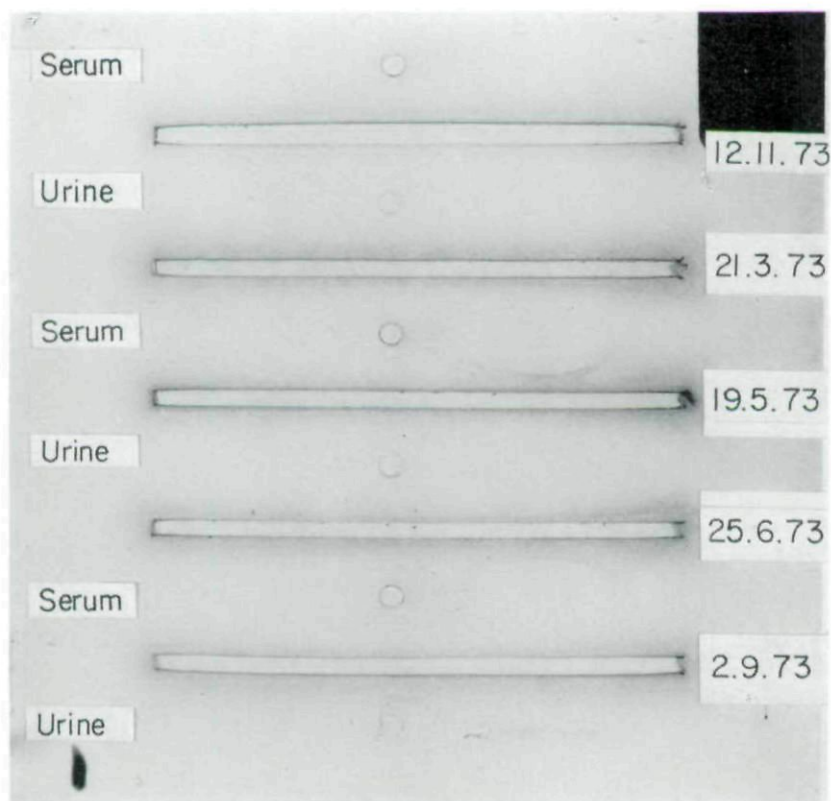
Results

Skin tests

Tests with the common allergens gave only a weakly positive reaction to the grass pollen extract. No reactions were given to the other extracts except for undiluted rat serum which gave a 6 mm weal. The Kveim test produced an acute papular reaction which showed non-specific acute inflammation histologically.

Table 3. Aerosol provocation inhalation tests

Text extract	Concentration	Duration of exposure	Result
Control Coca's solution		30 min	Negative
Sitophilus granarius	1 mg/ml	10 min	Negative
Sitophilus granarius	10 mg/ml	10 min	Negative
Weevil infested grain	1 mg/ml	10 min	Negative
Weevil infested grain	10 mg/ml	10 min	Negative
Clipping rat fur		30 min	Negative
Rat fur extract	5% w/v	10 min	Negative
Rat fur extract	5% w/v	30 min	Negative
Ampicillin powder	10:250 g lactose	30 min	Negative
Rat serum	1/10 dilution	10 min	Negative
Rat serum	1/10 dilution	30 min	Positive

**Fig. 6.** Immunoelectrophoretic test with patient's serum against rat serum and rat urine. Dates refer to successive blood specimens. The first specimen was taken at approximately the same time as Fig. 1.

Serological tests

Agar gel double diffusion tests. The freeze dried extracts of wheat, barley, maize and *Sitophilus granarius* gave no reaction. A single precipitin line developed with indiluted rat serum.

Immunoelectrophoretic tests. Electrophoresis of rat serum, rat urine and rat fur followed by tests against the patient's serum are shown in Fig. 6. Precipitin lines are present in the tests with both rat serum proteins and the proteins in rat urine. No precipitins were demonstrable to rat fur. Figure 7 shows rat serum, rat fur and rat pelt electrophoresed and tested against the rabbit antiserum to rat serum proteins. Precipitins are present in the tests with the rat serum and pelt extracts but not the rat fur extracts.

Provocation tests

Clinical exposure. (a) Clinical exposure of the patient to her working environment consistently produced worsening of her symptoms. Following a 3-day exposure at work, serial measurements of her DL_{CO} (Fig. 8) show the fall in DL_{CO} followed by a gradual return towards predicted levels over a period of 7 months. Following removal from her working environment a gradual return towards normality is seen. The change

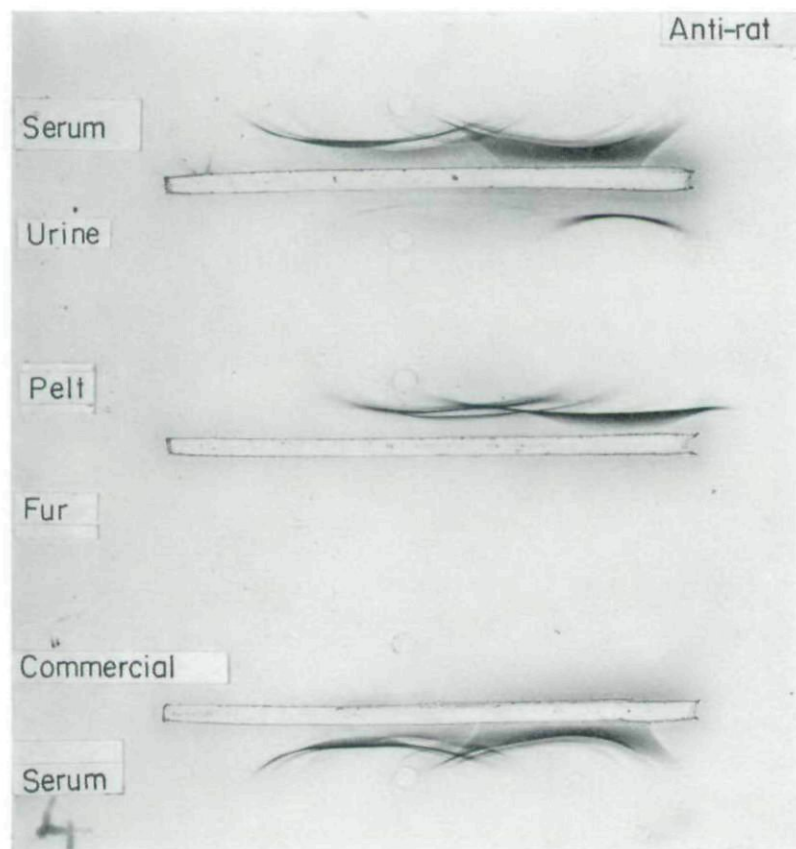


Fig. 7. Immunoelectrophoretic test with rabbit antirat serum protein antisera against (1) rat serum, (2) rat urine, (3) rat pelt and (4) rat fur. Rat fur does not show any precipitin lines.

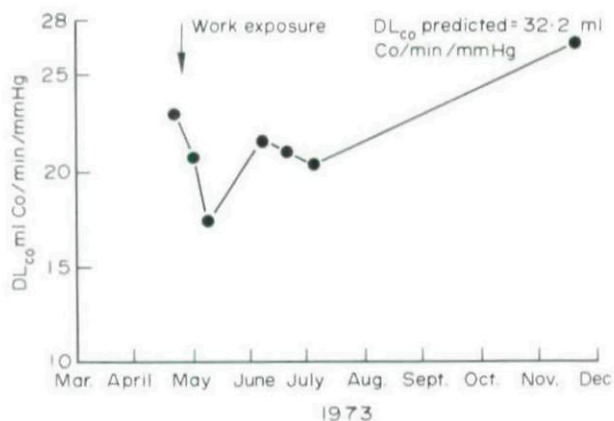


Fig. 8. Serial measurements of DL_{CO} (gas transfer, single breath) following a 3-day work exposure (arrowed).

in overall pulmonary function analysis (Table 4) shows improvement as well in FEV_1 , and FVC. (b) Table 3 shows the results of both the aerosol and occupational type inhalation tests. The positive reaction to the rat serum inhalation test made over a period of 30 min consisted of, malaise and aches, a temperature rise of $1.5^{\circ}C$, and a fall in DL_{CO} from 16.6 to 13.0 ml CO/min/mmHg, maximal at 6 hr and improving to 14.4 ml CO/min/mmHg in the following 12 hr. The total white blood cell count rose from 8100 cc/mm to 20,000 cc/mm at 6 hr and returned to 9200 cc/mm at 24 hr (Fig. 9).

Other investigations

Serum proteins—albumin 4.1 gm%, globulin 3.5 gm%. Individual immunoglobulins—IgA 341 mg%, IgG 1455 mg%, IgM 235 mg% performed in March 1973. Repeat values in September 1973 showed IgA 341 mg%, IgG 1500 mg%, IgM 244 mg%. Viral antibody tests on two occasions for influenzae A, B, C, adenovirus, respiratory syncytial virus, *R. burnetti*, psittacosis virus, *Mycoplasma pneumoniae* were all negative. Brucella agglutinin tests were negative on two separate occasions.

Table 4. Pulmonary function tests following a 3-day work exposure with comparable figures 6 months after cessation of exposure

	29.4.73	27.11.73	Predicted
FEV_1	2750	3080	3810
FVC	3025	3460	4380
$FEV_1/FVC\%$	91.0	89.0	87.0
VC	3100	2550	4380
FRC	2810	3412	3370
TLC	4910	5187	6180
$RV/TLC\%$	37.7	50.8	29.2
DL_{CO}	20.7	28.2	32.4
(gas transfer, single breath)			

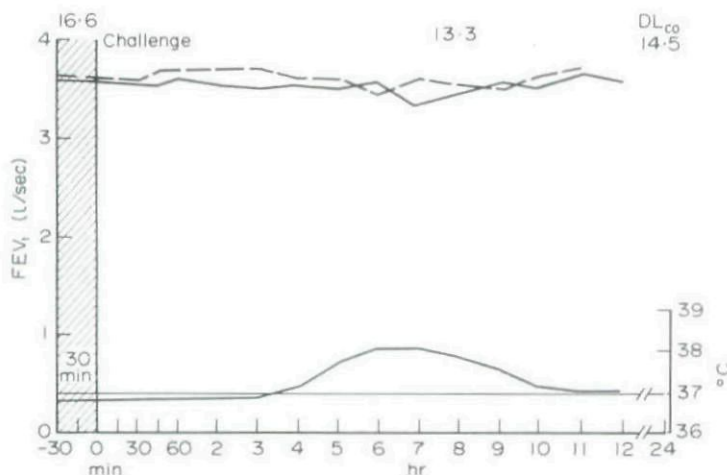


Fig. 9. Serial measurements of DL_{CO} (gas transfer, single breath), FEV_1 and temperature following 30 min exposure to extract of control Coca's solution (---) and 1/10 dilution of rat serum (—).

Discussion

The characteristic history of repeated episodes of cough, dyspnoea, malaise, weight loss associated with the radiographic and physiological findings are consistent with extrinsic allergic alveolitis. Extrinsic allergic alveolitis occurs mainly in non-atopics probably as a result of prolonged antigenic exposure, resulting in many instances in the appearance of precipitins. The precipitins are evidence of exposure and not necessarily diagnostic of disease. It is also not always possible to demonstrate precipitating antibody or to obtain positive skin tests which might serve as evidence of exposure. Under these circumstances or where there are doubts as to the relevance of the exposure provocation tests make possible the specific reproduction of the patient's symptoms under controlled hospital circumstances. In this case the patient's symptoms could only be reproduced by inhalation of the rat serum proteins. Evidence in support of this as the causal agent is given by the positive skin test reaction, the presence of specific precipitins against the rat serum albumen, improvement in her clinical condition on cessation of exposure and recurrence of symptoms on deliberate or inadvertent exposure to rats or mice.

The immunopathogenesis of the respiratory allergic disease in this case, is comparable to that of bird fancier's lung and pituitary snuff-taker's lung in which heterologous serum proteins are the cause of their disease and shows that their extrinsic alveolitis is a form of serum sickness, distinguished from the systemic disease in that the antigen enters the body through the respiratory tract where the reactions take place.

The possibility that the rat serum proteins may have become airborne from dried urine, in addition to the dander and hair particles, is important as a source of potent antigens in laboratory workers and merits examination in subjects allergic to rats and perhaps to other experimental animal species as well.

References

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