

Allergic respiratory reactions in bird fanciers provoked by allergen inhalation provocation tests

Relation to clinical features and allergic mechanisms

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Allergen inhalation tests were used to investigate respiratory allergy to birds in 81 persons. Allergic respiratory reactions were provoked in 36 bird fanciers with respiratory disease. Immediate reactions occurred in 9 patients and late reactions, usually commencing between 4 and 6 hours, in 31 patients. Four patients had dual, immediate followed by late, reactions. While the immediate reactions caused asthma, the late reactions had a variety of features that included pyrexia, polymorphonuclear leukocytosis, asthma, and features of a reaction in the peripheral respiratory tissues. The immediate reactions were associated with immediate skin reactions, while the late reactions were associated with late skin reactions and precipitating antibodies. The features of the immediate and late reactions corresponded to those expected of types I and III allergy, respectively. Immediate asthmatic reactions, occurring alone or as part of a dual reaction, occurred in patients with a history of attacks of wheezing consistent with asthma. Late asthmatic reactions were often unassociated with wheezing or rapid variability, and a history of asthma was difficult to obtain; these patients, however, had airways obstruction with or without extrinsic allergic alveolitis, and the airways obstruction was usually persistent, suggesting irreversible damage to airways. Late pyrexial and peripheral respiratory reactions occurred in patients with extrinsic allergic alveolitis who presented with similar clinical manifestations. The features of the respiratory reactions provoked by inhalation tests appear to reflect the different clinical manifestations of allergic respiratory disease.

Allergen inhalation tests are an important method for the study of allergic respiratory disease. They consist of the inhalation of an aerosol of the allergen extract under controlled conditions and of observing the response. The reactions can be divided into immediate, late, or dual in terms of their speed of appearance, and into bronchial or peripheral respiratory (farmer's lung type, extrinsic allergic alveolitis) reactions in terms of the site of reaction.

Immediate reactions have been observed most frequently and have received most study.¹⁻¹⁵ They are of rapid onset and begin within 10 minutes, reach a peak by 15 to 30 minutes, and resolve spontaneously within one to 3 hours. They occur in the airways and cause asthma without systemic features such as fever and leukocytosis. They may provoke a blood eosinophilia that is maximal at about 24 hours. They can be inhibited or reversed by beta receptor stimulants and inhibited by disodium cromoglycate given before the allergen challenge.^{15, 16}

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Antihistamines and corticosteroids¹⁶ have little or no effect. They are usually associated with immediate skin sensitivity and are attributed to a type I¹⁷ allergic reaction.

Late reactions begin between 3 and 13 hours, and usually between 4 and 6 hours, after allergen inhalation. They progress to a maximum more slowly, within one or over several hours, and are more prolonged, clearing usually within 24 to 48 hours, but they may last for several days. Their features include febrile attacks with polymorphonuclear leukocytosis, asthma, and features of peripheral respiratory reaction. These features tend to occur in one of two patterns, either as a pyrexial and peripheral respiratory reaction or as an asthmatic reaction in which the systemic features are less predictable.

Pyrexial and peripheral respiratory reactions appearing late were first described in patients with farmer's lung by Williams,¹⁸ and they were subsequently reported by others in patients with farmer's lung,¹⁹ bagassosis,^{20, 21} malt worker's lung,^{22, 23} bird fancier's lung,^{24, 25} mushroom worker's lung,²⁶ and fish meal worker's lung.²⁷ The features of these reactions are described in detail in the results of this study. They are inhibited by corticosteroids¹⁸ and, perhaps, by disodium cromoglycate.¹⁵ They are associated with precipitating antibodies, and in malt worker's lung and bird fancier's lung with late skin reactions, and they are thought to be due to type III¹⁷ allergy.

Asthmatic reactions appearing late may occur alone or, more frequently, as part of a dual reaction in which the late asthmatic reaction is preceded by an immediate asthmatic reaction. Carefully controlled studies have shown that the late asthmatic reaction is specific and is similar whether it is preceded by an immediate reaction or not. In dual reactions the immediate reaction resolves spontaneously before the onset of the late asthma, which tends to be as severe as or more severe than the immediate reaction. These late asthmatic reactions may be frequent because they have been observed following inhalation of a variety of particles, including house dust,^{9, 10, 16, 28-31} grass⁷ and ragweed^{14, 32, 33} pollen, *Aspergillus fumigatus*^{18, 34} and *A. clavatus*,³⁴ *Bacillus subtilis* enzymes,³⁵ cotton dust,³⁶ wood dusts,³⁷ including western red cedar and plicatic acid,³⁸ birds,¹⁵ toluene diisocyanate (Hargreave, unpublished), and aminoethylethanolamine.³⁹ In addition, they have been reported following positive tests with house dust extract in 26 per cent of patients,¹⁰ with grass pollen extract in 28 per cent,⁷ and with *A. fumigatus* extracts in 51 and 71 per cent of patients.^{18, 40} They may be inhibited by disodium cromoglycate and corticosteroids administered before the inhalation challenge^{15, 16} and may be reversed by beta receptor-stimulating drugs. Their mechanism is poorly understood. While in allergic bronchopulmonary aspergillosis and in the present study of bird fanciers, they can be closely related to dual skin reactions and precipitating antibodies, such a close correlation has not been found with the other inhaled particles just mentioned.

The present study was conducted in persons exposed to birds when it became clear that they could develop both extrinsic asthma and extrinsic allergic alveolitis and different reactions following inhalation challenge. It illustrates how the features of the reactions provoked by challenge are similar to the features of disease and adds evidence that the late reactions involve the participation of precipitating antibodies.

TABLE I. Patients exposed to birds and respiratory site affected by disease

Patient, exposure, and disease data	Respiratory allergy to birds	
	Present	Absent
Patient data		
Number of patients	39	42
Sex		
Male	20	24
Female	19	18
Age range, yr. (mean)	12-69 (44)	15-67 (46)
Exposure data		
Bird exposure		
Pigeon	14	7
Budgerigar	21	25
Other	4*	2
Number of birds		
1 or 2	22	22
5-14	3	4
20-100	14	8
Duration of exposure, yr. (mean)	0.58-48 (14)	1-50 (16)
Disease data		
Disease affecting airways	10	11
Disease affecting respiratory tissues	29	21
No respiratory disease	0	10

*Parrot in 2 patients, finches in one, and other different species in one patient.

MATERIALS

Patients

Eighty-one persons were investigated by inhalation tests with bird allergen extracts between 1965 and 1968. Following investigation they were divided into 2 groups, one of 39 patients with evidence to suggest respiratory allergy to birds, the other of 42 persons without this evidence. Allergy to birds was suggested by reactions provoked by inhalation tests with bird allergens, by a history of respiratory reactions following exposure, or by improvement when exposure to birds was discontinued. In the 39 patients with allergy to birds, inhalation tests provoked reactions in 36; in 34 of these avoidance of exposure was followed by clinical improvement. In the remaining 3 patients with negative inhalation tests, one had a history of respiratory reactions following exposure, and this one and another improved when exposure was discontinued. The third had clinical features of extrinsic allergic alveolitis but in retrospect may not have been allergic to birds.

Table I gives the sex, age, bird exposure, and respiratory disease of the 2 groups of patients. The respiratory disease was considered to affect the airways if there was a history of paroxysmal wheezing consistent with asthma or airways obstruction indicated by a reduced one-second forced expiratory volume (FEV_1)/vital capacity (VC). It was considered to affect the peripheral respiratory (alveolar) tissues if there was a history of febrile attacks with respiratory symptoms several hours after exposure consistent with the "acute" presentation of allergic alveolitis or if the chest radiograph showed nodular shadows, or if the lungs were small as indicated by total lung capacity (TLC) of less than 80 per cent of predicted normal, or if the CO transfer factor (TLCO) was reduced to less than 80 per cent of predicted normal in the absence of airways obstruction.

In the 39 patients with respiratory allergy to birds, all were exposed to birds and were investigated because they presented with respiratory symptoms. Ten had disease affecting the airways; while 29 had disease affecting the respiratory tissues, 6 of them also had airways obstruction. Their clinical features are presented in detail elsewhere.^{41, 42} Histopathology was available in one with airways disease and in 7 with peripheral respiratory disease.

In the 42 patients without allergy to birds all except 8 were exposed to birds. Four of these 8 and 6 of the others had no respiratory disease. Eleven had disease affecting the

airways only (extrinsic asthma, 5, intrinsic asthma, 2, chronic bronchitis, 1, acute bronchitis, 1, and chronic airways obstruction, 2). Twenty-one had disease affecting the respiratory tissues (panacinar emphysema, 1, mycoplasma pneumonia, 1, lymphangitis carcinomatosa, 1, extrinsic allergic alveolitis of other etiology, 3, cryptogenic fibrosing alveolitis, 9, eosinophilic granuloma, 1, sarcoidosis, 4, and Caplan's syndrome, 1).

Bird extracts

The chief materials used for allergen inhalation, skin, and precipitin tests were the serum and extracts of the skin and droppings of budgerigars and pigeons. The extracts were prepared in carbol-saline (Coca's) after defatting with ether and were then Seitz filtered and freeze-dried. Both sera and extracts were sterilized by heating at 56° C. for 20 minutes and filtered through a membrane. Other materials used in appropriate cases were the sera of hens, parrots, and canaries and extracts of the droppings of parrots. Egg and feather extracts were discarded early on because they gave only weak precipitin and skin reactions.

METHODS

Inhalation tests

These were conducted in hospital so that the patient was in a known environment, physically and mentally at rest, and so that frequent observations could be carried out over 24 hours. Eggs and egg products were omitted from the diet in case these provoked respiratory symptoms, and no smoking was permitted. Drugs that might interfere with the reactions, for example, antihistamines, bronchodilators, and corticosteroids, were not administered if the patient was well enough. However, 3 patients with asthma required bronchodilators, and one of these 3 and 4 others with allergic alveolitis were being treated with prednisone.

The tests were begun between 9 A.M. and 10 A.M., and only one test was performed daily. The patient's symptoms and physical signs, including the oral temperature, were noted. Blood was drawn for the white blood count (WBC) and absolute eosinophil count. The FEV₁ and VC were repeated carefully 3 times, and the variation in FEV₁ did not exceed 0.2 L. The patient was told that these depended on effort and cooperation and had been taught and was familiar with the procedure. In some patients the peak expiratory flow (PEF), specific conductance (SGaw), and TLco were measured.

The aerosol inhalation was then carried out. Administration was based on an open technique used by Citron and associates⁷ in asthma and by Williams¹⁸ in farmer's lung. The aerosols were produced by a Wright nebulizer,* which is designed to produce droplets of 5 μ in diameter, and none greater than 8 μ in diameter. It was passed through tubing into a rebreathing bag connected to a face mask, and the patient was instructed to breathe normally through the mouth. The oxygen flow (8 L. per minute), the length (6 inches), and bore (5 mm.) of the tubing, and size of the rebreathing bag (1 L.) were kept constant. The aerosol was administered in divided doses for up to a total of 5 minutes in the first instance. It was given first for one minute; if no symptoms were noted after 10 minutes, for a further 2 minutes, and if no symptoms were noted after 10 minutes, for another 2 minutes. Immediate allergic reactions began within 10 minutes, so that by using these intervals the inhalation test was discontinued if a reaction occurred, and in this way vigorous reactions were avoided.

The FEV₁ and VC were measured if symptoms developed or at 10 minutes after the final dose. They were repeated at 10 minute intervals for the first hour and one or 2 hour intervals up to 12 hours after the tests and again at 24 hours. They were carried out only once if they were within the pretest range, but if lower were repeated once or twice. In some patients measurement of PEF, SGaw, and TLco was performed between 6 and 14 hours and at 24 hours after the tests. The oral temperature was taken every one or 2 hours, and the WBC and absolute eosinophil count were repeated at 8 and 24 hours. If a reaction had not reversed at 24 hours, observation was continued until it had.

The patient was not told the nature of the aerosols so as to minimize any functional

*Aerosol Products Ltd., Colchester, England.

subjective response. On the first day the control solution, carbol-saline, was nebulized to exclude any nonspecific response and to obtain diurnal variation of observations. If the FEV₁ varied by more than 0.4 L. above or below the initial values, an oral bronchodilator was given every 6 or 8 hours to try and reduce the variability, and the control test was repeated.

Antigen extracts were administered on subsequent days. The serum of the appropriate bird was usually used initially, and the initial strength was determined by prior prick tests. It was found that severe immediate reactions could be avoided if the first solution used for inhalation was that which caused an immediate skin wheal of not more than 2 or 3 mm. If the skin tests were negative, then the starting dilution was 1/100. If the test caused no reaction, the strength of the extract was increased 10 times and the test repeated the next day. The maximum dose employed was serum diluted 1/10 and inhaled for 10 minutes. If this was negative, tests were carried out in a similar way with extracts of the appropriate bird droppings up to a strength of 10 mg. per milliliter inhaled for 20 minutes.

Reactions were interpreted as "immediate" if they developed within 10 minutes and as "late" if they developed after 3 hours. The following changes were regarded as significant: a fall in the FEV₁ or VC of 0.2 L. and of more than 10 per cent below values recorded in the control tests, a temperature increase to above 37° C., a rise in the WBC of more than 50 per cent or to above 11,000 per cubic millimeter, a change in the differential WBC of more than 10 per cent, a rise in the absolute eosinophil count to above 400 per cubic millimeter, and the appearance of crepitant rales if they had been absent before the test. Goldman and Becklake⁴³ have showed that in cooperative subjects an acute change in FEV₁ of 5 per cent can be statistically significant. The immediate reactions in this study were usually reversed by isoproterenol inhalation (0.14 mg.) because the possibility of a further late reaction in such cases was not recognized at the time. In the late reactions changes in ventilatory capacity were interpreted as "in the airway" if the falls in FEV₁ and VC could be reversed by isoproterenol inhalation (0.14 mg.) or by epinephrine subcutaneously (0.5 ml. of 1/1,000 solution), or as "in the peripheral respiratory tissues" if falls in the FEV₁ and VC were associated with no change in PEF or SGaw and could not be reversed by isoproterenol and epinephrine. As a precaution against troublesome immediate reactions, epinephrine 1/1,000 was always drawn up ready for use before any aerosol inhalation.

Tests of pulmonary physiology

FEV₁ and VC were determined with a low-inertia water-sealed spirometer of the type described by Bernstein.⁴⁴

The peak expiratory flow (PEF) was estimated by a Wright peak flowmeter.* The inspiratory airway resistance was measured using a whole body plethysmograph fitted with pneumotachygraph, mouth and box pressure transducers, and a mouth shutter operated by remote control.^{45, 46} The airways resistance was related to lung volume at which it was measured and expressed as the reciprocal of the resistance per liter of thoracic gas volume, which is the specific conductance (SGaw).

Lung volumes were measured by the closed-circuit helium dilution method modified from the method of McMichael.⁴⁷ Normal values were taken from a summary of existing data related to sex, age, and height of the patient prepared by Goldman and Becklake.⁴³ The transfer factor for carbon monoxide (TLCo) was measured by the steady state method with end tidal sampling at rest.^{48, 49} Predicted values were taken from Bates, Woolf, and Paul.⁵⁰

Skin tests

These were carried out by the modified prick and intracutaneous methods. In prick tests the extracts used were chiefly the appropriate bird serum and droppings extract (10 mg. per milliliter). The tests were read at between 10 and 20 minutes for immediate reactions. Intracutaneous tests were carried out by the injection of about 0.02 ml. of the extract. The appropriate concentration of the extract was determined by prior prick tests. If prick tests produced

*Airmed Ltd., Harlow, England.

TABLE II. Inhalation tests: Allergen and dose used to provoke reactions

Bird allergen			Reactions		
Type	Strength	Time (min.)	Immediate	Dual	Late
Serum	1:1,000	3		1	
		5	1		
	1:100	2	1		1
		5			11
	1:10	1		1	
		2	1		1
		3		1	
		5			8
		8			1
		10			2
Skin extract	1 mg./ml.	11	1		
Droppings extract	10 mg./ml.	3		1	
		10			2*
	15 mg./ml.	9	1*		
		20			1†

*Did not react to bird serum.

†Bird serum was not available to test this patient.

no wheal, undiluted bird serum and bird droppings extract (10 mg. per milliliter) were used, but if a wheal occurred, concentrations giving small wheals, up to 3 to 4 mm. in diameter, were used for the intracutaneous tests. The intracutaneous tests were carried out to detect late reactions. The tests were examined at between 10 and 20 minutes for an immediate reaction, at about 6 hours for a late reaction, and later at 24, 48, and 72 hours for any suggestion of a delayed reaction. Interpretation of immediate reactions to intracutaneous tests may be difficult because of the whealing effect of some of the test materials in nonexposed subjects. For the present purposes, wheals 6 mm. or larger were taken as suggestive of an immediate reaction.

Serologic tests for precipitins

Precipitin tests were performed by agar gel double diffusion^{51, 52} and immunoelectrophoresis techniques.⁵³ The antigen materials used were pigeon and budgerigar serum, skin and droppings extracts (30 mg. per milliliter), and, in 2 patients exposed to parrot, parrot serum and droppings extract (30 mg. per milliliter). If the tests were negative or if precipitin arcs were demonstrated to the droppings extract but not to the bird serum or skin extract, the patient's serum was concentrated 2.5 times and the test was repeated. In those patients whose serum did not react to the bird serum proteins but only to the bird droppings extract, the reaction will be called the "droppings only reaction."

RESULTS

Allergen inhalation, provocation, tests

These provoked reactions in 36 patients. The reactions were immediate only in 5 patients, late only in 27, and dual, immediate, and late, in 4. Table II shows the type and dose of bird extract used to provoke these reactions.

Immediate reactions

Fig. 1 illustrates a typical immediate asthmatic reaction like that observed in 9 patients. Falls in the FEV₁ of 0.45 to 1.25 L. (14 to 68 per cent) were

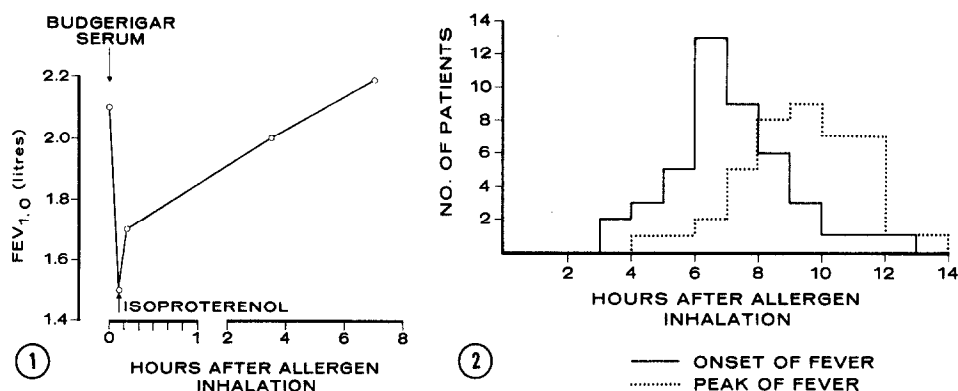


FIG. 1. Allergen inhalation test. Immediate asthmatic reaction.

FIG. 2. Allergen inhalation test. Late reactions; frequency of time of onset and time of peak of fever.

TABLE III. Inhalation tests with bird allergens: Features of late reactions in 31 patients

Features	Number of patients	Per cent
<i>Systemic</i>		
Fever	29	94
Systemic symptoms	25	81
Leukocytosis	22	71
<i>Respiratory</i>		
No respiratory features	8	26
Respiratory symptoms or signs only	9	29
Asthmatic reaction	8	26
Peripheral respiratory reaction	6	19

recorded. A blood eosinophilia occurred, usually at 24 hours, in 5 of 8 patients tested. In 4 of the 9 patients, the immediate reaction was followed by a late reaction and was therefore part of a dual response.

Late reactions

Table III shows the features of the late reactions. Fever tended to be the first sign except in those patients with late asthma. Its time of onset and peak are shown in Fig. 2. It usually cleared within 24 hours, but in 5 patients it persisted for between 30 and 72 hours. The highest temperature ranged from 37.1° C. to 39.9° C. Fig. 3 shows a typical febrile response.

Systemic symptoms usually accompanied the fever. They consisted of chills, shivering, feeling hot or sweating (22 patients), malaise (16 patients), anorexia (13 patients), nausea (5 patients), vomiting (1 patient), headache (10 patients), and body aches (2 patients). The febrile symptoms tended to precede or occur at the onset of fever, and the vomiting and body aches occurred with the more vigorous reactions.

Leukocytosis was observed usually in association with febrile late reactions, and it occurred in 67 per cent of febrile late reactions. It was usually seen be-

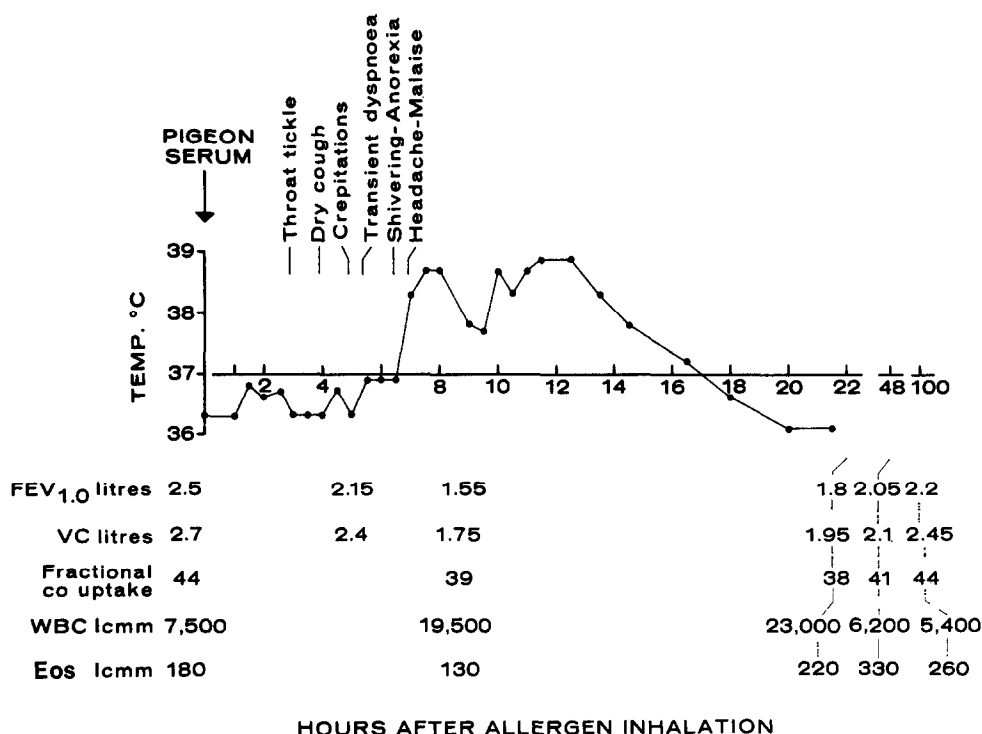


FIG. 3. Allergen inhalation test. Late pyrexial and peripheral respiratory reaction.

tween 6 and 14 hours after the inhalation test. The WBC then tended to fall, but was still raised at 24 hours in 35 per cent. In a few tests (16 per cent), the counts were unchanged at 6 to 14 hours, but raised at 24 hours. The WBC returned to pretest levels within 48 hours. The highest WBC recorded was 22,500 per cubic millimeter in a patient whose initial count was 10,500 per cubic millimeter. The percentage differential WBC showed that the polymorphonuclear leukocytes increased while the lymphocytes decreased. There was no change in the monocyte or basophil count. The absolute eosinophil count was raised before antigen inhalation in 2 patients and after antigen inhalation in 11 patients, usually at 24 hours. Immediate as well as late asthmatic reactions occurred in 3 of these 11 patients. The highest count recorded was 800 per cubic millimeter.

The respiratory features were mild in degree because the method of allergen challenge was arranged carefully to provoke only a febrile or an asthmatic reaction. In 9 patients symptoms and signs occurred without change in ventilatory capacity and consisted of cough in 5 patients, dyspnea in 5, and crepitant rales in 4.

Fig. 3 illustrates a late peripheral respiratory reaction of the type observed in 6 patients. Falls in the FEV₁ of between 0.2 and 0.95 L. (14 to 38 per cent) and in the VC of between 0.15 and 0.95 L. (7 to 37 per cent) occurred that could not be reversed by isoproterenol inhalation. The PEF measured in 5 of these patients and the SGaw in one had not fallen from pretest values. No change was

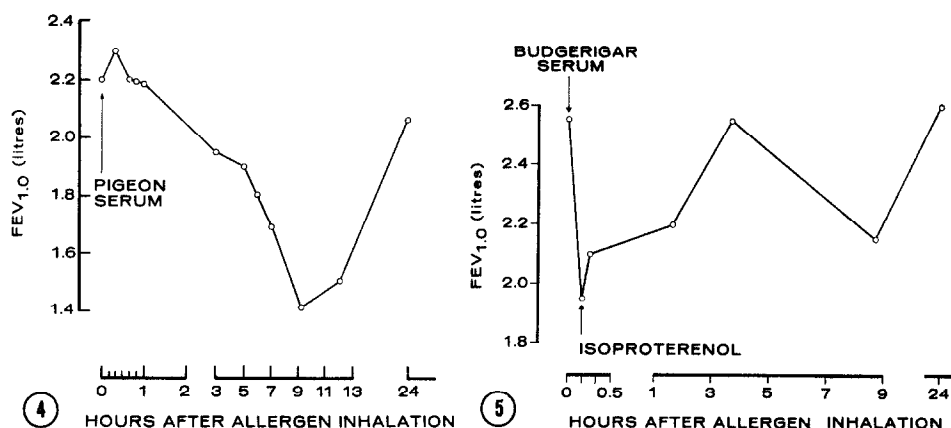


FIG. 4. Allergen inhalation test. Late asthmatic reaction.

FIG. 5. Allergen inhalation test. Dual asthmatic reaction.

detected in the TLco (steady state) at rest. The falls in the FEV₁ and VC tended to resolve in 24 hours, but in 2 patients they persisted for 2 and 4 days. Respiratory symptoms did not develop in 2 of these patients. Dry cough occurred in 3 and dyspnea without wheezing in 4. Crepitant rales developed in 2 patients in whom they had not been heard initially, and these cleared between one and 2 days. In the other 4 patients, the crepitant rales were present before the test, and it was not possible to say whether these had increased at the time of reaction. No rhonchi were heard.

Fig. 4 illustrates a late asthmatic reaction of the type observed in 8 patients. Falls in the FEV₁ of between 0.3 and 1.0 L. (14 to 54 per cent) occurred. In 3 of the 8 patients, the late asthmatic reaction was preceded by an immediate asthmatic reaction and was therefore part of a dual response. In all of the 8 patients, except 2 with a dual response, fever was also present.

Dual reactions

Fig. 5 illustrates a dual reaction consisting of an immediate asthmatic reaction followed by a late asthmatic reaction. Similar reactions were observed in 3 patients. In one patient the dual reaction consisted of an immediate asthmatic reaction followed by a late pyrexial reaction.

No reactions

In 45 patients the tests were negative. Three of these with clinical features of extrinsic allergic alveolitis were thought to be allergic to birds for reasons given in MATERIALS.

Relation of the type of allergic respiratory reaction provoked by inhalation tests to the clinical features of disease

The features of the respiratory reactions and of the site of respiratory disease are analyzed further in Table IV.

TABLE IV. Allergen inhalation tests: Relation of type of reaction to type of respiratory disease

Inhalation test reactions	Total number of patients (36)	Type of respiratory disease		
		Airways obstruction		Peripheral respiratory, alveolitis 26 patients
		Reversible, asthma 10 patients	Irreversible 8 patients	
Immediate Asthma	5	5	—	—
Dual				
Immediate asthma and late asthma	3	3	—	—
Immediate asthma and late fever	1	1	—	1
Late				
Fever only	7	—	—	7
Fever and respiratory symptoms and signs only	9	—	2	9
Fever and asthma	5	1	5	3
Fever and peripheral respiratory symptoms	6	—	1	6

All 9 patients in whom allergen inhalation tests had provoked immediate asthmatic reactions presented with a history of asthma. In 4 of the 9 patients dual reactions were observed, immediate followed by late asthmatic reactions in 3, and immediate asthma followed by late pyrexial reactions in one. The latter patient gave a history of both asthma and febrile attacks 5 or 6 hours following exposure to his pigeons, possibly indicating that reactions were occurring both in the bronchi and in the alveolar regions.

In 27 patients in whom the allergen inhalation tests caused late reactions that were not preceded by immediate asthmatic reactions, all except 2 presented with alveolitis. These 2 patients (and a further 6) also had airways obstruction as indicated by an FEV₁/VC below 66 per cent, which was not reversible by isoproterenol at the time of investigation, nor by the avoidance of bird exposure. A history of asthma was obtained in one of the 2 patients with irreversible airways obstruction. Late respiratory reactions consisting only of fever occurred in 7 patients with alveolitis.

Of 9 patients who gave late systemic and respiratory reactions consisting of fever and respiratory symptoms and signs, but who showed no change in ventilatory capacity, all had alveolitis and 2 also had airways obstruction. One of these 2 patients had a past history of asthma in childhood unrelated to bird exposure, and this may have been the cause of the airways obstruction (case reported by Godfrey⁵⁴).

Of the 5 patients with late reactions consisting of fever and asthma, all had irreversible airways obstruction. The late asthmatic reactions were slow in onset and unassociated with wheeze, and this may explain why a history of asthma was obtained in only one patient. Nevertheless, this late asthmatic reaction provoked by inhalation tests was reversible, and the patients recalled similar attacks at home. Three patients with a late asthmatic reaction on challenge had not only

TABLE V. Allergen inhalation tests: Relation of type of reaction to skin reactions and precipitins

Inhalation test reaction	Total number of patients	Skin test reactions						Precipitins		
		Prick, immediate		Intracutaneous						
				Immediate		Late		Bird serum		Droppings only (No.)
		No.	%	No.	%	No.	%	No.	%	
Immediate	5	5	100	3/3	100	1/3	33	1	20	0
Dual, immediate, and late	4	3	75	4	100	4	100	2	50	1
Late	27	7	26	15/26	58	22/26	85	24	89	3
No reaction	45	0	0	1/22	5	2/22	9	5	11	7

irreversible airways obstruction but also alveolitis. In these 3 patients, the measurements of ventilatory capacity carried out during the inhalation tests were not suitable for the detection of a "peripheral respiratory" reaction because of the airways obstruction that was also present.

Of the 6 patients with late respiratory reactions consisting of fever and a peripheral respiratory reaction, all had alveolitis and one had an FEV₁/VC of 65 per cent and normal PEF suggestive of borderline airways obstruction.

When bird exposure was discontinued, all except 2 of the patients who gave reactions improved,⁴¹ suggesting that these inhalation test reactions were clinically relevant.

Relation of the type of allergic respiratory reaction provoked by inhalation tests to the antibodies

Table V shows that reaginic antibodies, corresponding to the immediate skin reactions to prick tests, were detected in 8 of the 9 patients who also developed immediate asthmatic reactions. By contrast, they were demonstrated in only 7 of 27 (26 per cent) who did not give immediate asthmatic reactions, but who gave late respiratory reactions, and in none of the patients with no respiratory reaction. Furthermore, the skin reactions in patients giving immediate asthmatic reactions were more vigorous than the weak skin reactions given by those patients who gave late respiratory reactions to inhalation tests.

Immediate skin reactions caused by intracutaneous tests were observed in 15 of 26 patients (58 per cent) who had a late respiratory reaction but no immediate respiratory reaction. This implies that the prick technique, in which smaller amounts of antigen are injected, is a better technique to use in the clinical evaluation of immediate asthma.

Precipitating antibodies, in contrast, were found in 30 of the 31 patients with late respiratory reactions, less commonly but in one of the 5 with an immediate respiratory reaction, and in 12 of 45 with no respiratory reaction. This correlation is even closer if one subdivides the precipitins into those to the bird serum and those to droppings extract only, and if one analyzes the group with precipitins and no respiratory reactions. Thus, the higher number of "droppings

only" precipitins in the patients with no respiratory reactions suggests that these precipitins correlate less closely with the respiratory sensitivity to birds. In fact, 6 of these 7 patients had no evidence of bird sensitivity. Of the remaining 6 patients, 3 were considered to be sensitive. In these 3 the inhalation challenge may have been negative in one because he kept finches, and the tests were carried out with budgie and pigeon extracts that may not have contained the specific antigens. In the other 2 the inhalation challenge may have been negative because they were exposed to large numbers of birds, and the inhaled dose may have been too low.

The late skin reactions began to appear between 2 and 4 hours after the injection; they tended to reach a peak at about 5 to 8 hours and then to clear within 24 to 48 hours. In 2 patients they were associated with late systemic and respiratory symptoms similar to those observed following inhalation tests. They were almost always preceded by an immediate skin reaction, and the more vigorous the immediate reaction the more vigorous the late reaction.⁴¹ In addition, they correlate closely with the presence of precipitins.

DISCUSSION

Allergen inhalation tests are not practiced widely because they are time consuming. The procedure used in this study illustrates how to elicit controlled, mild to moderate reactions that are acceptable and not distressing to the patient.

In future work the method could be modified to exclude the re-breathing bag so that the aerosol is delivered directly to the face mask. If this is done the dose will be much higher, and it will be necessary to use weaker extracts, probably diluted at least ten times.

The reactions provoked by inhalation tests included all of those that have been reported previously. Care was taken to ensure that they were specific. While the observations used to detect these reactions, the temperature and FEV₁ and VC, were adequate and simple, the FEV₁ and VC are relatively insensitive measurements, and other tests are necessary to study the respiratory reactions more carefully.

In asthma of immediate onset, Colldahl and associates¹³ demonstrated that the following physiologic measurements are useful in decreasing order of sensitivity: the airways resistance, N₂ washout, maximum midexpiratory flow (MMF), FEV₁, PEF, and VC. Tse,⁵⁵ using flow volume curves in addition to MMF and FEV₁ has shown that the flow at 25 per cent and 50 per cent of VC is more sensitive. When the immediate reaction is carefully elicited it does not have to be reversed by isoproterenol, and its natural course can be followed. This point is relevant in the interpretation of dual, immediate followed by late, reactions which have been analyzed in other studies.^{15, 16, 35, 38}

In asthma of late onset more detailed studies of lung mechanics have not yet been carried out. In the pyrexial reactions of late onset, which were unassociated with asthma as detected by the FEV₁ and VC, changes in ventilatory capacity were detected in only 6 of 21 cases. While this probably was due to the small quantity of allergen inhaled, it is possible that changes in pulmonary function may have been detected by more sensitive tests. In patients with

farmer's lung, Williams¹⁸ demonstrated that the fall in static lung compliance was more marked than the fall in VC. In addition, the functional changes of the peripheral respiratory reactions need to be analyzed more carefully. Initially they were thought to be due to changes only in the respiratory tissues, that is, the respiratory bronchioles, alveolar ducts, and alveoli. However, histopathologic studies have shown involvement of small bronchi and bronchioles,^{42, 56} and airways reactions have been demonstrated in persons with alveolitis. Therefore, it seems probable that in some of these "peripheral respiratory reactions" the predominant reaction is occurring in small airways. Schlueter and associates⁵⁷ present evidence to support this possibility in a pigeon fancier following environmental exposure to his pigeons. More physiologic studies are required.

The clinical relevance of the respiratory reactions provoked by inhalation tests is strongly supported by two points. The first is that the clinical features of disease are similar to those of the respiratory reactions provoked by inhalation tests, and the second is that the clinical features of disease improved (in all except 2 patients) when exposure to birds was discontinued.

Bearing in mind that the provoked reactions were mild and that the physiologic measurements were limited, the following conclusions may be made. Patients with disease affecting the airways and not the respiratory tissues developed an airways reaction to challenge. Patients with disease affecting the peripheral respiratory tissues (alveolitis) and who had no airways obstruction developed a pyrexial and peripheral respiratory reaction. Patients with both alveolitis and airways obstruction developed both pyrexia and an airways reaction.

All of the airways reactions provoked by allergen inhalation tests were asthmatic. While a history of asthma was obtained in all 9 patients who had immediate asthmatic reactions, it was present in only one of 5 patients giving late asthmatic reactions not preceded by immediate asthmatic reactions. This difference may be attributed to the different features of the immediate asthmatic reactions that were of rapid onset and short duration, while the late asthmatic reactions had a slower onset and longer duration, or to the association of asthma with alveolitis in 3 of the remaining 4 patients, or perhaps to a difference in the site of reaction in the airways, with the late reactions chiefly affecting the small airways.

Febrile symptoms occurring 5 to 6 hours or so following exposure are characteristic of the acute presentation of extrinsic allergic alveolitis that follows intermittent exposure in persons who keep large numbers of birds away from their home, for example, pigeon fanciers, and are simulated by the inhalation test. Febrile symptoms are less consistent and milder in the insidious presentation of extrinsic allergic alveolitis when the exposure is over a longer period to relatively smaller doses of allergen, and these conditions occur in persons keeping a bird in the home, for example, budgerigar fanciers.^{41, 42}

The close correlation of immediate asthmatic reactions to reaginic antibodies demonstrated by prick tests and of late respiratory reactions with precipitating antibodies supports, first, the specificity of the respiratory reactions and, second, suggests the participation of types I and III allergic mechanisms, respectively.

In the immediate asthmatic reactions, there is much circumstantial evidence to suggest the involvement of type I allergy. Thus they correlate with the finding of reaginic, skin-sensitizing antibodies that have recently been identified as chiefly IgE immunoglobulins. In further work⁵⁸ on the sera of 8 of the 9 patients with immediate asthmatic reactions reported in this study, IgE was identified in 5 by the radioallergosorbent technique (RAST) of Wide and associates.⁵⁹ The timing of the immediate reactions is consistent with reagin-mediated type I allergy. Type I allergic reactions are characterized by eosinophil cell infiltration, and in this study a blood eosinophilia was provoked by inhalation challenge in 5 of the 8 patients tested. Recent immunohistochemical studies by Gerber and associates⁶⁰ in patients with bronchial asthma have shown nonspecific IgE characteristically in 3 locations: the respiratory epithelium of small bronchi and particularly bronchioles, along the epithelial aspect of the bronchial basement membrane, and in the intrabronchial mucus. Further studies need to be performed to determine the localization of specific IgE.

The allergic mechanism(s) involved in the late asthmatic and respiratory reactions is less clearly defined. In this study these patients had precipitating antibodies that were shown in subsequent work by Faux and associates⁵⁸ to be IgA, IgG, or IgM antibodies. Specific IgE was identified only in 2 negative prick test reactors. The IgG and IgM antibodies could mediate type III allergic reactions in the lungs, and this possibility is supported by the timing of the reaction and by the provocation of a blood polymorphonuclear leukocytosis, which is a characteristic cellular response of type III allergy, following inhalation challenge.

The late skin reactions also have a time course similar to the late respiratory reactions. Their occurrence, also correlated with the demonstration of precipitins and immunohistopathologic studies by Pepys and associates,⁶¹ supports the view that they are mild type III reactions. Their histology consists of edema and of infiltration and perivascular cuffing initially with polymorphonuclear leukocytes and later with mononuclear cells, and some eosinophils. Deposition of immunoglobulins and complement (β IC) was found in the cytoplasm of the perivascular inflammatory cells and also within the vascular endothelium. These late skin reactions were related to or dependent upon the preceding immediate reaction. The more vigorous the immediate reaction, the more vigorous the late reaction.⁴¹ A similar relationship has been found between late and immediate skin reactions to *Aspergillus* antigen in man^{34, 62, 63} and in experimental animals.⁶⁴⁻⁶⁶ In atopic subjects the immediate reaction is mediated by the IgE, "long-term," antibody (type I) mechanism, while in nonatopic subjects it may be due to IgG, "short-term," antibody described by Parish.⁶⁷ If this is true, then this study shows that "short-term" IgG antibody does not mediate immediate asthmatic reactions.

The pathology of late asthma has not been studied. However, IgG, IgM, and complement have been shown to be deposited along the epithelial surface of the bronchial basement membrane^{68, 69} and could be associated with bronchial allergic reactions.

The histopathology of extrinsic allergic alveolitis is consistent with type II, type III, or type IV allergic reactions. It shows an infiltration with mononuclear

cells, consisting of histiocytes, plasma cells, and lymphocytes, of granulomas of the sarcoid type, and of a vasculitis.⁵⁶ Mononuclear cells are seen in the later stages of all of these reactions. In the type III allergic reaction, the characteristic infiltrate of polymorphonuclear leukocytes occurs only in the early stages; in an early acute fatal case of farmer's lung, Barrowcliff and Arblaster⁷⁰ observed an infiltrate in which polymorphonuclear leukocytes were prominent. Granulomas are formed in the type III allergic reaction when insoluble immune complexes in antigen-antibody equivalence are formed.⁷¹ The vasculitis does not particularly involve the small venules, which is characteristic of the type III reaction, and it is not a pronounced feature. Although this may be the result of chronic mild reactions occurring predominantly in the tissues, Wenzel and associates⁷² suggest that it may be because the reaction is mediated by a type II, cytotoxic, mechanism. Type IV allergy mediated by lymphocytes has also been considered, especially because of the mononuclear cell infiltration and granulomas. Some evidence to support this possibility has been presented by Bach and associates,⁷³ who have demonstrated blast transformation of lymphocytes in culture and inhibition of leukocyte migration in response to pigeon and budgerigar serum.

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