# PIGEON BREEDER'S DISEASE - THE CLINICAL SPECTRUM AND HUMORAL RESPONSE.

An investigation of the nature and extent of extrinsic allergic alveolitis due to pigeon-derived antigens among pigeon fanciers continuing to pursue the pastime, with particular reference to early clinical and humoral-mediated responses following exposure

by

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"Here we have an intercept between the internal and external environments, and the complexity and diversity of factors making up the final outcomes that we recognise as diseases of the lung are truly dazzling!"

Peter A Ward M.D.
1979
Department of Pathology
University of Michigan, Ann Arbor.

## DECLARATION

The principal work comprising this thesis was initiated and completed due to my own efforts, but I have enjoyed the support and assistance of the Department of Respiratory Medicine, Glasgow Royal Infirmary throughout. These studies were undertaken as a member of a research team investigating extrinsic allergic alveolitis, under the overall direction of Dr Gavin Boyd MD (Hons) FRCP (Edin. & Glas.) Consultant Physician.

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## SUMMARY

This thesis examines the relationships between the clinical manifestations of Pigeon Breeder's Disease among active pigeon fanciers, and the associated humoral responses to pigeon-derived antigens. Clinical data was obtained in questionnaire format from a large scale field study interviewing 100 pigeon fanciers. A wide range of skin tests were performed and serum antibodies were estimated by sensitive and quantitative radioimmunoassay techniques. Selected pigeon fanciers also underwent detailed pulmonary physiological assessments including monitoring of lung function after antigen challenge. In particular, immediate components of the condition both clinical and immunological have been investigated.

Simple criteria have been formulated to categorise pigeon related clinical responses and a wider spectrum of Pigeon Breeder's Disease was identified. Modifications to the current classification are suggested to encompass -

1) **acute progressive disease** - the typical acute hospital referred case

2) **acute recurrent disease** - recurrent,febrile, alveolitis episodes but without short or medium term clinical deterioration. Affected persons continue active participation in the hobby and therefore this is probably more common than acute progressive disease with a prevalence of approximately 10% in the present

studies. Self regulation of exposure is an important aspect.

3) **Immediate Response** - consisting of 3 or more immediate symptoms; commonly recorded among pigeon fanciers, often with delayed symptoms forming an indistinct group that did not represent a specific clinical or immunological entity

4) **chronic PBD** - evidence was found suggesting that chronic respiratory symptoms are an important part of the clinical spectrum occurring in up to 20% of non-smokers.

The pulmonary function data supported these clinical distinctions and lung permeability studies showed disturbed physiological integrity even where routine pulmonary function parameters were normal. This technique merits further investigation as a potentially sensitive indicator of physiological abnormality in extrinsic allergic alveolitis.

Relationships between avian-specific IgG, exposure factors and clinical response have been extended. There was a progressive tendency towards an altered immune reactivity (immunisation) as certain parameters of exposure increased,but this did not correlate with a major likelihood for finding extrinsic allergic alveolitis. High antibody responders are a group determined by host immunological responsiveness. An intense IgG response correlated with the presence of Pigeon Breeder's Disease independently of exposure,and those having >60ug/ml serum IgG to pigeon globulin evident within 10 years of pigeon keeping were particularly likely to report acute Pigeon Breeder's Disease.

The skin test data indicate that the humoral immune response routinely includes reaginic activity which relates to IgG antibody rather than IgE and such responses were associated with the presence of extrinsic allergic alveolitis independently of the late,6 hour,intradermal response.

These studies redefine the clinical spectrum of Pigeon Breeder's Disease and establish that there is a dynamic interaction between exposure and symptoms constituting a self-regulation of the condition particularly relevant in those persons with acute recurrent disease. A close relationship between avian-specific IgG and Pigeon Breeder's Disease has been reaffirmed and shown to relate primarily to factors other than intensity of exposure,and to have a functional capability including reaginic activity. The findings favour an active role for antibody in the immunopathogenesis of Pigeons Breeder's Disease and it is postulated that avian-specific reaginic activity within the pulmonary compartment may initiate or enhance other immune events leading in susceptible persons to the disease entity of extrinsic allergic alveolitis.

# CHAPTER I

## Introduction and review of the literature

### PULMONARY DEFENCES

The primary function of the lung is to achieve gas exchange and this is very effectively accomplished by means of the 50 - 100 square metres of blood-gas interface formed by the respiratory zone of some 300 million alveoli distal to the conducting airways (West, 1974a). However, this huge surface also constitutes an interface between the internal and external environments which is 50 times more extensive than the skin (Lancet editorial, 1978). Exposure to a wide range of micro-organisms, smokes, dusts and other aerosols at this surface is continually occurring and it has been estimated that 2.5 mg of atmospheric dust alone, is inhaled daily by a city dweller in the 10 - 20,000 litres of air breathed (Brain & Valberg, 1979). Accordingly the respiratory system is equipped with a number of physical, pharmacological, cellular and immunological mechanisms to defend the body from the harmful effects of these contaminating particles.

The nature and extent of this defence system is still under intensive intestigation, but it is evident that cellular and immune methods play a prominent role in maintaining the body's

integrity at this surface (Kaltreider, 1976; Hunninghake et al., 1979). This is particularly the case distal to the mucociliary blanket formed by the tightly packed ciliated epithelial cells lining the respiratory tract from trachea to terminal bronchioles and which forms a physical and pharmacological barrier effecting rapid removal of deposited particles (Cohen & Gold, 1975; Gail & Lenfant, 1983). Inhaled particles larger than 10um rarely penetrate beyond the terminal bronchioles, but 50% of 3um particles may be retained in the respiratory zone (Lippmann & Albert, 1969). This material may also eventually exit via the mucociliary blanket but initial handling involves the poorly understood bronchoalveolar clearance mechanisms. The alveolar macrophage has a major role in removing particulate material from the pulmonary compartment (Morrow, 1971; Green, 1973), but many elements of the immune system including circulatory and localised immunoglobulin, complement components, and pulmonary lymphoid tissue are also represented and involved in "detoxification" (Kaltreider, 1976).

There is also evidence that in addition to maintaining the cleanliness of the air spaces the alveolar macrophage plays an active role in pulmonary inflammatory and immune processes (Schwartz & Bellanti, 1977; Yeager et al., 1980; Kaltreider, 1982). The lungs therefore possess a complex and varied immune capability which involves both the expression of systemically generated immune reactions and local phenomena where the lung functions as an autonomous immuno-competent organ (Schuyler et al., 1978; Daniele et al., 1975). The immune response depends,

in part, on the route of access and localisation of particular antigen.

## DISORDERS OF PULMONARY IMMUNITY

Although developed for host protection, disorders of the pulmonary immune system are thought to play a part in the pathogenesis of many respiratory diseases, and all 4 types of allergic tissue damage have been identified (Schatz et al., 1979). A Type I reaction occurs in bronchial asthma, a Type II reaction in Goodpasture's syndrome, a Type III reaction in some of the pulmonary complications of systemic lupus erythematosis and a Type IV reaction in pulmonary tuberculosis.

However, at present the best defined clinical conditions involving allergic tissue injury result from hypersensitivity to inhaled external allergens. There are 2 quite different types of disorder recognised:-

### 1. Hay fever and asthma.

The immediate hypersensitivity reaction is characterised by antigen-induced release of mediators (granule and membrane derived) from mast cells previously sensitised with specific IgE antibody producing rapid onset of symptoms. In asthma there is bronchoconstriction, oedema of airways wall, and an influx of inflammatory cells due to release of chemotactic factors. The complex multifactorial nature of bronchial asthma has been

increasingly recognised in recent years but the mast cell and IgE mediated hypersensitivity retain an important role (Holgate & Kay, 1985).

## 2. Extrinsic allergic alveolitis (EAA).

In this group of conditions antigen inhalation provokes an immunopathological process in the peripheral gas-exchanging areas of the lung and results in characteristic clinical and physiological abnormalities which develop 6-12 hours after contact. The terms extrinsic allergic bronchiolo-alveolitis (Scadding, 1974) and hypersensitivity pneumonitis (North American literature) have also been applied to these disorders, but offer no particular advantage. A steadily growing number of antigens are recognised which can cause EAA, including a number of chemicals encountered in occupational settings, such as isocyanates used in the plastics industry (Charles et al., 1976) and the laboratory reagent sodium diazobenzenesulphate (Evans & Seaton, 1979). Although each variety has its own characteristics there is a close similarity in symptomatology between the different types and reproduction of the typical acute symptom complex by inhalation provocation, even where chronic symptoms predominate, is the most reliable method of establishing the diagnosis (Fink, 1974; Muittari et al., 1980; Harries et al., 1980).

The most constant histological features are an interstitial and alveolar inflammation where lymphocytes predominate, but with a varying degree of plasma cell, histiocyte, and

polymorphonuclear cell infiltrate (Emanuel et al., 1964). Non-caseating granulomas are also often found and the inflammatory process may involve the bronchioles (Reyes et al., 1982). These findings are patchily distributed in biopsy specimens, and this is also characteristic of the fibrosis and bronchiolitis obliterans found in more advanced cases (Tukianen et al., 1980).

Theories as to immunopathogenesis have centred mainly on either an immune complex-mediated basis (Pepys, 1969), or a cell-mediated process (Hansen & Penny, 1974). The delayed onset of symptoms, which includes fever and arthralgia, is consistent with the known clinical manifestations of immune complex tissue injury and accords with the delayed (6 hours) skin reaction, where deposits of complement and immunoglobulin have been demonstrated (Pepys et al., 1968a). Numerous studies have reported an association between detection of serum precipitating antibodies to an antigen and EAA (Salvaggio et al., 1967; Parratt et al., 1975; Faux et al., 1971a). However, there is little direct evidence for immune complex involvement from the biopsy findings, as only a few studies have shown acute polymorphonuclear cell inflammation with oedema and vasculitis (Seal et al., 1968; Barrowcliff &Arblaster, 1968), and immunofluorescence has very rarely demonstrated deposits of complement and immunoglobulin (Ghose et al., 1974). The histological findings are more in keeping with cell-mediated tissue injury, although granuloma formation can result from antigen-antibody complexes in the

equivalence zone (Brentjens et al., 1974). It is probable that a series of immunological events are necessary to the development of EAA (Reynolds, 1982; Richerson, 1983).

### 3. Comparison of immediate hypersensitivity and EAA.

There are several differences between the antigens and circumstances of exposure characteristic of these responses. The antigens responsible for immediate hyersensitivity are generally >10 um in size and therefore impact on the mucosa of the upper airways, whereas the antigens associated with EAA are <5um and can penetrate more distally into the respiratory zone. Whilst ordinary everyday exposure may result in immediate hypersensitivity reactions, periods of intense or protracted exposure are typically required to produce EAA. These differences in the site and intensity of antigenic stimulus are relevant to the nature of the humoral response produced. Antigens encountered in the upper airways stimulate a predominantly IgE antibody response in the local lymphoid tissues, whilst antigens deposited beyond the terminal bronchioles induce an IgG antibody from the hilar lymph nodes, bronchoalveolar cells and other lymphoid tissues.

Although a single antigen may produce both types of antibody response, for example avian proteins, a dual clinical response is exceptional (Karr et al., 1978). Pepys (1969) found the atopic status of individuals an important determinant of response with atopy predisposing to immediate hypersensitivity but rare in

alveolitis. Faux and co-workers (1971a) in a study of responses to budgerigar antigen also found that atopics developed asthma and non-atopics alveolitis. At present no characteristics have been found which identify those "at risk" for EAA. Flaherty et al. (1975) reported an increased frequency of the HLA-B8 tissue type amongst 20 patients with farmers' lung but a detailed study by Rodey et al. (1979), has failed to show any association between tissue type and Pigeon Breeder's Disease (PBD).

## 4. Avian-related EAA.

A number of varieties of EAA have been identified which relate to contact with avian species, and these are summarised in Table 1. The antigenicity of avian proteins has routinely been assessed by their ability to elicit a serum IgG antibody response and usually antibodies are detected to a variety of material, such as avian serum proteins, egg yolk (and white), feathers, and pigeon dropping extracts (PDE). Cross-reactions between proteins of different species occurs, but mainly relate to avian serum albumin, with the gammaglobulin fraction containing the most species specific antigens (Faux et al., 1971b; Walbaum & Biquet, 1971).

Four types of protein have been identified which account for the major part of the precipitin reactions to pigeon-derived material:- gammaglobulin, pigeon serum albumin, a B-globulin, and another protein which cross-reacts with gammaglobulin, and termed protein XPGG by Edwards et al. (1969). This latter protein was

TABLE 1 Avian-related EAA

| AUTHORS | CLINICAL DETAILS | AVIAN SPECIES |
|---|---|---|
| Plessner (1960) | Description of fever in workers handling duck and goose feathers occurring 3 - 4 hours after exposure and improved by wearing masks. | Geese/Ducks |
| Pearsall, Morgan, Tesluk & Beggs (1960) | Febrile illness with breathlessness and CXR infiltrates in 59 yr. old man who kept 400 parakeets (budgerigars). Precipitins to parakeet dander demonstrated. | Budgerigars (parakeets) |
| Reed, Sosman & Barbee (1965) | Definitive description of Pigeon Breeder's Disease in 3 pigeon breeders. Precipitins found, challenge tests undertaken. | Pigeon/Turtle doves |
| Korn, Florman & Gribetz (1968) | A 6 year old boy with recurrent febrile, breathless episodes resolving away from home. CXR infiltrates and hilar adenopathy. Patient lived near a chicken market and positive precipitin to hen litter. | Chickens |
| Boyer, Klock, Schmidt, Hyland, Maxwell, Gardner & Renzetti (1974) | Several persons with delayed-onset symptoms compatible with extrinsic allergic alveolitis in a study of 205 workers in turkey raising or processing. | Turkeys |
| Muller, De Haller, & Grob (1976) | Review of 15 cases of Bird Fancier's Disease from 213 sera referred for analysis. One 22 year old female with a pet parrot for years developed alveolitis 2 weeks after acquiring budgerigars. Precipitins to parrot antigens present, not absorbed by budgerigar serum | Parrot (?) |

subsequently shown to be pigeon IgA (Tebo et al., 1977) and is a major component of droppings and external secretions with the antigenicity of this protein retained even in "old" droppings. Goudswaard et al. (1978) suggest that antibody to IgA light chains could account for cross-reactions with other pigeon immunoglobulins.

The source of antigens relevant to PBD is generally considered to be pigeon droppings and Edwards et al. (1970) considered this the most complete source of antigens with which pigeon fanciers came into contact. McCormick et al. (1982) have identified an antigenic component of PBD against which an antibody response was only evident in symptomatic persons. However, clinical experience suggests that there is no one antigen invariably involved. For example Reed et al. (1965) found that one patient did not react to inhalation of coop material but developed the typical acute alveolitis syndrome when challenged with a pigeon feather preparation. A similar finding was reported by Warren & Tse (1974) in a case of EAA due to chickens, when challenge with chicken serum was ineffective but a feather preparation caused symptoms.

Possible differences in the type and quantities of materials inhaled could account for some of the observed differences between the avian-related alveolitis syndromes. EAA amongst budgerigar keepers is often a rather insidious illness (Faux et al., 1971b; Warren, 1972) and this has been attributed to the

low-grade, but protracted exposure occurring with a single bird kept in the home. In contrast, pigeon fanciers are intermittently but intensely exposed to their birds and discrete clinical episodes following exposure are characteristic. Boyd (1978) has drawn attention to the abundant dust which comes directly off the pigeons to be deposited on the hands and clothes of their owners. Antibodies against this waxy powder, pigeon bloom, have been found in high titre in the sera of fanciers with PBD (Banham et al., 1982). Partial identity with some of the pigeon feather antigens has been found (C. McSharry, personal communication) and this material could provide an alternative to pigeon droppings as a "natural" source of antigen. Poultry workers are regularly exposed to large numbers of birds yet alveolitis is rare (Warren & Tse, 1974) and perhaps a lack of certain avian material, such as bloom, is relevant.

PIGEON BREEDER'S DISEASE

An illness affecting the lung parenchyma, distinct from ornithosis but linked to contact with pigeon excreta was reported by Feldman & Sabin in 1948. However the first definitive report of pigeon-related EAA was by Reed et al., from North America in 1965. The disorder has provided a convenient model for the investigation of these illnesses because the disease is essentially limited to a well-defined population, i.e. pigeon breeders (referred to as pigeon fanciers in Great Britain) and furthermore, suitable antigen preparations are readily available

for clinical and laboratory use. The condition has been reported from many different countries, wherever pigeons are kept for racing or other purposes, including Australia (Maloney, 1967); Portugal (Avila, 1967); France (Molina et al., 1969), and more recently Chile (Ullou et al., 1978). The first cases of PBD recognised in Great Britain, where there are an estimated 200,000 pigeon fanciers (Schlueter, 1974) were reported by Hargreave, et al. in 1966.

Reports in the literature identify a number of different clinical subgroups of PBD, but 2 main types of classification are recognised. Firstly, a system based on the predominant symptom complex, **acute** - where typical febrile episodes are the main feature, **sub-acute** - a rather ill defined group with predominantly respiratory symptoms but sometimes progressing quite rapidly, and **chronic** - the insidious onset of breathlessness only evident after many years of exposure. Alternatively, the illness has been reported in terms of the severity of symptoms, ranging from a **severe** rapidly progressive illness, including childhood cases where failure to thrive is common (Cunningham et al., 1976), to those with **mild** hardly noticeable clinical features.

1. **Acute alveolitis.**

The typical clinical presentation is with fever, chills, arthralgia and general malaise accompanied by cough, chest tightness and breathlessness developing between 6 - 12 hours

after exposure. Inspiratory basal crackles and in severe cases central cyanosis (Boyd, 1979) are the associated clinical findings. A brisk leukocytosis accompanies the acute febrile episode and chest radiograph may show a diffuse reticular mottling most marked in mid zones. Clinical progress after the onset of symptoms appears quite variable. Amongst the original 7 cases reported in detail in North America and Great Britain (Reed et al., 1965; Hargreave, 1967; Godfrey, 1967; Boyd, et al., 1967) deterioration continued until medical intervention occurred or the pigeon fanciers spontaneously reduced or discontinued exposure. However, in a group of 10 fanciers with acute symptoms reported by Fink et al. (1968a), 4 had experienced symptoms for between 10 and 20 years without obvious deterioration in health. In a subsequent report on lung function (Schlueter et al., 1969) these fanciers had only minor abnormalities demonstrated. Apart from the variable clinical course after developing symptoms, a range of susceptibility is apparent from the duration of exposure before symptoms develop, which varies from a few months to many years (Hargreave et al., 1966).

Six of 146 pigeon fanciers studies by Elgefors et al. (1971) reported upper respiratory symptoms of sneezing in a survey where the overall prevalence of pigeon-related symptoms was 8% (11 persons). This type of symptom was found in 61% of poultry workers (Boyer et al., 1974) and largely attributed to an immediate hypersensitivity reaction. However, 4 persons in the 1971 study and 13 poultry workers also had delayed, alveolitis-like, symptoms. Other authors have also described PBD

accompanied by rhinitis (Siegal & Ouellette, 1969; Redmond et al., 1975) but the incidence of this dual response reported by Christensen and co-workers (1975) when 9 of 10 people with PBD had rhinitis and/or conjunctivitis appears exceptional. Pelikan & Pelikan-Filipek (1983) performed nasal provocation tests in fanciers with allergic rhinitis and found that some developed a late reaction of fever, general malaise and nasal haemorrhages which they considered a nasal form of PBD.

2. **Other symptoms.**

Berrill et al. (1975) reported a close association between bird fanciers' lung (BFL) and coeliac disease but the diagnosis of BFL rested largely on detecting serum precipitins to bird serum. Faux et al. (1978) have shown that coeliac disease is independently associated with a precipitin response to bird serum where the antigen concerned is different from those specific to BFL and probably dietary in origin. Another study by this group (Hendrick et al., 1978) failed to demonstrate any major association of BFL with coeliac disease. More ususual symptoms linked to PBD are impotentia coeundi (Lamers & Maesen, 1976) and psychological disturbance (Misur & Takac, 1978)!

3. **Sub-clinical disease**

There are a few reports of individuals with very minor symptoms but definite evidence of PBD. Allen et al. (1975) gave

details of an asymptomatic girl aged 7 years with an abnormal radiograph, reduced lung volumes and decreased carbon monoxide uptake. Metzger et al. (1978) described 2 persons considered to represent an early stage of chronic PBD where antigen challenge was necessary to establish the diagnosis. These reports are consistent with the accepted view that chronic PBD develops insidiously over a considerable period and therefore has a sub-clinical phase.

4. **Treatment.**

Avoidance of further contact is the cornerstone of therapy and a good clincal recovery can be anticipated even in severe, acute cases. A satisfactory clinical recovery usually occurs in chronic PBD as well, but some irreversible impairment of function may persist (Hensley et al., 1969). The rare fatalities associated with PBD have occurred in such persons following the development of cor pulmonale (Molina, 1976). In a longitudinal study of pigeon fanciers reviewed 8 - 30 months after diagnosis and cessation of exposure (Allen et al., 1976) the extent of clinical and functional recovery related to the duration of exposure following the onset of symptoms, and to age. In one young patient there was a steady improvement in lung function over a 2.5 years period following cessation of exposure. Their findings accord with those in a follow-up study of farmers' lung disease (Braun et al., 1979), where the degree of pulmonary function abnormality correlated with the number of acute episodes

of alveolitis. Antigen avoidance should also include other avian species since pigeon fanciers with PBD have developed symptoms when exposed to budgerigars (Boyd et al., 1967) and chickens (Sennecamp et al., 1974).

Whilst complete antigen avoidance is desirable, it is apparent to those observing pigeon fanciers that they are extremely reluctant to relinquish their hobby. Experience with respect to lesser degrees of antigen avoidance such as wearing a mask, has varied. Reed et al., (1965) observed that one fancier was able to resume the hobby without difficulty by wearing a mask. Siegal & Ouellètte (1969) found that a mask which filtered particles >0.5um in size prevented recurrence of symptoms in an affected laboratory worker. Metzger et al. (1978) also reported improvement in pulmonary function in a fancier who began to wear a mask, and Boyd & Walker (1985) found a reduction in antibody level plus clinical recovery in affected fanciers persuaded to wear masks. In contrast Fink et al. (1968a), found permanent lung damage developing in a person who was regularly wearing a mask.

Corticosteroids have been widely used to hasten the clinical recovery, and to limit permanent damage arising from acute PBD, and Metzger et al. (1978) even observed an improvement in lung function on steroids despite continued exposure. However, no controlled trials have been done and some authors are not convinced steroids are of value (Nash et al., 1967; Allen et al., 1976). As with "natural" recovery, those with a long

history of symptoms or chronic PBD pose the greatest problem. Schlueter (1974) recommends steroids to relieve symptoms in chronic PBD and Stokes & Mitchell (1980) also consider a trial worthwhile even when the chest radiograph and pulmonary function suggest irreversible disease. Finally, disodium cromoglycate administered prior to aerosol challenge with pigeon serum inhibited a late asthmatic reaction (Pepys et al., 1968b) and Elgefors et al. (1971) described a fancier with systemic symptoms who found taking disodium cromoglycate for 2 days before exposure and 1 day after visits to his dovecote reduced his symptoms. This fancier considered the medication impractical, and indeed this type of therapy has not been widely explored.

5. **Prevalence.**

The frequency with which these various forms of PBD occur in the general population of pigeon fanciers, and their prevalence at any one time has been difficult to ascertain. One reason is undoubtedly an anxiety amongst pigeon fanciers that medical "interest" may jeopardise their pursuit of this pastime, and no properly selected sample of pigeon fanciers has been obtained. Various surveys have been carried out, either by questionnaire or interview, with a considerable discrepancy in findings, although as with other types of EAA only a minority of those exposed were affected. No cases of "typical" PBD were identified amongst 200 pigeon fanciers interviewed at a convention (Fink et al., 1972), whereas 11 of the 53 fanciers studied by Christensen et al (1975)

were considered to have the disease (21%). This prevalence is higher than generally reported, and other surveys suggest approximately 6% are affected (Jongerius, 1969; Caldwell et al., 1973). The prevalence of chronic symptoms has not been recorded, but a proportion of those interviewed by Fink et al. (1972), may have had chronic PBD since cough and breathlessness were common and some had compatible radiographic and pulmonary function abnormalities.

Further investigation of the spectrum and severity of PBD among the general population of pigeon fanciers is one of the elements comprising this thesis.

## 6. Pulmonary Function

The predominant abnormalities are a restrictive ventilatory defect, reduced lung compliance and disordered gas transfer similar to other interstitial lung disorders (Scadding 1974). This pattern is particularly evident in the acute progressive cases seen in hospital practice. Amongst 25 cases of mainly acute avian-related alveolitis reported in 3 series (Dinda et al., 1969; Allen et al., 1976; Warren et al., 1977) routine tests revealed a reduced vital capacity in 56% and a low carbon monoxide transfer factor in 68%. The total lung capacity was less often reduced (24%). Only 20% had evidence of airflow obstruction ($FEV_1/FVC$ ratio <70%), but the residual volume was above 120% predicted normal in 26%. Arterial blood gases were detailed in one of these series (Dinda et al., 1969) and resting

hypoxaemia was found in 7 patients (78%). These patients also had a reduced partial pressure of carbon dioxide with a normal blood pH, thereby suggesting chronic hyperventilation. More detailed gas-exchange data was provided by Allen et al. (1976) who measured the alveolar-arterial oxygen tension gradient in 7 patients and found this increased in 6 cases. Interestingly the individual with a normal gradient had a decreased vital capacity (65% predicted) which improved after cessation of exposure.

These authors also investigated pulmonary mechanics and small airways function, and although $FEV_1/FVC$ ratio was reduced in only 1 subject, evidence of small airways obstruction was found in all cases. In some cases the changes were reversible with avoidance of antigen but in several instances, notably persons with an increased compliance, the abnormalities persisted. These latter persons had a pattern of lung function abnormality similar to that described by Schlueter et al. (1969) in chronic PBD

Warren et al. (1978) investigating pulmonary mechanics considered a disturbance of small airways function common to both the acute inflammatory process with broncholitis and the fibrotic destruction of lung units evident in longstanding avian-related alvolitis (Hargreave et al., 1972). A different view was expressed by Sovijarvi et al. (1980) investigating farmer's lung disease. Since small airways dysfunction did not correlate with a reduced carbon monoxide transfer factor it was concluded that the 2 measurements reflected bronchiolitis and alveolitis

respectively, occurring as separate events. Schofield et al. (1976) considered that there was a loss of lung units rather than a generalised stiffness of the lungs or narrowing of functioning small airways and the patchy distribution of inflammatory change evident on biopsy specimens would seem to support this view.

Findings with respect to those with milder, but recurrent episodes of PBD also vary. Schlueter et al. (1969) found no consistent abnormality in such a group other than an elevated residual volume in 62.5%. Two patients had a minor abnormality of gas-exchange with an increased tidal volume to dead space ratio, and 2 had reduced compliance. In contrast another study of 24 persons with avian-related alveolitis (Petro et al., 1978) in whom mean lung volumes and airways resistance were normal, did show significantly reduced mean volumic compliance. The mean carbon monoxide transfer factor was also significantly reduced, but the mean transfer coefficient was not, therefore, indicating restriction of ventilation as the cause of this dysfunction. The authors concluded that carbon monoxide transfer factor was a valuable investigation in the assessment of the disease and there was a correlation between the reduction in transfer factor and the duration of exposure.

Inhalational provocation tests provide the most direct evidence of pulmonary physiological change in EAA. However, as demonstrated by Hendrick et al. (1980) reporting on a large series of such challenges, the most sensitive and reliable positive findings are the clinical features of general malaise,

pyrexia and a peripheral blood leukocytosis. A reduction in forced vital capacity (and proportionate fall in $FEV_1$) was the only consistent change in routine pulmonary function parameters. Carbon monoxide transfer factor decreased significantly (>15%) only in a few cases with a severe reaction. In contrast Muittari et al. (1980) found a decrease in the diffusing capacity a sensitive index of response in a series of challenge tests investigating EAA related to hot water. Data from the pulmonary function assessments performed in the present studies, reported in preliminary form (Banham et al., 1979) also suggest that the diffusing capacity for carbon monoxide can alter in the absence of a major clinical response to challenge. Detailed lung function studies following challenge in 2 pigeon fanciers with mild symptoms were reported by Schlueter et al. (1969). There was a fall in forced vital capacity maximal 7 hours after exposure and a decrease in the forced expiratory flow between 25 and 75% of vital capcity. No consistent alteration in pulmonary mechanics was found and diffusing capacity was not measured.

In contrast to these findings, other studies have reported large airways obstruction as an important feature of PBD in circumstances other than in the chronic disease already described. Hargreave & Pepys (1972) reported a variety of responses to inhalation challenge amongst 36 bird fanciers, where a significant feature was the combination of airways obstruction with symptoms of alveolitis. Subjects with a history of avian-related asthma characteristically developed immediate airways

obstruction but, in some instances, this was followed by late asthma, and in one person delayed fever only. More surprising was the finding of 5 subjects with late airways obstruction and fever without preceding early bronchospasm, of whom 3 were considered to have alveolitis. Moore et al. (1974) also observed the development of airways obstruction with fever and leucocytosis after inhalation challenge in 2 atopic pigeon fanciers and considered the response compatible with PBD Dual asthmatic and alveolitis reactions were found in 6 of 7 persons with suspected occupational EAA challenged by Harries et al. (1980), with only one experiencing alveolitis alone.

Another study in poultry workers found a correlation between intensity of serum precipitins to avian antigen and presence of large airways obstruction (Wuthe et al., 1980). This was considered an early feature of EAA, because the carbon monoxide diffusing capacity was normal or high but these results conflict with an earlier report from the same group (Petro et al., 1978) when diffusing capacity was affected more than pulmonary mechanics. Warren et al. (1977) closely monitored 6 persons with avian-related alveolitis for an early response to challenge but no evidence of airflow obstruction was detected by spirometry, flow-volume loops or single breath nitrogen washout. All the subjects developed late systemic symptoms and reduced vital capacity beginning 3 - 4 hours after challenge.

In summary, no single pulmonary function parameter provides an accurate guide to diagnosis, severity or progression of PBD,

but a reduced vital capacity and decreased carbon monoxide transfer are the commonest findings on routine tests and after inhalational challenge. Altered compliance, low lung volumes and small airways dysfunction may indicate an early stage in the development of chronic PBD. Finally, whilst large airways obstruction is not characteristic of PBD this finding should not preclude the diagnosis and may occasionally be an important element of the functional disturbance.

## 7. Immune Response.

While involving an artificial separation of what are increasingly recognised as inter-related events, for convenience, and because of the historical background, the immune responses to pigeon-derived antigens are considered here under the headings of humoral immunity and cell-mediated immunity.

### a) Humoral immunity.

An association between humoral immune mechanisms and avian-related alveolitis has been recognised since the first detailed report of this type of condition (Pearsall et al., 1960), when precipitins to parakeet dander were found in the serum of a budgerigar keeper with an unexplained febrile illness. Immediate and late skin test reactions to the dander preparation were also evident. Precipitins to pigeon-derived antigens are a usual feature of PBD. The antibody is primarily of the IgG class and milligram per ml. quantities of specific antibody have

been reported (Fink et al., 1969; Boren et al., 1977).

In addition to immunoglobulins directed against specific antigens the acute progressive cases are commonly associated with a generalised hypergammaglobulinaemia. Amnestic reactions in low titre to a variety of viral antigens have been reported (Boyd et al., 1967; Bach et al., 1971) and although some of these reactions may be cross-reactions due to preparation in egg media (Newman-Taylor et al., 1977) such reactions also occur with viral antigens prepared in non-avian media (Boyd, 1978). Serum rheumatoid factor has also been detected in some cases and may reflect a generalised increase in plasma cell activity (Banaszak & Thiede, 1974), although Turner-Warwick & Haslam (1971) found the incidence of these auto-antibodies similar to a healthy population.

A further auto-antibody directed against the $P_1$ antigen of the P blood group system has been found in over half of the $P_1$ negative pigeon fanciers compared to only 6% of similar non-exposed persons. This anti-$P_1$ activity is due to IgM antibody and completely removed by absorption with pigeon serum, indicating a cross-reaction between the $P_1$ antigen and avian protein (Radermecker et al., 1975; Munro et al., 1980). No correlation between anti-$P_1$ activity and susceptibility to disease has so far been found by those investigating this phenomenon. Antibody production in those exposed to various relevant antigens has been found to occur less often among

smokers (Morgan et al., 1973; Andersen & Christensen, 1983). Furthermore, Warren (1977) reported a negative correlation between smoking and EAA compared to other interstitial lung disease. Whether smoking interferes with antigen penetration or modulates the immune response itself is uncertain.

Only a few well-substantiated cases of PBD lacking serum antibody have been described (Allen et al., 1975; Sennekamp et al., 1978; Canet et al., 1980), but specific antibody is not confined to those with symptoms and all workers in this field report a humoral response to pigeon-derived antigens in a proportion of apparently healthy fanciers. Such antibodies are not found in non-exposed persons (Schribner et al., 1980). The percentage of unaffected pigeon fanciers with antibody ranges from 7.8% (Verbeke et al., 1971) to 62% (Christensen et al., 1975). Differences in the methods used to detect antibody and in the antigens tested accounts for some of the variation seen. For example, in a group of pigeon fanciers where 40% had precipitins to dropping extact only 20% had precipitins to pigeon serum (Barboriak et al., 1973). Dropping extracts develop a greater number of precipitin lines compared to pigeon serum, but also produce non-specific reactions (Berrens & Maesen, 1972).

Simple immunodiffusion has been the most routinely used method of detecting antibody, but other more sensitive methods include a radioimmunoassay (Nielsen et al., 1974), indirect immunofluorescence of avian intestine (Sennecamp et al., 1976), an avian erythrocyte agglutination test (Diment & Pepys, 1977)

and an enzyme-linked immunosorbent assay (Andersen & Christensen, 1983). All these methods will detect antibody not evident by precipitin methods, but in studies involving relatively small numbers of pigeon fanciers, differences in the incidence of avian-specific antibody are probably largely due to bias in the selection of those tested. In general, more sensitive assay methods do not improve the correlation between antibody and disease.

In several early studies Fink et al. (1967, 1968b) reported serum precipitins to pigeon-derived antigens that were found only in symptomatic pigeon fanciers, but subsequent work has failed to consistently demonstrate a response which is unique to those with PBD. Nevertheless higher titres of antibody were found in subjects with PBD and furthermore no correlation was found between the presence of precipitins and the amount of exposure to pigeons. Antibody was therefore considered to have an important role in the development of the disease. However, in a later study this group (Fink et al., 1972) reported an association between both the presence and intensity of precipitin response to pigeon protein and an increasing exposure "index". None of the pigeon fanciers in this survey was considered to have PBD and the authors, therefore, suggested that production of specific antibody primarily reflects the degree of exposure. These latter findings have been widely reported, and accepted, although there is in fact surprisingly little precise information concerning degree of exposure, antibody response and symptoms.

The correlation between a specific antibody response and EAA

varies considerably between the different syndromes, but is closest in budgerigar fancier's lung where antibody production is rare in the absence of symptoms and poorest in poultry workers, up to 50% of whom may have antigen-specific antibodies but in whom EAA is unusual (Pepys, 1973).

Early studies defined some of the functional capability of the serum avian-specific antibodies by demonstrating passive cutaneous anaphylaxis (Fink et al., 1967) and an avid complement fixing capacity (Caldwell et al., 1973; Moore & Fink, 1974). More recently, with the development of better immunological techniques, further categorisation of the humoral response has been possible. Faux et al. (1971b) were the first to show immunoglobulin of several classes against avian antigen in their studies of budgerigar fanciers. Higher levels of avian-specific IgG, IgM and IgA antibodies were found in those with budgerigar fanciers' lung, whereas unaffected persons had mainly IgG antibodies. Patterson et al. (1976) found higher levels of IgA and IgG antibodies in an individual with severe PBD using a polystyrene tube radioimmunoassay, than in a group of less affected pigeon fanciers. Subsequently using the same method a larger group was studied including estimation of avian-specific IgG and IgA antibodies in broncho-alveolar lavage fluid (Patterson et al., 1979). There were significantly higher mean levels of serum and lavage IgG and IgA in those with PBD but considerable overlapping with asymptomatic fanciers was evident. In all cases IgA activity was higher in broncho-alveolar lavage

fluid than in serum but this was not the case for IgG.

There has been no convincing report of significant avian-specific IgE production in relation to PBD, although Patterson et al. (1976) found evidence of reaginic antibody activity which was destroyed by heating to 56°C in one patient but the radioimmunoassay was unable to detect IgE. A few studies have also found indirect evidence in support of IgE involvement. Redmond et al. (1975) reported a young boy with severe PBD who had a very high level of serum total IgE, but without a history of Type I allergy, and where the IgE level declined after cessation of exposure. Keith et al. (1981) reported similar findings in another child. Faux et al. (1971a), however, investigating budgerigar fanciers found avian-specific IgE essentially confined to persons with avian-induced asthma. Cohen et al. (1979) also failed to identify specific IgE in patients with PBD.

Evidence of reaginic antibody activity of some type is readily apparent from the results of skin testing with pigeon-derived material. Faux et al. (1971a) and Hargreave & Pepys (1972) suggested this might be the short-term skin sensitising antibody (S.T.S.-IgG) now thought to be of the $IgG_4$ sub-class (Parish, 1974). Supporting evidence that this reaginic activity is not IgE has come from passive transfer studies in man. Warren et al. (1977) found that reaginic activity responsible for immediate intradermal skin test reactions with avian material could be transferred in serum, but unlike IgE activity was

retained for only a short period (4 hours) and was resistant to heat or 2-mercaptoethanol. Freedman et al. (1981) reported similar findings with respect to immediate skin test responses using farmers' lung disease antigens.

Skin test responses to pigeon-derived material have also been regarded as a possible reflection of immune events occurring within the lungs and attention has focused on the late, 6 - 12 hour skin test reaction which coincides with the onset of the clinical syndrome. Biopsy of this oedematous swelling demonstrates an acute perivascular inflammation with polymorphonuclear leucocytes predominating, and immunofluorescence has identified localised immunoglobulin and complement at the injection site (Pepys, 1969; Caldwell et al., 1973). This type of skin test reaction was first observed by Maurice Arthus (1903) and this Arthus-type response has been widely used to describe local immune-complex mediated tissue injury involving precipitating antibody (Gell & Coombs, 1968).

The late-onset skin test reactions have been considered the most characteristic cutaneous responses and by extrapolation of such findings to the lungs, an immune-complex mediated basis for the disease was postulated (Pepys, 1969). The preceding immediate response appears to have been somewhat overlooked although a review of 60 cases of PBD where adequate details of skin test responses are given (Table 2) shows that there were 80% with an immediate intradermal response to pigeon-derived antigens and 63.3% with a late-onset reaction. Very few cases (3.3%) had a

TABLE 2 Skin test findings in P B D

| AUTHORS | NUMBER STUDIES (PBD) | ANTIBODY POSITIVE | * ATOPIC | Skin tests, avian-antigen EARLY RESPONSE | Skin tests, avian-antigen LATE (6 hr) RESPONSE | OTHER IMMUNE RESPONSE |
|---|---|---|---|---|---|---|
| Fink, Sosman, Barboriak, Schlueter & Holmes (1968) | 13 | 13 | 4 | 10 | 12 | 7 patients with hypergamma-globulinaemia |
| Elgefors, Belin & Hanson (1971) | 11 | 7 | No details | 5 | 2 | No Type IV reaction detailed |
| Moore, Fink, Barboriak, Ruff & Schlueter (1974) | 5 | 5 | 3 | 5 | 5 | No Type IV skin test reactions. 4 persons had lymphocytes sensitised to P.D.E. |
| Christensen, Schmidt & Robbins (1975) | 11 | 9 | 5 | 11 | 6 | |
| Redmond, Thomas, Magill Lowry & Stanford (1975) | 2 | 2 | 1 | 2 | 0 | 2 children with P B D, both raised serum IgE, but neither very atopic. |
| El-Hefny, Ekladious, El-Sharkawy, El-Ghadban, El-Heneidy & Frankland (1980) | 15 | 9 | 7 | 12 | 10 | 7 patients also reported to have Type IV reactions. All had sensitised lymphocytes. |
| Keith, Holsclaw & Dunsky (1981) | 3 | 3 | 1 | 3 | 3 | All hypergammaglobulinaemia |

* Atopy variously defined from history and prick test responses to common allergens or avian antigens.

late-onset reaction without a preceding immediate response. More late-onset reactions might have occurred with higher concentration of antigens, since a relationship has been found between intensity of the early reaction and likelihood of a subsequent late-onset reaction (Pepys, 1969). In those studies reporting the skin test responses amongst the wider population of pigeon fanciers (Elgefors et al., 1971; Christensen et al., 1975; El Hefny et al., 1980) both immediate and late-onset reactions have been found in asymptomatic persons, so that again there is no absolute separation between healthy and affected fanciers.

Two quite recent studies using farmers' lung disease antigens have reported a positive correlation between the presence of antigen-specific serum precipitins and a positive immediate intradermal skin test (Freedman et al., 1981; Edwards & Davies, 1981). Since the methods used did not discriminate between IgG sub-classes, these findings are in keeping with the view that $IgG_4$ is responsible for this skin test response. Unfortunately relatively few papers have given information concerning IgE reaginic antibody in PBD (atopic status, prick tests to avian antigens, serum IgE level) but the findings detailed in Table 2 suggest that a significant proportion of those affected may be atopic, and in such cases avian-specific IgE might be present.

A number of studies have investigated the possible involvement of immune-complex tissue injury more directly by measuring circulating immune complexes or complement activation.

Moore et al. (1974) monitored serum complement ($CH_{50}$) following aerosol challenge with pigeon serum in 4 people with PBD and a similar number of unaffected fanciers. Only the unaffected subjects showed any alteration in complement levels, being reduced. It was concluded that antigen only gained access to the peripheral circulation in healthy persons resulting in "non-toxic" immune complexes as part of the antigen clearance mechanism. Schatz et al. (1976a) reported similar findings but a symptomatic person also had a significant fall in serum complement some 8 hours after challenge. The dynamic nature of the complement system and the temporal variability of PBD makes interpretation of this kind of data difficult and similar considerations apply to measurements of circulating immune complexes. Yang et al. (1978) found complexes in only 2 of 10 people with BFL and similarly in 2 of 10 unaffected fanciers, although the immune complex levels were much higher in those with disease. Studies undertaken at Glasgow Royal Infirmary (McSharry et al., 1981) commonly detected complexes in the serum of pigeon fanciers. Complexes correlated with circulating avian-specific antibody rather than PBD, but in several patients monitored during recovery a rapid decrease in circulating complexes was found in advance of the declining antibody level.

Direct activation of the complement system (alternative pathway) has also been demonstrated (Edwards et al., 1974; Berrens and Guikers, 1972b), but the most potent complement activators in pigeon dropping extracts are "non-specific"

antigens also responsible for false-positive precipitin tests. Whether direct antigen activation of complement is important in the development of PBD remains speculative.

b) Cell-mediated immunity

Jean et al. (1970) and Bach et al. (1971) were the first to report lymphocyte stimulation by pigeon-derived antigen in PBD. Further studies using both the technique of measuring macrophage migration inhibition resulting from antigen stimulation of lymphocytes (Caldwell et al., 1973) and antigen induced lymphocyte proliferation estimated by uptake of tritiated thymidine (Hansen & Penny, 1974) have confirmed the presence of specifically activated circulating lymphocytes in PBD. These studies did not find activated lymphocytes in a small group of asymptomatic pigeon fanciers or non-exposed controls.

Allen et al. (1975) demonstrated macrophage migration inhibition in several precipitin-negative fanciers with PBD and also demonstrated, for the first time, that specific T-lymphocyte activation was not confined to symptomatic persons. Schatz et al. (1976b) using a lymphocyte culture method also found evidence of lymphocyte stimulation in an unaffected pigeon fancier and another person not exposed to pigeons but who had undergone skin testing with pigeon serum. The lymphocyte responsiveness in this lattter subject increased following cutaneous challenge. These investigators observed the lymphocyte stimulation at intervals after cessation of exposure in the individual with PBD and found a significant decline in antigen-induced proliferation

coincidental with the clinical recovery. Sennecamp et al. (1978) also reported a decline in lymphocyte transformation following cessation of contact in a precipitin-negative person with PBD. The preservation of phytohaemagglutin stimulation was considered an indication that concurrent steroid therapy was not responsible for the decline in lymphocyte transformation. However, this conclusion needs to be reviewed as Toogood et al. (1980) found a depression of lymphocyte transformation to pigeon antigens persisting for 24 hours after a single intravenous injection of 250 mg hydrocortisone, yet phytohaemagglutin responsiveness was unaltered. These authors discuss a number of other factors which may influence lymphocyte responsiveness to antigen, such as age, and non-steroid drugs. Considerable experience is required to give reliable results with these tests and care is required in their interpretation.

Despite the firm evidence which has accumulated indicating specific cell-mediated immunity to pigeon derived antigens, a delayed 24 - 72 hour skin test response typical of cell-mediated immunity has rarely been found using pigeon derived material (Roberts & Moore 1977; Pepys 1977). Sennecamp et al. (1978) reported an isolated Type IV reaction in a patient with PBD lacking avian-specific antibodies. Warren & Woolf (1972) found that Type IV reactions could be identified in PBD if the immediate and 6 hour responses were inhibited by antihistamines. The late-onset reaction usually resolves within 24 hours but may sometimes persist for longer (Stiehm et al., 1967) and might

thereby occasionally mask a delayed, Type IV response. The investigation of cell-mediated immunity, like the humoral immune response, has been extended with the development of the technique of broncho-alveolar lavage via the fibreoptic bronchoscope. Moore et al. (1980) reported lymphocyte responsiveness to pigeon serum from peripheral blood and bronchoalveolar fluid in both systomatic and asymptomatic pigeon fanciers. Circulating lymphocytes from 5 of 8 persons with PBD demonstrated antigen-induced proliferation, but so too did 5 of 9 people without symptoms. A better correlation was found in respect to broncho-alveolar fluid since 7 of 8 persons with PBD had a positive test compared to only 2 of 9 without symptoms. The significance of such findings is uncertain, especially as some persons had antigen-reactive cells in the peripheral blood but not in the lungs and others vice versa. A common feature of these studies on lavage fluid, also reported by other workers (Reynolds & Newball, 1974; Voisin et al., 1981) is the large number of T-lymphocytes in bronchoalveolar fluid obtained from persons with EAA.

Investigation of lymphocyte subsets has shown an increased suppressor (OKT8) to helper (OKT4) ratio in lavage fluid from persons with EAA (Semenzato et al., 1986), including PBD (Leatherman et al., 1984). These findings contrast with the increased lymphocyte content of lavage fluid in sarcoidosis which is predominantly due to helper cells (Hunninghake et al., 1984).

The significance of these findings is uncertain but there is a great deal of current research into immunoregulatory aspects of EAA and consequent modulation of the pulmonary inflammatory response (Salvaggio & deShazo, 1986).

8. **Immunopathogenesis.**

From the preceding review of the literature regarding avian-specific immune responses it is evident that exposure to pigeons can result in a wide range of immunological reactions, and activation of humoral and cell-mediated events is usually found in those with PBD. Whilst evidence of cell-mediated immunity probably correlates best with the presence of symptoms, the techniques used have not been applied to large numbers of pigeon fanciers. It is clear that no single immune mechanism provides a satisfactory explanation for all the known clinical, immunological and histopathological findings.

A number of animal models of EAA have been developed to investigate immunopathogenesis. Both complement-dependent, humoral-mediated (immune complex) pulmonary inflammation and cell-mediated lung damage have been elicited by aerosol challenge with pigeon-derived antigens in previously immunised animals (Barboriak et al., 1976; Roska et al., 1979). In general, animals with a predominantly humoral response develop an acute haemorrhage inflammation with a polymorphonuclear infiltrate, whereas specific lymphocyte activation is associated with a more chronic interstitial pulmonary inflammation with granulomas (Richerson, 1974). This difference between the lung lesions

produced by humoral-dependent or cell-mediated mechanisms is supported by studies of the passive transfer of hypersensitivity using serum or cells. These experimental models require both adjuvants and intense antigenic stimulation to induce an inflammatory response, necessitating caution in extrapolating to EAA in humans.

Studies using a monkey model of PBD which is more closely related to the disease in humans, indicate that elements of both humoral and cell-mediated immunity contribute to the condition (Hensley et al, 1974). Other authors (Roberts & Moore, 1977; Bernado et al., 1979) also consider a combination of specific immune responses are involved in the development of the condition with humoral mechanisms likely to be relevant in the early phase and cell-mediated events subsequently. In contrast Burrell & Rylander (1981) postulated that the early inflammatory changes in EAA were due to non-specific activation of complement by antigen which persists in the lung leading to lymphycyte and alveolar macrophage stimulation and development of the typical histopathological findings. The alveolar macrophage has a functional capability including phagocytosis, processing of antigens and release of a variety of chemotactic factors which suggests an important role in the effector mechanism of EAA (Stankus et al., 1978; Schatz et al., 1979).

As was discussed earlier further characterisation of the lymphocytic response in serum and lavage fluid has focused recent attention on immunoregulatory events in EAA. Keller and co-workers (1982a) reported increased peripheral blood suppressor T-lymphocyte activity in both asymptomatic monkeys exposed to

pigeon antigens and in asymptomatic pigeon fanciers (Keller et al., 1982b), postulating that suppressor activity could inhibit the development of EAA. This group subsequently reported finding similar OKT8 lymphocyte counts and ratios amongst symptomatic pigeon fanciers (Keller et al., 1984) but there were differences in blastogenic activity between lavage lymphocytes from symptomatic compared to asymptomatic persons. This heterogeneous nature of OKT8 lymphocyte populations was also evident in a similar study of farmer's lung disease patients (Semenzato et al., 1986), and further clarification of lymphocyte function and interaction is awaited. Downward modulation of inflammatory responses by suppressor factors or cells does not however, appear to involve classic immunological tolerance since animal models subjected to repeated challenge demonstrate waning histological responses without diminution of cell-mediated or humoral reactivity (Schuyler & Schmitt, 1984).

In summary the immune mechanisms involved in the development of PBD are now recognised to be much more complex than originally postulated and the present view is of an early phase of acute inflammation after antigen inhalation mediated via humoral-dependent events or non-specific alternative pathway complement activation followed by a predominantly cell-mediated process, but with an expanding population of suppressor T-cells and their products which may modulate the degree of granulomatous inflammation.

## 9. Outline of present studies

### a) Clinical

There are several differences between the type of patient with PBD encountered in hospital practice and cases identified during surveys of active pigeon fanciers. A detailed assessment of symptoms amongst active pigeon fanciers was undertaken (1979/80) with particular emphasis on the relevance of immediate responses to exposure, and the occurrence of chronic symptoms. Criteria for categorising symptom status which might be applicable to future survey work have been formulated and validated. The spectrum of PBD is reappraised and a modified classification proposed

### b) Exposure

Criteria established from the symptom study were retrospectively applied to a wider survey of the pigeon keeping population. A radioimmunoassay was used to determine IgG antibodies against pigeon globulin and the relationships between this humoral response, exposure factors, and clinical status have been clarified and extended.

### c) Immediate hypersensitivity

In view of an interest in those events occurring in the early phase after avian contact as possible "trigger" or "permissive" mechanisms for other immunological phenomena leading to the full clinicopathophysiological disorder, immediate hypersensitivity aspects of the humoral response were

investigated. A large series of skin tests were performed employing a range of common and avian-specific antigens, and the responses examined with respect to associated clinical and serological findings.

d) Physiological measurements

A series of loft and laboratory-controlled challenge tests were undertaken to support the clinical findings and to investigate any early post-exposure alteration in lung function. Tests included sensitive parameters of airflow obstruction (flow-volume loop) and of ventilation perfusion inequality (alveolar-arterial oxygen tension gradient). Finally lung permeability has been measured as a sensitive indicator of physiological integrity in some of the affected but actively exposed, type of pigeon fancier.

# CHAPTER II

## Methods

### CLINICAL ASSESSMENT

The clinical information upon which a medical diagnosis is normally based is obtained from a detailed medical interview and physical examination of the patient, but for the purposes of many clinical studies a structured, standardised method of documenting clinical details is required. One such method, the medical questionnaire enables information to be obtained from large numbers of patients and is especially important for epidemiological studies where access to people can be limited. The pigeon fanciers investigated in this thesis came from several sources including both persons referred to Glasgow Royal Infirmary Respiratory Unit by their G.P.s or other specialists, and those contacted in various field studies. The clinical information in all cases has been documented in a questionnaire form and the same criteria regarding clinical status (Chapter III) have been applied throughout.

#### 1. Questionnaire.

A large scale clinical study of pigeon fanciers was carried out under the direction of Dr G Boyd during 1976 and 1977 with

the enthusiastic support and co-operation of the Scottish National Flying Club. A series of meetings was arranged at selected pigeon racing clubs and all local pigeon fanciers were actively encouraged to participate. They were asked to complete and return a questionnaire and to provide a 20 ml venous blood sample. There were 277 data sets obtained, and although not involved with the field study, an analysis of this clinical material performed by myself from the raw data is presented in Chapter III.

This study lacked detail in some aspects of symptomatology and a further questionnaire-based field study was designed by myself. The problems in obtaining and utilising data from medical questionnaires or clinical forms have been discussed in a series of articles by Wright & Haybittle (1979), and a number of factors were considered in the form of the questionnaire:

a) Form-filling.

The 1976/77 study illustrated some of the difficulties encountered with a self-completed questionnaire. There were 32 questionnaires (11.5%) with unsatisfactory replies to questions about symptoms. Sometimes the question had been misunderstood and contradictory answers given, but the commonest problem was uncertainty about answers where the form-filler had been required to delete inappropriate replies. In most instances the correct interpretation could be deduced, but necessitated a laborious checking procedure. In order to reduce these problems the new clinical study forms were completed in a direct interview either by myself, or in my presence, but a questionnaire format was retained.

b) General design.

The questions were kept as simple as possible with the answers mainly 'Yes' or 'No', and although the form filling was well controlled the replies consisted of ticks to appropriate boxes to minimise error in completion. The form was designed to be completed in about 10 minutes and the draft questionnaire was tested on a group of pigeon fanciers (Stenhousemuir Racing Club), and their comments sought before finalising the format.

c) Information requested

The full questionnaire is detailed in Appendix 1. Clinical information concerned the following topics:-

**Questions 1 - 7,** the intensity, diversity and character of avian exposure.

**Questions 8a - f,** symptoms experienced during or immediately after exposure.

**Questions 13a - h and 15,** symptoms experienced 6 - 12 hours after contact

**Questions 9 - 12, 14, 16, 17, 18a - d and 19,** severity of symptoms

**Questions 20 - 27,** chronic respiratory symptoms

**Questions 28a - f, 29 and 30,** background medical history

**Questions 31a - g, and 32 - 35,** smoking and occupational dust exposure

**Questions 36a - d, 37 and 38,** personal or family history of allergy

d) Data handling.

The information was collected in such a way as to enable translation to a code suitable for transferring the data to the disc storage of a PDP 11/45 computer. This coding procedure also provided a useful check as to the correctness of completed forms.

## IMMUNE RESPONSE

Several different aspects of the humoral immune response were studied. A radioimmunoassay for serum IgG antibody to pigeon globulin is described first followed by a paper radioimmunosorbent test for total serum IgE. A range of skin tests directed at detecting atopy, specific pigeon-related Type I and Type III responses, are described,along with the Tine test as an indication of cell-mediated immunity.

### 1. Preparation of pigeon-derived antigens.

Blood was drawn from ether anaesthetised pigeons (between 20 - 30 mls from each bird) by cardiac puncture, and left at room temperature for several hours to clot. The serum was separated by centrifugation at 3000 r.p.m. for 20 minutes.

a) Antigens for skin tests and challenge tests.

Fresh pigeon serum was heated for 20 minutes at 56$^{o}$C in order to inactivate complement, and then passed through a 0.22 um millipore filter. Samples of serum were then tested for non-specific pyrogen reactions (Blood Transfusion Services, Law Hospital). Thereafter 2ml aliquots were freeze-dried, capped in

sterile containers, and kept at 4°C until used.

b) Antigens for serology.

The globulin fraction of fresh serum was precipitated by adding dropwise, saturated ammonium sulphate solution to a final concentration of 35% (Herbert, 1974). The precipitate was separated by centrifugation at 3000 r.p.m. for 20 minutes and then washed with 35% ammonium sulphate solution prior to further centrifugation. The pellet was redissolved in a small amount of 0.15M saline and dialysed in Visking tubing (Medicell Ltd) against several changes of 0.1M phosphate buffered saline, pH 7.2. The protein solution was concentrated to 20 mg/ml and passed through a Sephadex G-200 (Pharmacia Ltd) column (100 x 2 cm) in 5ml aliquots. Four protein peaks emerged and the second, containing the main gammaglobulin antigens (Walbaum & Biquet, 1971; Fink et al., 1969), was pooled and concentrated to 20 mg/ml by pressure dialysis (Amicon, Ltd). This antigen preparation was stored at -20°C until used.

## 2. Estimation of avian-specific IgG (radioimmunoassay).

The method described by Nielsen et al. (1974) was used to measure serum IgG antibody to pigeon gammaglobulin antigens.

a) Preparation of solid-phase antigen.

One gram of cyanogen bromide-activated Sepharose 4B beads (Pharmacia Ltd) was mixed with 3ml of 0.1M bicarbonate buffer, pH 9.6, and 2mls of pigeon gammaglobulin solution (40 mg protein). This mixture was left overnight at 4°C continuously agitated by a

magnetic stirrer. After centrifugation the supernatant with unbound protein was removed and the Sepharose with covalently linked protein washed twice with 0.1M bicarbonate buffer. The Sepharose-globulin was then treated with 0.1M ethanolamine, pH 8.0 for 2 hours at room temperature to inactivate any unreacted protein binding sites. The solid phase antigen preparation was washed with 3 cycles of an acid rinse (0.1M acetate buffer, pH 4.2) and an alkali rinse (0.1M bicarbonate buffer, pH 8.6), and finally with 2 washes of asay buffer (0.1M phosphate buffered saline, pH 7.4 - containing 0.01% Triton-X). The antigen preparation was diluted to 30 ml with wash buffer plus 0.02% azide preservative and stored at $4^{o}C$ until use.

b) Radiolabelling of anti-human IgG

Anti-human IgG, gamma chain specific, raised in sheep was donated by the Scottish Antibody Production Unit (Law Hospital, Carluke). The gammaglobulin fraction was obtained by precipitation via the dropwise addition of saturated ammonium sulphate until 40% saturation was achieved. The precipitated globulin was collected by centrifugation, dissolved in a minimal volume of 0.15M saline and dialysed in Visking tubing (Medicell Ltd) for 24 hours against several changes of saline. The protein solution was concentrated by pressure dialysis (Amicon Ltd) to 30 mg/ml and divided into 2ml aliquots. Each aliquot was radiolabelled with 500uCi of iodine-125 ($I^{125}$)(carrier-free, code IMS30, Amersham) by the method of McConahey & Dixon (1966). Briefly the anti-human globulin and $I^{125}$ are mixed with 150 ul of

Chloramine-T at 2mg/ml and the reaction terminated after 1 minute by the addition of 200 ul of sodium metabisulphite. Unbound radio-iodine was separated by passing the mixture through a 30 x 1cm Sephadex G-25 fine column (Pharmacia Ltd) and retaining the first high molecular weight peak, which usually had an uptake of 70% of the radiolabel.

c) Procedure.

By micropipette 30 ul of test sera was added to test tubes in duplicate, and 250 ul of Sepharose-antigen added. After 30 to 60 minutes incubation at room temperature unbound protein was removed by 3 successive cycles of washing with assay buffer and centrifugation at 3000 r.p.m. for 10 minutes. The Sepharose-antigen-anti-pigeon globulin antibody complex was incubated with 100 ul of $I^{125}$ for 2 hours and the unbound radiolabel removed by 3 washings with the supernatant collected in a large glass reservoir for appropriate disposal. Serial dilutions of a standard serum for which the IgG specific for pigeon gammaglobulin had been measured by quantitative precipitation were included. The samples were counted in a gamma counter (Gammaset) for 2 minutes, along with 2 tubes to which no radiolabel had been added (background radioactivity) and 2 tubes containing only 100 ul of radioiodine (total activity). The specific IgG antibody titre was expressed in micrograms per millilitre by interpolating the counts (computer programme, log: log regression; Dr. W. Gray) onto the standard curve constructed for the known samples.

Control serum from non-exposed persons has no detectable antibody with a value of <1 ug/ml. In a series of control samples no level above 3ug/ml was found (Boyd, 1975). Therefore a value of >4ug/ml of antibody has been considered a significant humoral response i.e. immunisation as defined by Herbert & Wilkinson (1977).

3. **Estimation of IgE (paper radioimmunosorbent test.)**

The serum total IgE (U/ml) was measured using a paper radioimmunosorbent test (P.R.I.S.T.). Paper discs with anti-IgE covalently bound (Phadebas kits, Pharmacia) are incubated with test samples along with a series of known IgE Standard solutions. The IgE in the unknown samples and the Standard solutions reacts with the paper disc linked anti-IgE. After washing, the discs are incubated with radiolabelled anti-IgE to form a complex of paper anti-IgE + IgE + radiolabelled anti-IgE. After further washing the retained radioactivity is measured in a gamma counter and comparison of the counts between the known Standards and serum samples enables the IgE levels for the latter to be calculated.

In detail, serum samples were diluted 1:10, doubling dilutions of the IgE Standard were prepared to give 100, 50, 10, 2.0, 1.0 and 0.5U IgE/ml respectively. In duplicates, 100 ul of either an IgE Standard or test serum was added to test tubes containing the anti-IgE paper discs. After incubation for 3 hours at room temperature the tubes were aspirated and 2.5 ml

aliquots of saline added. The tubes were aspirated again after 10 minutes and this washing procedure repeated a further twice. Then 100 ul of $I^{125}$ labelled anti-IgE was added to the tubes and left for 12 hours. The tubes were aspirated and washed 3 times with saline as previously described. After the final aspiration the radioactivity retained by the paper discs was measured by a gammacounter (Gammaset), which read each tube for 2 minutes. Two tubes containing paper discs to which $I^{125}$ IgE had not been added were read, as were 2 tubes containing only 100 ul of $I^{125}$ anti-IgE. These measurements provide the background radioactivity and total radioactivity respectively.

The standard curve was constructed on a graph of IgE level (U/ml) against % total activity plotted on a log-linear scale. The % total activity for the known IgE values was calculated according to the formula:-

$$\%\text{total activity for IgE Standard} = \frac{\text{Mean count rate of IgE Standard x 100}}{\text{Mean count rate of total activity}}$$

A typical curve is shown in Figure 1. Serum samples were tested in several batches with some samples repeated on each occasion to check the reproducibility of the results.

Although atopy may be suspected in persons with a total serum IgE level above 100 U/ml, in the present investigations only a value of >200 U/ml has been considered significantly elevated in keeping with the range used by the routine laboratory service (Department of Bacteriology, Western Infirmary, Glasgow).

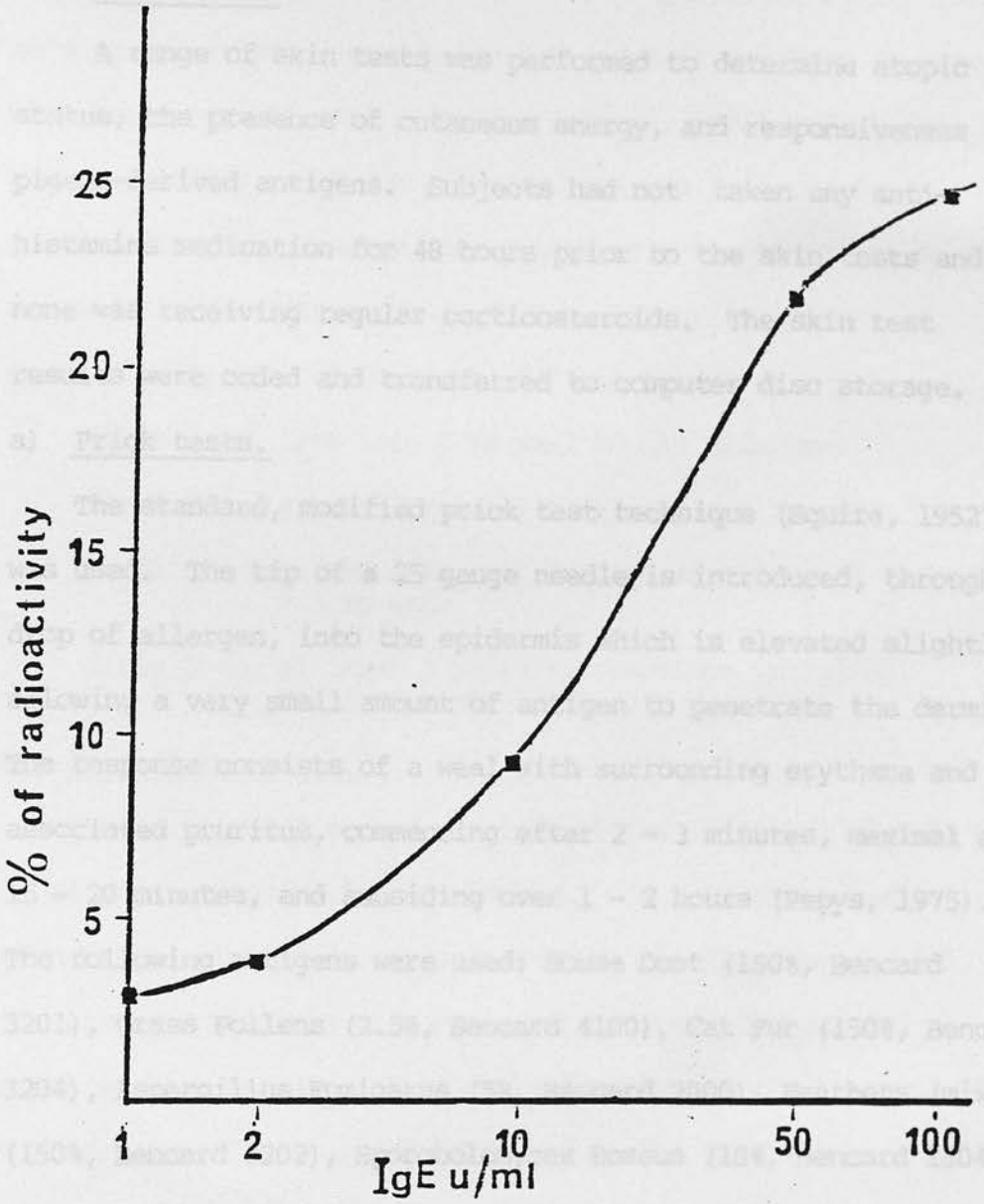



FIGURE 1 Standard curve for serum IgE (u/ml) by paper disc, radioimmunosorbent test.

A plot of the % total radioactivity obtained for known Standard IgE samples. Test samples interpolated onto curve and serum IgE level (u/ml) obtained by multiplying x10, due to dilution factor. The 0.5 u/ml Standard not shown because of the scale of the illustration.

4. **Skin tests.**

A range of skin tests was performed to determine atopic status, the presence of cutaneous anergy, and responsiveness to pigeon-derived antigens. Subjects had not taken any anti-histamine medication for 48 hours prior to the skin tests and none was receiving regular corticosteroids. The skin test results were coded and transferred to computer disc storage.

a) Prick tests.

The standard, modified prick test technique (Squire, 1952) was used. The tip of a 25 gauge needle is introduced, through a drop of allergen, into the epidermis which is elevated slightly allowing a very small amount of antigen to penetrate the dermis. The response consists of a weal with surrounding erythema and associated pruritus, commencing after 2 - 3 minutes, maximal at 15 - 20 minutes, and subsiding over 1 - 2 hours (Pepys, 1975). The following antigens were used; House Dust (150%, Bencard 3201), Grass Pollens (2.5%, Bencard 4100), Cat Fur (150%, Bencard 3204), Aspergillus Fumigatus (5%, Bencard 2000), Feathers (mixed) (150%, Bencard 3202), Sporobolomyces Roseus (10%, Bencard 1804), Pigeon Feathers (6%. Merck Ltd), Pigeon Serum (50mg/ml protein, reconstituted sterile freeze-dried, pyrogen free, preparation), and a Control solution (Bencard 1908).

Tests were performed on the volar aspect of a forearm in a regular pattern and the weal diameter measured at 15 minutes using a Bencard perspex graduation chart. Since control tests cause weals only in dermatographic individuals (1-2% of the population), very small responses of 1mm weals have been

considered significant (Davies, 1979). However, the correlation between prick test reaction and IgE reaginic antibody is closest with large weal diameters (Davies & Pepys, 1976) and in the present studies a minimum of a 2mm weal was required for a positive response. Atopy, determined from the reactions to 5 common allergens (House dust, Pollen, Asper. fumigatus, Spors.roseus, and Cat fur), was further graded as -

Grade 0 - less than 2 mm weal to all allergens

Grade 1 - a 2 or 3 mm weal to a single allergen

Grade 2 - a 2 or 3 mm weal to several allergens or a 4 mm weal

Grade 3 - at least 3 positive reactions including one 6 mm weal.

b) Intradermal tests.

The intradermal method consists of the injection of 0.02 ml of allergen solution intracutaneously via a 25 gauge needle, producing a pinhead size papule without bleeding (Pepys, 1975). The response consists of a weal, surrounding erythema and sometimes pseudopodia. This early reaction, maximal around 20 minutes, subsides in 1 - 2 hours but may be followed by a late reaction developing 4 8 hours after antigen injection and consisting of an oedematous swelling which resolved slowly over 24 - 36 hours (Pepys et al., 1968a). Tests were performed on the volar surface of the forearms and were colour coded. The early reactions were measured at 15 minutes using a Bencard perspex graduation chart. The following preparations were used - Pigeon Feathers (0.1%, Bencard 3514), Pigeon Serum (1:5 dilution, 10 mg/ml. protein), and a Control solution (Bencard 1909). Because

of the larger quantity of antigen administered by the intradermal method, prick tests were performed first, to identify any persons highly sensitised to the pigeon-derived antigens. When a >5mm weal occurred on prick testing the concentrations of the intradermal antigen preparation were halved. (Pigeon Serum diluted to 1:10; and Pigeon Feather to 0.05%). The threshold concentration of histamine required to produce a weal is 1,000 to 10,000 times less for the intradermal test than the prick test, and non-specific weals due to irritants in the allergen solutions commonly occur with the former method (Pepys, 1975). Various criteria for positive intradermal skin tests with pigeon-derived antigens have been applied. Hargreaves & Pepys (1972) regarded only a 6 mm weal as positive, whereas Christensen et al., (1975) considered any reaction, in the presence of a negative control test, significant. In the present work control tests were omitted on one of the field studies due to a delay in laboratory supplies and a 7 mm weal has been chosen as the minimum positive response. The reactions were further graded as -

Grade 0 - a less than 2 mm weal

Grade 1 - a 2 or 3 mm weal

Grade 2 - a 4, 5 or 6 mm weal

Grade 3 - a 7, 8 or 9 mm weal

Grade 4 - a 10 mm weal or greater

In order to document any late reaction, subjects were given a card on which to indicate the reaction evident 6 to 12 hours after testing. Illustration of varying sized papules were

included and subjects asked to tick the most appropriate form (1) -a less than 5 mm raised area, (2) - a 5 to 10 mm papule, (3) - a 1 to 2 cm lump, or (4) - a larger than 2 cm swollen area. A stamped addressed envelope was provided to return the form and subjects were also asked to comment on the prick test sites at 6 to 12 hours.

c) Tine test

The 4 pronged disposable Tine test (Lederle) containing the equivalent of 5 T.U. was performed on the forearm in subjects who had not recently received a BCG vaccination and who were not on anti-tuberculous drugs. Subjects were given a return-card (Lederle) to be completed 72 hours after the test and which had illustrations of the possible responses. There has been some debate concerning the acceptability of the Tine tuberculin test but Sinclair & Johnston (1979) found a good correlation between Tine tests applied for 2 seconds and Mantoux test by regarding a Grade 2 response (ring of papules) as positive. The same technique and grading were used in the present studies.

## CLINICAL "FIELD STUDY"

A field study was conducted at several pigeon shows during the winter of 1979/80 and concerned a detailed documentation of the symptoms experienced by active pigeon fanciers and their relation to immune responses. A group consisting of myself, Dr G Boyd and Dr P Lynch with nurses and technical staff attended

major pigeon shows at Edinburgh, Ayr and Blackpool. Pigeon fanciers were informed of the proposed attendance of the research team at a particular venue through announcements at local pigeon racing clubs and publicity in the national pigeon racing press (British Homing World).

Emphasis was placed on the desire to interview individuals who had symptoms which they suspected could relate to their hobby. Nevertheless other pigeon fanciers who were willing to undergo the complete series of investigations were included, and altogether 102 people were interviewed. Those describing major symptoms were also encouraged to consult their local doctors.

1. **Clinical data.**

The questionnaire described earlier was completed by each subject in a direct interview. The classification of response is detailed in Chapter III, but 3 major categories were identified.

| | | |
|---|---|---|
| Immediate Response | - | 3 or more symptoms during or immediately following exposure |
| PBD | - | the combination of a delayed systemic and delayed respiratory symptoms or 3 delayed systemic symptoms |
| Chronic PBD | - | undue exertional breathlessness or chronic cough (>3 months of year) in non-smokers. |

2. **Skin tests.**

The full range of skin tests was performed on 100 pigeon

fanciers either personally or under my direct supervision at the time of their clinical interview in an area adjacent to the pigeon show hall. Ninety people returned the forms giving details of late skin test reactions and Tine test results, and only these cases where full data were available are presented (Chapter IV).

3. **Serological investigation.**

A 20ml venous blood sample was obtained from 100 subjects. The sample was collected into plain tubes and bottles containing potassium E.D.T.A., the latter for complement studies (C.McSharry). Serum was separated by centrifugation and divided into 2ml aliquots, which were stored at -20°C until used. The serum IgG (ug/ml) to pigeon globulin was measured using the R.I.A. and serum total IgE (U/ml) estimated by the P.R.I.S.T. (Pharmacia).

PHYSIOLOGICAL MEASUREMENTS

1. **Ventilation.**

Measurements of static and dynamic lung volumes were made with the subjects seated and all values were corrected to body temperature and pressure saturated with water vapour (B.T.P.S.).

a) Spirometry

The vital capacity (VC), forced vital capacity (F.V.C.) and forced expiratory volume in 1 second ($FEV_1$) were measured

directly using a low-inertia spirometer (Bernstein et al., 1952; P.K. Morgan Ltd.) according to standard practice where the best of 3 maneouvres is accepted. The results were compared to the predicted normal range of Grimby & Soderholm (1963), with the derived $FEV_1/FVC$ ratio compared to the predicted values of Cotes (1979).

A maximum inspiratory maneouvre and fast expiratory spirogram were also performed using a pneumotachograph with flow integrated to volume and displayed on an X-Y plotter to produce a maximum flow-volume curve, breathing air. The best of 3 comparable curves was accepted. Measurements were made of peak expiratory flow rate (P.E.F.R.), V.C. and ratio of maximum expiratory flow at 25% of V.C. to flow at 75% of V.C. ($\dot{V}max^{25}/\dot{V}max^{75}$). (Figure 2).

b) Helium dilution lung volumes.

The functional residual capacity (FRC) was determined by the closed-circuit helium dilution method (Gibson & Hugh-Jones, 1949; TLC tests, P.K. Morgan Ltd.). The expiratory reserve volume (ERV) was measured by recording a maximal expiratory manoeuvre at the end of helium breathing and the residual volume (RV) derived by subtracting ERV from FRC, i.e. RV = FRC - ERV.

The total lung capacity (TLC) was calculated by adding the V.C. determined from spirometry to the R.V., i.e. TLC = VC + RV. Values were compared to predicted normal (Grimby & Soderholm, 1963).

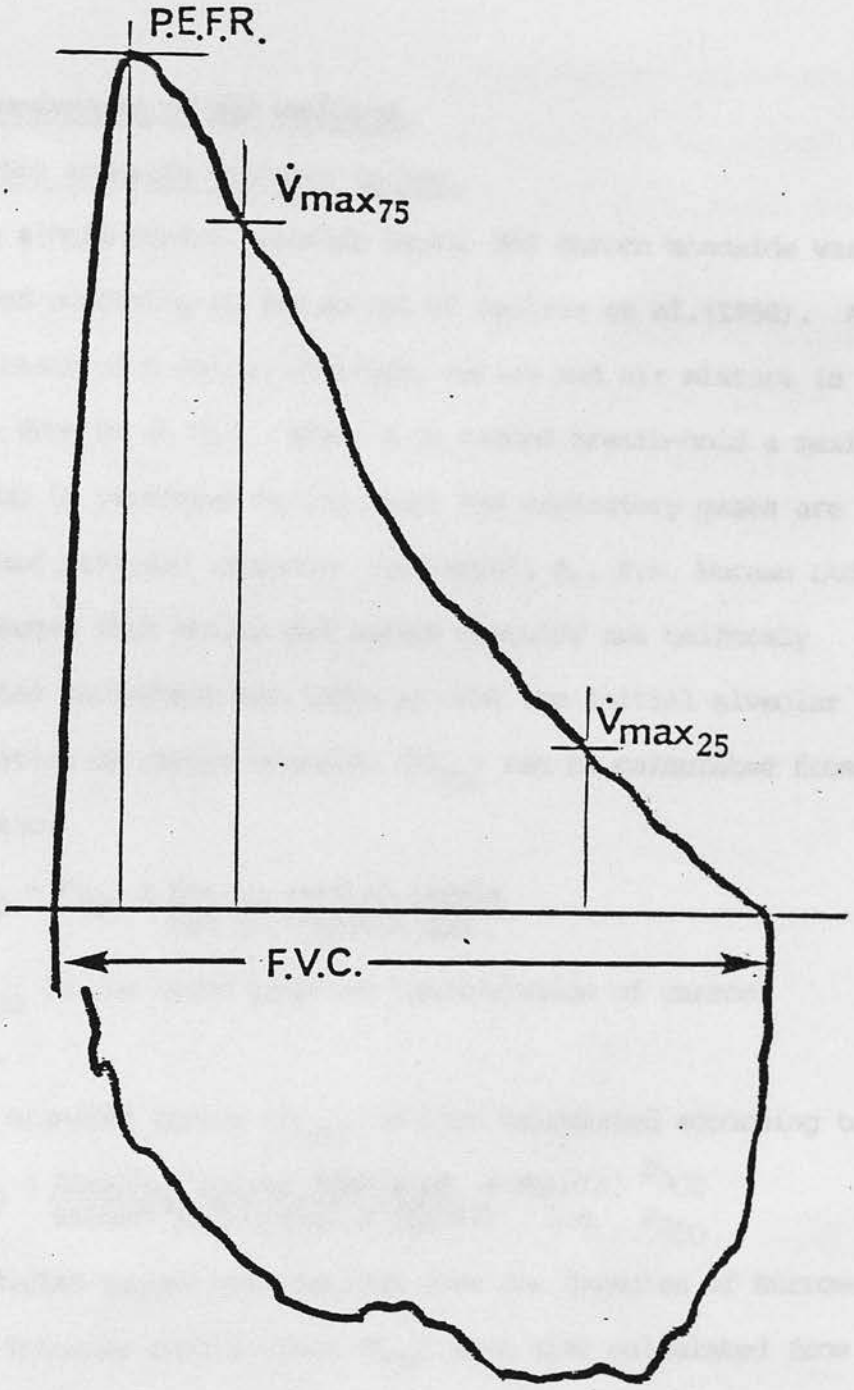



FIGURE 2 Flow-volume loop
An example of a maximal effort flow-volume loop obtained during challenge studies. The measurements were made from the best of 3 comparable recordings.

## 2. Measurements of gas exchange.

### a) Carbon monoxide transfer factor.

The single breath transfer factor for carbon monoxide was determined according to the method of Ogilvie et al.(1958). A single breath of a carbon monoxide, helium and air mixture is inspired from RV to TLC. After a 10 second breath-hold a maximum expiration is performed during which the expiratory gases are sampled and analysed (Transfer test Model, A., P.K. Morgan Ltd.). It is assumed that helium and carbon monoxide are uniformly distributed throughout the lungs so that the initial alveolar concentration of carbon monoxide ($FA_{CO}$) can be calculated from the equation

$$F_{A_{CO}} = F_{I_{CO}} \times \frac{\text{He\% in expired sample}}{\text{He\% of inspired gas}}$$

where $F_{I_{CO}}$ is the known inspired concentration of carbon monoxide.

The transfer factor ($T_{L_{CO}}$) is then calculated according to,

$$T_{L_{CO}} = \frac{\text{Alveolar volume STPD} \times 60}{\text{Breath hold (secs)} \times (P_B - 47)} \times \text{Natural } \log \frac{F_{A_{CO}}}{F_{E_{CO}}}$$

Predicted values were derived from the formulae of Burrows, (1961). Transfer coefficients ($K_{CO}$) were also calculated from $T_{L_{CO}}$ and VA .

### b) Arterial blood gases.

An indwelling catheter (Quik-cath, Travenol Laboratories Ltd) was inserted into the radial artery by percutaneous puncture under local anaesthesia with 2% lignocaine. A three-way tap was connected to the catheter and glass sterile syringes containing a

drop of heparin (1,000 units/ml) were attached for the withdrawal of 2 to 4 ml samples. Catheters remained in situ for around 4 hours and full aseptic technique was employed throughout. The catheter was intermittently flushed with 1ml of heparin (100 units/ml) to maintain patency. The arterial oxygen and carbon dioxide tensions were measured along with pH and the derived values for bicarbonate and base excess in a fully automated blood gas analyser (I.L.613). The alveolar-arterial oxygen tension difference ($A\text{-}aO_2$) was calculated using the alveolar-gas equation (West 1974b).

### 3. Lung permeability

The barrier between the alveolar spaces and pulmonary capillary vasculature facilitates the rapid exchange of gas but severely restricts the diffusion of large solutes. This barrier consists of surfactant, alveolar epithelium, capillary endothelium, basement membranes and interstitial space but it has been calculated that 90% of the resistance to diffusion of hydrophilic solutes lies within the alveolar epithelium which has pores of 0.8 - 1nm in radius (Jones et al. 1983). In recent years the integrity of the alveolar-epithelial barrier has been investigated by estimating the transport of suitable tracer solute molecules from alveolar to vascular space. The method developed by Jones et al. (1980) for the half-time clearance of an inhaled aerosol of 99 m-technetium labelled diethylene triamine pentacetate (99m-TcDTPA) with a molecular weight of 492 daltons from lung to blood ($T\frac{1}{2}LB$) has been most widely adopted.

The lung permeability in a group of pigeon fanciers was measured using this technique.

Subjects inhaled the output for a nebuliser system containing 200 megabecquerels (MBq) 99mTcDTPA in 5 ml isotonic saline and the breathing circuit is illustrated diagramatically in Figure 3. An important feature is the separater consisting of an array of 3 mm ballbearings through which the aerosol from a simple jet nebuliser driven at a flow rate of 8 - 10 l/min air is passed. This arrangement has been shown to produce an aerosol with particles of mean mass aerodynamic diameter 0.9 um which are predominantly retained by the peripheral gas exchanging regions of the lungs. A reservoir bag, one way valve and expired air filter are also incorporated.

The subjects inhaled for 3 - 5 minutes and estimation of residual radioactivity in the nebuliser and associated apparatus suggest a 10% uptake of the radiolabelled aerosol which is similar to the figure calculated by Jones et al. (1983). External scintillation detection was performed using a gamma camera fitted with a high sensitivity, parallel hole collimator and interfaced to a dedicated mini-computer. With the subject seated, posterior images of 60 seconds duration were obtained of the lower half of both lung fields and the inter-renal area for 30 minutes following cessation of aerosol administration. An intravenous bolus of 20MBq 99m-TcDTPA in 1ml. isotonic saline was then given via a peripheral vein and scanning continued for a further 10 minutes. Regions of interest are drawn over the lung

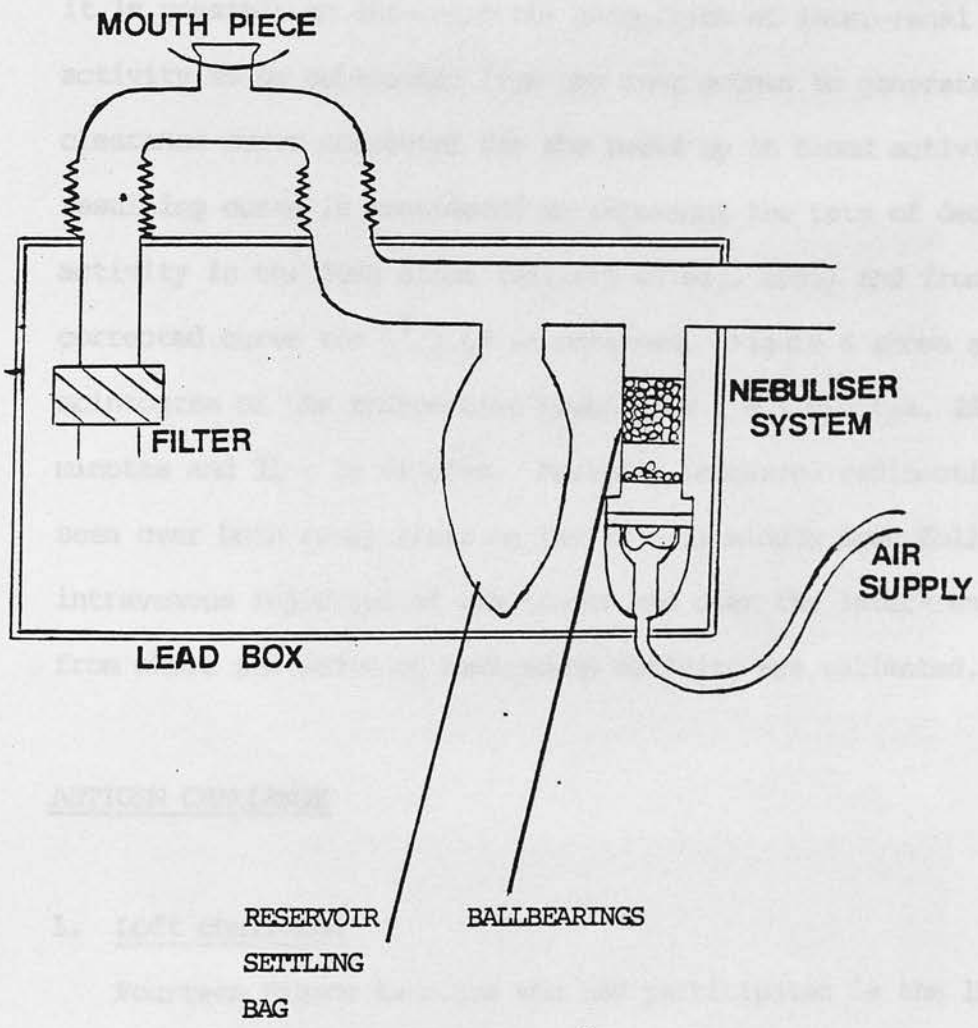


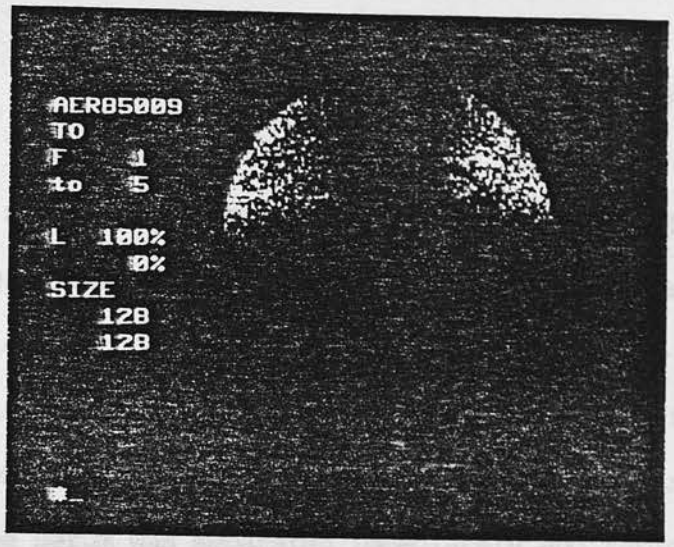

FIGURE 3 Diagragm of breathing circuit for lung permeability studies

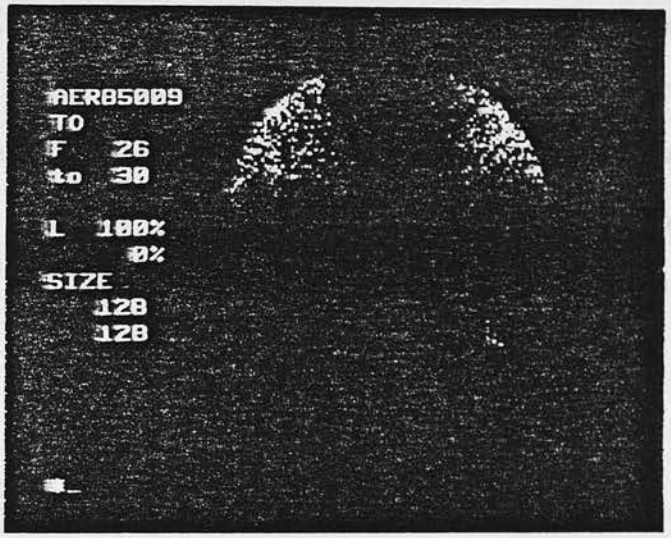
tissue and the inter-renal area, and the fall in activity is measured. The build up of blood activity within the field of the lung scan (background radioactivity) is corrected by using activity in the inter-renal area as a measure of blood activity. By measuring the ratio of the rise in activity over the lung and inter-renal areas following the injection of 20 MBq 99 m-TcDTPA it is possible to determine the proportion of inter-renal activity to be subtracted from the lung counts to generate a clearance curve corrected for the build up in blood activity. The resulting curve is considered to represent the rate of declining activity in the lung alone (Gellert et al., 1985) and from this corrected curve the $T^{1}/2$ LB is obtained. Figure 4 shows a scintogram of the radioactive count from 1 - 5 minutes, 26 - 30 minutes and 31 - 35 minutes. Markedly increased radioactivity is seen over both renal areas on the 31 - 35 minute scan following intravenous injection of the tracer and over the inter-renal area from where the index of background activity was estimated.

ANTIGEN CHALLENGE

**1. Loft challenge**

Fourteen Pigeon fanciers who had participated in the 1976/77 survey study and who reported delayed symptoms after contact with their pigeons were studied following an hour spent in the loft environment. A temporary field centre was established at a local GP's branch surgery within a short distance of all the subjects homes in the Stenhousemuir area and a further 2 pigeon fanciers



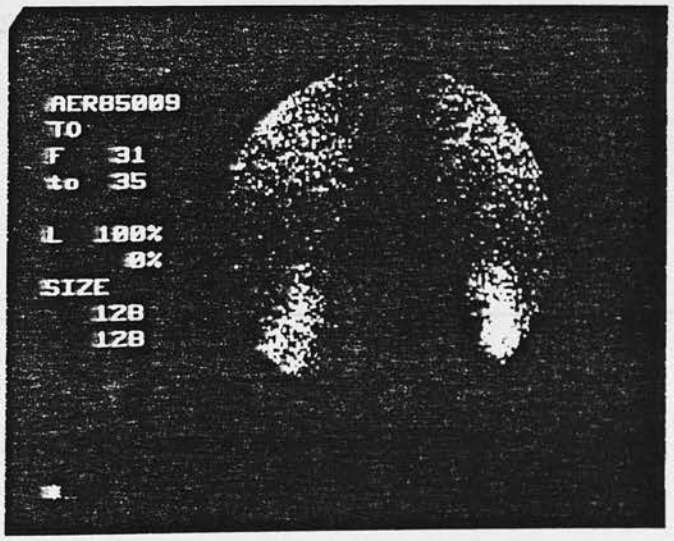



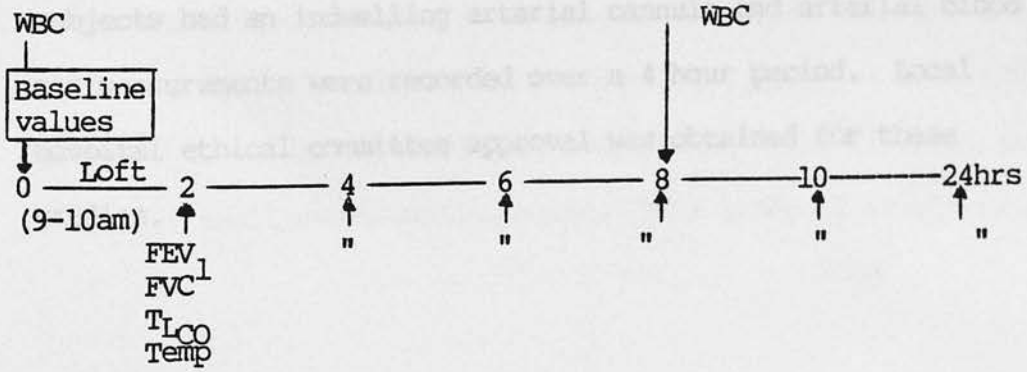

FIGURE 4

Scintigrams of 99m-TcDTPA uptake.

who fulfilled the criteria for PBD living locally also participated. All subjects were immunised to pigeon globulin (>4ug/ml) and 7 had PBD. Routine spirometry (FEV1, FVC and FEV1/FVC) and single breath $TL_{CO}$ with RV (Transfer Test Model 'A', P.K. Morgan Limited) measurements were performed immediately prior to challenge and monitored over the subsequent 8 - 10 hours with final values obtained the next morning, 24 hours after the challenge. Venous blood samples were obtained at regular intervals. The serum was separated immediately and rapidly frozen using a mixture of "dry ice" (CO2) and alcohol. The peripheral white blood cell count was determined at Time 0 and again 8 hours after challenge using an improved Neubauer counting chamber with a 1/20 dilution of blood and counting the white cells seen in the four corner 1 x 1 mm squares using a medium power lense. The white cell count/cu mm is then calculated as follows:-

$$\frac{\text{White cells seen}}{\text{Squares counted}} \times 200$$

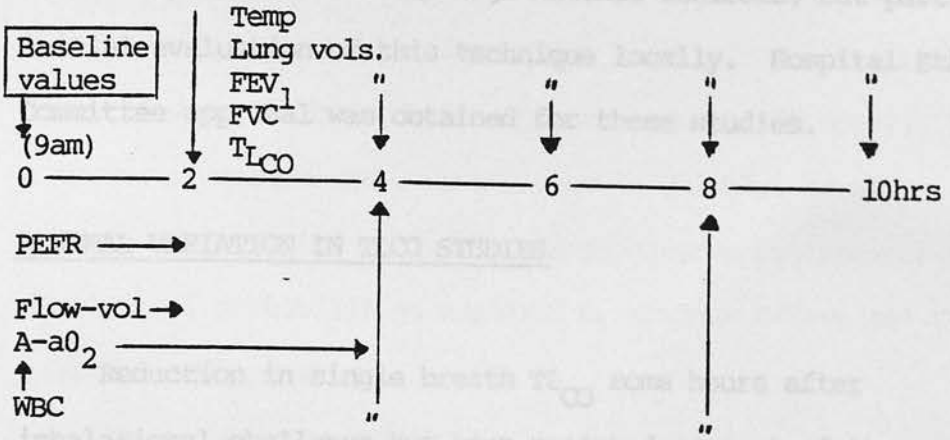
Temperature (oral) was taken at Time 0 and 2 hourly after completion of the loft challenge.



## 2. Laboratory inhalation provocation tests

Ten pigeon fanciers with PBD underwent a laboratory controlled inhalation challenge with nebulised, sterile, pyrogen free, pigeon serum. The antigen dose was determined by first performing a skin prick test with the antigen preparation in order to identify any individuals at risk for severe immediate hypersensitivity reactions (Hendrick et al., 1980). For skin test weals of <5mm then 0.25ml - 0.5ml of pigeon serum was given (50mg/ml protein). When skin test response was greater then 0.1 - 0.25ml of pigeon serum was used. The antigen was made up to a volume of 5ml with sterile water for injection and nebulised with air at 8L/min.via a mini-nebuliser face mask (Life Care Hospital Supplies Limited).

Following baseline tests serum was nebulised for 2 minutes after which PEFR was checked (mini Wright, Air Med) and if there was no significant reduction a further 3 minutes of nebulisation was given before checking PEFR again. A period of 15 - 20 minutes was required to complete the nebulisation. The standard measurements were helium dilution lung volumes, single breath $TL_{CO}$, spirometry (including flow-volume loop), temperature (oral), and peripheral white cell count. In addition some subjects had an indwelling arterial cannula and arterial blood gas measurements were recorded over a 4 hour period. Local hospital ethical committee approval was obtained for these studies.




LUNG PERMEABILITY STUDIES

Nine pigeon fanciers had a lung permeability study. These fanciers had only recently come to the attention of the Department, either through clinical referral or via contact established at a pigeon fanciers' gathering. Pigeon-related symptoms were suspected but they did not all fulfill my criteria for P B D. The studies were conducted during the afternoon in the Department of Nuclear Medicine, G.R.I. under my direct supervision and between 4 - 6 hours after the fanciers' usual brief morning contact with their pigeons. A clinical history, pulmonary function tests and immunological data were obtained but due to constraints of individual travel arrangements were not always completed at the same time as the permeability study. All the pigeon fanciers studied were non-smokers since this has been found to profoundly affect lung permeability (Minty et al., 1981) and the $T\frac{1}{2}LB$ values obtained were compared with the findings in a group of 7 healthy non-smoking males. This group of volunteers

were hospital staff and not age-matched controls, but part of an initial evaluation of this technique locally. Hospital Ethical Committee approval was obtained for these studies.

## DIURNAL VARIATION IN TLCO STUDIES

Reduction in single breath $TL_{CO}$ some hours after inhalational challenge has been reported as part of the positive response characteristic of EAA. However Cinkotai & Thomson (1966) reported a diurnal variation in $TL_{CO}$ amongst a group of normal adults and the relevance of this finding does not appear to have been pursued by other workers in this field. The single breath $TL_{CO}$ was therefore monitored under closely controlled conditions between 9 am and 5 pm in a group of 17 healthy non-smoking adults. Helium dilution lung volumes, spirometry and single breath $TL_{CO}$ were normal prior to the study. The tests were performed with the subjects sitting and having rested for 10 minutes before each set of measurements which were performed at 9 am, 11 am, 1 pm, 3 pm and 5 pm. The inspired volume was kept to within 10% of that predicted for each subject to minimise any variation caused by an altered alveolar volume. The haemoglobin, packed cell volume (Coulter counter) and blood carboxyhaemoglobin (1L282 Co-oximeter) were estimated at 9am and 5pm.

## STATISTICS

A number of simple statistical methods were used in data

analysis. Where appropriate the mean, standard deviation (SD) and standard error of the mean (SEM) were calculated for each group of data using a digital computer (PDP 11/45 deck). A computer programme was also employed in calculation of correlation coefficient (linear least squares regression) and statistical probabilities assessed by student t-test and the t-test for reduced data.

Non-parametric tests using a chi-squared analysis included contingency tables with Yate's continuity correction (2 x 2 table) and a chi-squared test for the order of proportions.

# CHAPTER III

## The clinical spectrum of PBD and inter-relations with avian exposure and avian-specific IgG response.

### INTRODUCTION

The established classification (Fink, 1983) divides PBD into acute, sub-acute and chronic forms, but these descriptions are based largely on clinical cases and their relevance to the overall population of pigeon fanciers is uncertain. Review of the literature (Chapter 1) suggests a somewhat different pattern of response in the few survey studies that have been undertaken. There are 3 particular areas that require clarification and have been investigated in the present studies. Firstly, upper respiratory tract pigeon-related symptoms which are frequently encountered in survey studies are rarely mentioned in reviews of PBD or are referred to as a separate immediate hypersensitivity reaction among atopic subjects. Secondly, the overall contribution to morbidity from sub-acute and chronic forms has remained largely undetermined due to their similarity with the symptoms of chronic bronchitis. Thirdly, the frequent finding in survey studies of PBD manifest as recurring episodes of EAA but without apparent functional deterioration has not been adequately explained in terms of the immunological and exposure factors necessary for this situation to pertain.

The lack of data in regard to the general pigeon-keeping community may partly relate to a suspicion among pigeon fanciers that medical interest will result in their being advised to give up the sport. There is indeed, a clear impression that the commitment both emotional and financial which pigeon fanciers have to their pastime is not sufficiently appreciated by those not involved, including the medical profession. However as a result of the efforts of Dr Gavin Boyd, consultant physician, and Dr Philip Lynch, a general practitioner who is himself a pigeon fancier, close links have been established with pigeon fanciers in Central Scotland, thereby providing the necessary co-operation for large scale community-based studies.

This chapter concerns 2 large scale studies of pigeon fanciers. One study conducted during the winter of 1979/80 involved a questionnaire-based interview of 100 pigeon fanciers (Chapter II) and was orientated towards detailed documentation of symptoms. Clinical criteria formulated from these data were then utilised in a further retrospective analysis of the raw data from another less detailed but potentially more representative survey of 277 pigeon fanciers, conducted during 1976/77.

## PRELIMINARY ANALYSIS OF CLINICAL DATA

### 1. "Quality control" assessment

One hundred and two people were interviewed at pigeon shows during the winter of 1979/80 but 2 individuals declined the

associated skin tests and have been excluded from the analysis. Although orientated towards persons who suspected symptoms arising from pigeon contact some who denied pigeon-related symptoms were also included.

A preliminary analysis of the clinical data was performed to check for major inconsistences in the pattern of questionnaire replies (Table 3), prior to considering criteria for clinical significance with respect to pigeon-related symptoms. The anticipated association between smoking habit and the presence of chronic respiratory symptoms, and between a family history of allergy and a personal history of hay fever and/or asthma was confirmed. Logical symptom clustering was also evident in that 23 of the 27 persons reporting immediate breathlessness also recorded one of the closely associated symptoms of cough, chest tightness or wheeze. Further as would be expected there was a trend towards increasingly frequent episodes with increasing number of symptoms. The interview replies, therefore appear to have been free of major distortion or inconsistency.

## 2. Classification of the clinical response

The clinical response to a discrete period of exposure was divided into those symptoms:- (1) during or immediately after contact (2) respiratory symptoms developing 6 - 12 hours later and (3) systemic symptoms developing after 6 - 12 hours.

TABLE 3 "Quality-control" assessment for clinical data

a) Smoking versus chronic symptoms

| | | |
|---|---|---|
| Smoking habit (>5 pack years) | 38 | 21 with chronic cough |
| Non-smoker | 62 | 9 with chronic cough |
| $\chi^2$ value 2 x 2 table * | | 18.6, $p<0.001$ |
| Smoking habit ( 5 pack years) | 38 | 22 with regular sputum |
| Non-smoker | 62 | 10 with regular sputum |
| $\chi^2$ value 2 x 2 table * | | 18.8, $p<0.001$ |
| Smoking habit ( 5 pack years) | 38 | 20 with undue breathlessness |
| Non-smoker | 62 | 13 with undue breathlessness |
| $\chi^2$ value 2 x 2 table * | | 10.6, $p<0.005$ |

Chronic cough - question 22, Appendix 1 - morning cough on most days for 3 months each year

Regular sputum - question 25, Appendix 1 - morning sputum on most days for 3 months each year

Undue breathlessness - question 26, Appendix 1 - becomes short of breath when walking on level ground compared with others of a similar age.

* Chi-squared value calculated without Yate's correction

b) Family history of allergy versus hay fever and/or asthma

| | | |
|---|---|---|
| Hay fever and/or asthma | 26 | 10 also F.H. of allergy |
| Others | 74 | 9 also F.H. of allergy |
| $\chi^2$ value 2 x 2 table ⊕ | | 7.0 $p<0.01$ |

c) Frequency of immediate symptoms versus number of symptoms

| | | |
|---|---|---|
| 1 - 2 symptoms | 33 | 7 **(22.2%)** occurred x 2 per week |
| 3 - 4 symptoms | 18 | 7 **(38.8%)** " " " " " |
| 5 - 6 symptoms | 6 | 3 **(50%)** " " " " " |

⊕ Chi-squared value calculated with Yate's correction

Clinical data for the 100 pigeon fanciers is detailed in Appendix 2, Section 1.

Seventy two fanciers reported a clinical response to a discrete episode of avian contact and the frequency of reporting for each symptom is shown in Table 4a. The majority of subjects (57 persons) reported two or more symptoms and included both immediate and delayed in 42. Where both types of symptom were reported these were usually first experienced at approximately the same time, but if not (7 persons) then immediate symptoms appeared first, the interval varying between 2 and 11 years.

Although many fanciers described both immediate and delayed symptoms the pattern of reporting differed when comparing increasing number of symptoms of each type (Table 4b). Whereas pure immediate responses mostly (11 of 15) consisted of a single symptom, 8 of 15 similar delayed responses included 3 or more symptoms.

Since no single symptom is diagnostic for PBD a combination of features are sought, with necessarily most certainty as to diagnosis where a full medical history, clinical examination, radiology and immunological data are available. In survey work data is restricted and there are no universally agreed criteria for diagnosis, but in general a combination of delayed onset, systemic and respiratory symptoms are considered most indicative of PBD with immediate or early onset symptoms noted separately. The classification adopted here follows these principles and

TABLE 4 Symptoms following avian contact

a) Frequency of each symptom among 72 fanciers with symptoms following exposure to pigeons

| IMMEDIATE SYMPTOMS | Number affected | Percent |
|---|---|---|
| cough | 26 | 36.1 |
| breathlessness | 27 | 37.5 |
| wheeze | 20 | 27.7 |
| chest pain | 23 | 31.9 |
| sneezing | 26 | 36.1 |
| itchy eyes | 17 | 23.6 |
| **DELAYED RESPIRATORY SYMPTOMS** | | |
| cough | 23 | 31.9 |
| breathlessness | 20 | 24.7 |
| wheeze | 7 | 9.7 |
| chest pain | 17 | 23.6 |
| **DELAYED SYSTEMIC SYMPTOMS** | | |
| general malaise* | 24 | 33.6 |
| "influenza-like" | 35 | 48.6 |
| shivering | 17 | 23.6 |
| perspiring | 20 | 27.7 |
| feverishness | 14 | 19.4 |

* Question asked if tiredness, muscle pain or headache experienced

b) Pattern of reporting for immediate and delayed symptoms according to increasing number of symptoms

| IMMEDIATE SYMPTOMS | Total affected | Immediate symptoms alone |
|---|---|---|
| 1 | 22 | 11 |
| 2 | 11 | 4 |
| 3 | 12 | 0 |
| >3 | 12 | 0 |
| **DELAYED SYMPTOMS** | | **Delayed symptoms alone** |
| 1 | 13 | 2 |
| 2 | 17 | 5 |
| 3 | 9 | 3 |
| >3 | 17 | 5 |

groups persons who share common features without necessarily having exactly the same symptoms or number of symptoms.

When respiratory and systemic symptoms developed 6 - 12 hours after exposure on at least 3 occasions this was regarded as definite acute PBD. Although individually non-specific delayed-onset constitutional symptoms are a prominent feature even after inhalational challenge (Hendrick et al., 1980) and where 3 such symptoms were reported this has also been considered to represent acute PBD.

Unlike delayed-onset symptoms the immediate symptoms do not readily sub-divide and therefore a definite response has been simply defined as those with 3 or more immediate symptoms (termed an Immediate Response).

Information was also sought concerning chronic respiratory symptoms, and these were all more common among smokers (Table 3). When such symptoms occurred without obvious cause they have been considered potentially attributable to avian contact. For simplicity, unexplained cough for 3 months of the year and/or undue exertional breathlessness have been designated chronic PBD.

## RESULTS

### 1. Immediate pigeon-related response.

The Immediate Response group, those with 1 or 2 immediate symptoms (possible Immediate Response) and those denying any immediate or delayed pigeon-related symptoms (no acute response)

have been compared with respect to several indices of exposure that might indicate severity of response :- (1) leaving the loft prematurely, (2) frequent symptoms (x 2 weekly), (3) less than average (20 hours) weekly contact and (4) wearing or having worn a mask (Table 5). The groups were also compared with respect to associated history of hay fever and/or asthma, and the presence of chronic PBD. Significant differences between definite and possible Immediate Response groups were found with respect to leaving the loft prematurely, reporting a history of hay fever and/or asthma and in regard to the presence of chronic symptoms (chi-squared $p<0.005$, $<0.05$, $<0.05$ respectively).

2. **Acute PBD**

As already indicated responses were considered in terms of the usual symptom-complex of EAA rather than examining the significance of individual symptoms per se. Two delayed symptoms were considered sufficient to establish a diagnosis of PBD when both respiratory and systemic symptoms were represented, as suggested by Christensen et al. (1975), but including also any person reporting 3 or more systemic symptoms. A similar comparison to that undertaken for immediate pigeon-related response was performed for definite and possible PBD (Table 6). The definite PBD group was significantly more likely to have had a medical opinion expressing that symptoms could relate to pigeon keeping, and to have recorded chronic symptoms (chi-squared analysis $P<0.05$, $p<0.001$ respectively). The distribution of the

TABLE 5 Comparison of clinical features and exposure factors between Immediate Response (IR) groups

| Symptom group | Total | Wears or wore mask | 20 hours in loft/wk | Chronic PBD | Symptoms x2/wk | Leaves loft early | Hay fever and/or asthma |
|---|---|---|---|---|---|---|---|
| I.R. | 24 | 8 | 11 | 9 | 10 | 11 | 10 |
| Poss I.R | 33 | 9 | 12 | 3 | 8 | 2 | 4 |
| No acute response | 28 | 1 | 11 | 3 | | | 5 |
| $x^2$ value 3 x 2 table | | 8.0 $p<0.05$ | N.S. | 9.2 $p<0.01$ | | | 7.6 $p<0.005$ |
| $x^2$ value * 2 x 2 table | | N.S. | | 5.3 $p<0.05$ | N.S. | 10.3 $p<0.005$ | 5 $p<0.05$ |

*Chi-squared analysis with Yate's continuity correction. N.S. - not significant

TABLE 6 Comparison of clinical features and exposure factors between delayed response (PBD) groups

| Symptom group | Total | Wears or wore mask | 20 hours in loft/wk | Chronic PBD | Symptoms x 2/wk | Medical suspicion re pigeons | Hay fever and/or asthma |
|---|---|---|---|---|---|---|---|
| PBD | 32 | 12 | 19 | 12 | 10 | 15 | 9 |
| Poss PBD | 25 | 7 | 9 | 0 | 4 | 4 | 10 |
| No acute response | 28 | 1 | 11 | 3 | | | 5 |
| $x^2$ value 3 x 2 table | | 9.7 $p<0.01$ | N.S. | 15.1 $p<0.001$ | | | N.S. |
| $x^2$ value * 2 x 2 table | | N.S. | | 9.8 $p<0.001$ | N.S. | 4.8 $p<0.05$ | |

*Chi-squared analysis with Yate's continuity correction. N.S. - not significant

exposure indices was similar for the 3 groups although, wearing a mask was more evident in those reporting symptoms.

3. **Chronic PBD**

Undue exertional breathlessness or chronic cough were reported by 49 fanciers. Twenty two were non-smokers and although 7 had a history of occupational dust exposure none had pneumoconiosis. Five of these 22 reported bronchial asthma but symptoms were well controlled on inhaled therapy. One person requiring diuretic for cardiac failure was removed leaving 21 with unexplained symptoms designated chronic PBD. It can be seen from Tables 5 and 6 that the presence of chronic PBD is associated both with an Immediate Response and acute PBD, although the relationship was more marked with the latter. There was also an association between chronic PBD and (1) reduced weekly contact, (2) with a medical suspicion that symptoms were pigeon related and (3) with hay fever and/or asthma (Table 7, chi-squared analysis $P<0.05$, $P<0.01$, and $P<0.05$ respectively).

A summary of the symptom groups resulting from the foregoing sets of criteria is shown diagramatically in Figure 5. A comparison of the general characteristics and exposure histories for the main acute response categories (Table 8) demonstrates that the groups are similar with respect to age, smoking habit, occupational dust exposure, number of pigeons kept and hours spent in the loft each week. A separate analysis was not performed for the women fanciers, since only 4 were interviewed.

TABLE 7 Comparison of clinical and exposure factors between those with or without chronic PBD

| Symptom group | Total | Wears or wore mask | <20hours in loft/wk | Medical susp.re pigeons | Hay Fever/and or asthma |
|---|---|---|---|---|---|
| Chronic PBD | 21 | 8 | 14 | 8 | 10 |
| Others | 79 | 19 | 31 | 11 | 16 |
| $x^2$ value 2 x 2 table | | N.S. | 4.0 $p<0.05$ | 7.9 $p<0.01$ | 5.1 $p<0.05$ |

Chi-squared analysis with Yate's continuity correction.

FIGURE 5 Symptom grouping of pigeon fanciers in 1979/80 study

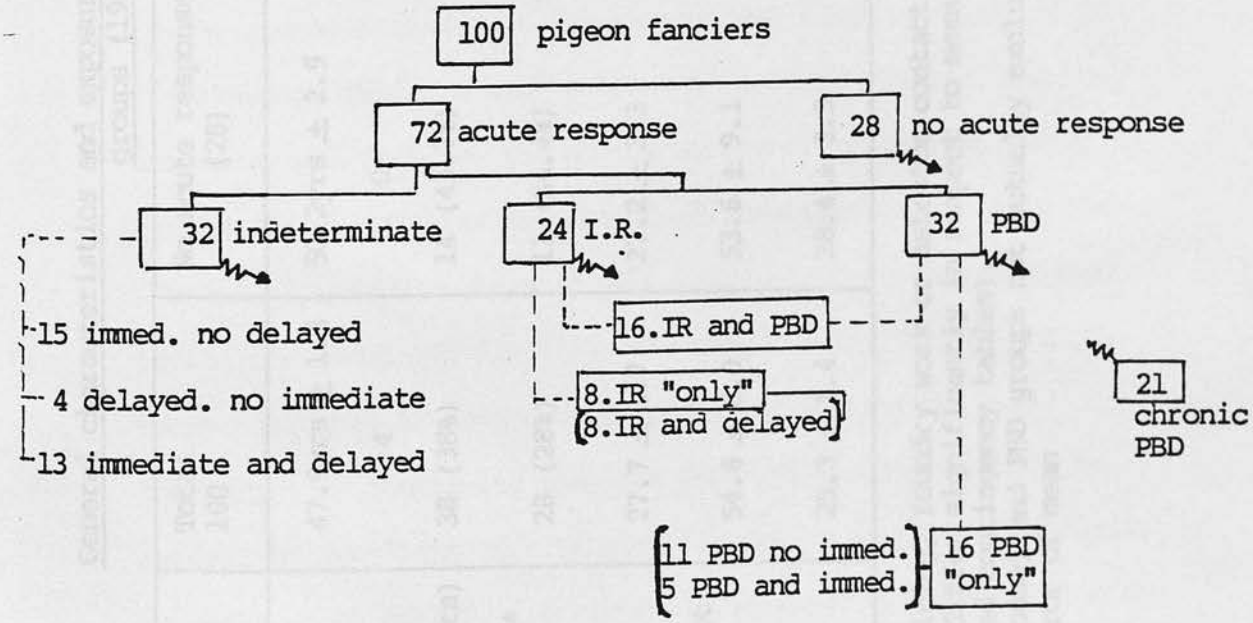


TABLE 8 General characteristics and exposure histories for acute response groups (1979/80)

| | Total 100 | No acute response (28) | Indeterminate response (32) | I.R. (24) | PBD (32) |
|---|---|---|---|---|---|
| Age (mean± S.E.M.) | 47.9yrs ± 1.4 | 50.2yrs ± 2.9 | 46.9yrs ± 2.6 | 45.1yrs ± 2.3 | 47.5yrs ± 2.0 |
| Females | 4 | 0 | 1 | 2 | 3 |
| Smoking (>5 pack yrs) | 38 (38%) | 14 (43.7%) | 13 (46.4%) | 8 (33.3%) | 10 (31.2%) |
| Occupational dust * exposure | 28 (28%) | 11 (34.4%) | 9 (32.1%) | 3 (12.5%) | 7 (21.8%) |
| Years exposed (Mean ± S.E.M.) | 27.7 ± 1.7 | 27.2 ± 3.3 | 29.5 ± 3.4 | 27.1 ± 2.6 | 27.3 ± 2.4 |
| No. of pigeons kept (mean ± S.E.M.) | 54.6 ± 6.0 | 53.6 ± 9.1 | 59.9 ± 15.5 | 48.7 ± 3.9 | 55.8 ± 8.7 |
| Hours in loft/wk (mean ± S.E.M.) | 25.3 ± 1.4 | 28.4 ± 3.3 | 24.0 ± 2.1 | 24.4 ± 2.6 | 22.8 ± 2.5 |

* Refers to coal mining, foundry work or asbestos contact.
The groups do not differ significantly in respect to mean values (t-test for reduced data) or distribution of factors (chi-squared contingency tables).
IR (Immediate Response) and PBD groups not mutually exclusive.
S.E.M. standard error of mean

## 4. Characteristics of pigeon-related responses

### a) Immediate Response

The 2 Immediate Response subgroups (Figure 5) were examined in relation to a history of hay fever and/or asthma, acute PBD, chronic PBD and with 2 immune responses characteristic of respiratory hypersensitivity disorders (antigen-specific skin prick test and antigen-specific serum IgG antibody) (Table 9). None of the relationships reached statistical significance with the small numbers involved, although the Immediate Response "only" category were more often atopic and less often had a significant serum avian-specific IgG antibody response (immunisation to pigeon globulin, >4ug/ml by radioimmunoassay - Chapter 2).

### b) PBD subgroups

The characteristics of the various PBD subgroups (Figure 5) are shown in Table 10 with respect to several clinical and immunological factors. The groups were similar in most respects except for the greater proportion of immunised fanciers in the PBD "only" and [PBD + chronic PBD] groups, although mean antibody levels for immunised fanciers were very similar in all groups. Those with both an Immediate Response and PBD showed less immunological reactivity than the PBD "only" subgroup both in respect to immediate hypersensitivity and as regards alveolitis. An early onset of PBD, i.e. within 10 years of commencing pigeon keeping was evident in 9 of 32 subjects with PBD, of whom 7 were in the PBD "only" category. The characteristics of those with

TABLE 9 Characteristics of Immediate Response subgroups (1979/80)

| Symptom Group | Total | Hay fever/and or asthma | P B D | Chronic P B D | PS/PF* prick test | IgG ⊕ (>4ug/ml) |
|---|---|---|---|---|---|---|
| I.R. "only" | 8 | 6 | 0 | 3 | 3 | 2 |
| I.R.+ delayed symptoms | 16 | 4 | 16 | 6 | 3 | 9 |
| $x^2$ value 2 x 2 table | | N.S. | N.A. | N.S. | N.S. | N.S. |

* - 2mm or more weal to pigeon serum (PS) and/or pigeon feathers (PF)
⊕ - Serum avian-specific IgG response >4ug/ml (immunisation to pigeon globulin)
N.A. - not applicable since IR subgroups defined on the basis of the number of associated delayed symptoms

TABLE 10 Characteristics of PBD Subgroups (1979/80)

| Symptom Group | Total | Hay fever and/or asthma | Onset* <10yrs | Duration+ of sympt. (yrs) | Mean IgG (Immunised) | PS/PF prick test | IgG (>4 µg/ml) |
|---|---|---|---|---|---|---|---|
| PBD "only" | 16 | 5 | 7 | 8 ± 1.8 | 64.3 ± 9.8 | 6 | 13 |
| IR + PBD | 16 | 4 | 2 | 5.6 ± 1.4 | 55.3 ± 9.5 | 3 | 9 |
| PBD+chronic PBD | 12 | 7 | 4 | 10 ± 2.4 | 56 ± 9.5 | 4 | 11 |
| PBD | 32 | 9 | 9 | 6.9 ± 1.2 | 60.6 ± 7.2 | 9 | 22 |
| Chronic PBD | 21 | 10 | N.A. | N.A. | 47.0 ± 8.9 | 9 | 16 |

* Refers to onset of acute P B D
+ Refers to duration (years) of acute P B D
Values are mean ± S.E.M. for durations of symptoms and IgG level
N.A. - Not applicable

early onset of disease are examined further in relation to the 1976/77 survey later in this chapter.

Immunisation to pigeon globulin afforded the most sensitive correlation with PBD identifying 22 people from that group (68.75%). The most specific factor for PBD was a medical suspicion of pigeon-related symptoms where 15 of the 19 fanciers indicating this (78.9%) fulfilled the symptom criteria for definite acute PBD. Seven of the 10 people describing PBD but lacking a significant antibody response were dual responders (IR + PBD). Of these 2 wore masks and 2 indicated a doctor had suggested their symptoms were pigeon-related. None described chronic PBD.

There were 20 pigeon fanciers who were immunised to pigeon globulin but did not fulfil the criteria for definite PBD of whom only 4 had a vigorous antibody response (>60ug/ml). No similarities were found in the type or number of symptoms recorded but 2 gave a history of wearing a mask in the loft and one had chronic PBD. No common features were evident among the 4 pigeon fanciers who did not fulfil the criteria for PBD but who indicated a medical suspicion of pigeon-related symptoms.

## 5. Prevalence data

### a) Pigeon-related responses

The preceding analysis concerns the spectrum of clinical syndromes among active pigeon fanciers but biased for symptomatic persons. The symptom groups defined were therefore applied to

the data from a large scale survey of pigeon fanciers undertaken in 1976/77 and centred on several discrete pigeon racing communities where a high degree of co-operation was sought. Self-completed questionnaires and a venous blood sample were obtained from 277 fanciers, of whom 132 reported at least one immediate symptom, 121 a delayed symptom and 100 denied any acute response to exposure. Although the clinical assessment was somewhat different (Chapter II), criteria for response defined for the 1979/80 study have been applied unaltered. Thirty two fanciers had incorrectly completed their questionnaires with respect to questions about symptoms but most answers could still be appropriately interpreted. In the 15 instances where uncertainty remained those questions have been ommitted from the assessment. There were 55 people with an Immediate Response, 29 with PBD and 51 with chronic PBD. Immediate Responses were therefore again common and their characteristics very similar to those found in the 1979/80 study, being associated with PBD and an avian-specific antibody response. The proportion of the survey population with PBD was lower in this less selected group, yielding a prevalence of 10.4%. Chronic PBD occurred at a similar frequency in both studies but in the large scale survey was less often associated with acute PBD (29% compared to 57%). The characteristics of the various PBD subgroups were broadly similar to the 1979/80 findings.

b) General characteristics

The general characteristics and exposure indices for the PBD

TABLE 11 General characteristics, exposure histories and immune reactivity for P B D and no acute response groups (1976/77)

| | Total (277) | No acute response (100) | PBD (29) |
|---|---|---|---|
| Age (mean ± S.E.M.) | 43.3 yrs±0.9 | 44.8 yrs±1.4 | 43.8 yrs±3.0 |
| Females | 36 | 15 | 0 |
| Smoking | 122 (44%) | 40 (40%) | 6 (20.6%) |
| Years exposed (mean ± S.E.M) | 20.4 yrs±0.9 | 20.6 yrs±1.5 | 21.4 yrs±5.0 |
| Number of pigeons kept (mean±S.E.M.) | 46.6 ± 1.5 | 47.1 ±2.6 | 49.2 ± 5.0 |
| Hours in loft/wk (mean ± S.E.M.) | Summer 29.6 hrs±1.1<br>Winter 10.6 hrs±0.55 | 27.3 hrs±1.5<br>10.3 hrs±0.8 | 18.7 hrs±2.3*<br>9.1 hrs±1.3 |
| Number immunised | 84 (30.3%) | 29 (29%) | 21 (72.4%) |
| IgG (immunised) (mean ± SEM) | 34.4ug/ml±5.4 | 16.4ug/ml±2.0 | 89.0ug/ml±15.8† |

Smoking history significantly more common among those without P B D (chi-squared analysis, $X^2 = 6.5$, $p < 0.05$).

*Similar exposure histories throughout, except for a significantly lower summer, weekly exposure in the P B D group ($p < 0.05$, t-test for reduced data).

All groups had a significant difference between summer and winter weekly exposure ($p < 0.05$, t-test for reduced data).

† The mean antibody level for immunised fanciers in the P B D group was considerably higher than for the other groups ($p < 0.001$, t-test for reduced data).

group, those with no acute response and the whole study population are presented in Table 11. Exposure factors were similar between the groups except for weekly contact. The weekly contact varied significantly for all groups between the winter months and the active racing months of summer. This peak contact was significantly less among those with PBD (t-test for reduced data, $P<0.05$). The PBD group was associated with the presence of an avian-specific IgG antibody response and the mean antibody level of 89 ug/ml ± 15.8 for immunised fanciers with PBD was significantly higher than the value of 34.4ug/ml ± 5.4 for the overall group of immunised fanciers (t-test for reduced data $P<0.001$).

## 6. The avian-specific IgG response

### a) Antibody and PBD

To extend the relationship between intensity of IgG antibody response and PBD, the proportion of PBD cases was calculated at increasing levels of antibody response as shown in histogram form in Figure 6. There was a progressive increase in the percentage of affected fanciers at each successive level of antibody response and all 7 pigeon fanciers in the study with >90 ug/ml of avian-specific IgG antibody fulfulled the criteria for definite PBD. Chi-squared analysis showed a significant relationship between PBD and antibody level >60ug/ml compared to those immunised fanciers with a less intense humoral response ($P<0.005$). A similar analysis for the 1979/80 data (Figure 7)

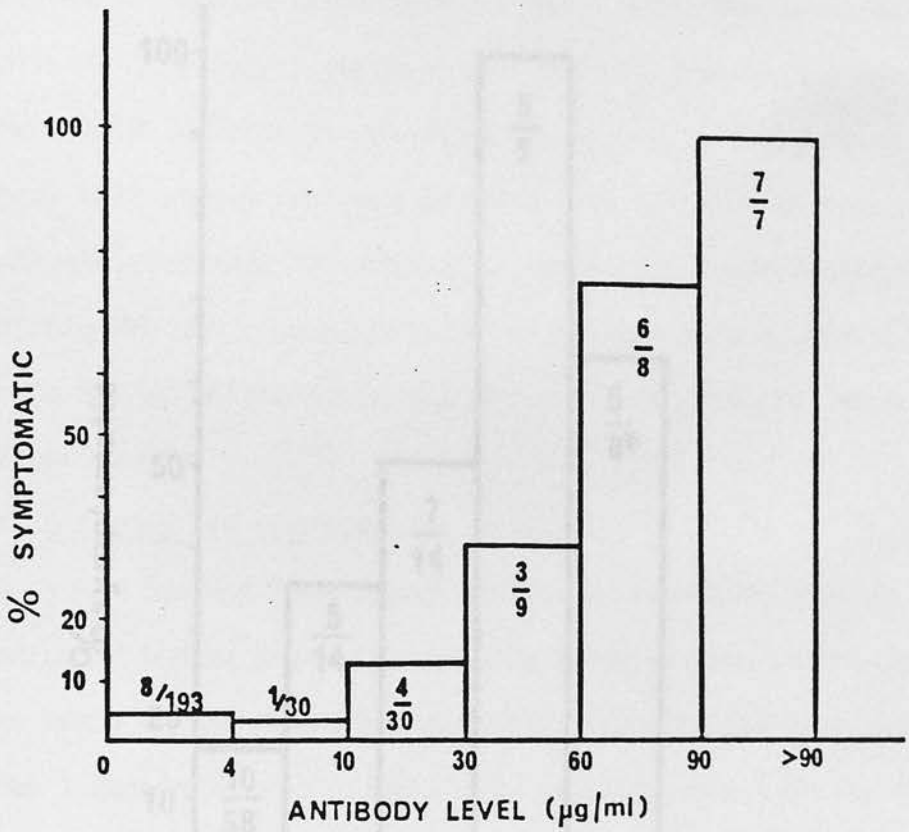



FIGURE 6 Histogram of proportion of subjects with P B D in groups of fanciers arranged by IgG antibody level to pigeon gammaglobulin (ug/ml) (1976/77).
Chi-squared fourfold contingency table with Yate's correction; P B D positive or negative and serum antibody>60ug/ml, or 4-60ug/ml $X^2 = 33.1, p<0.0005$

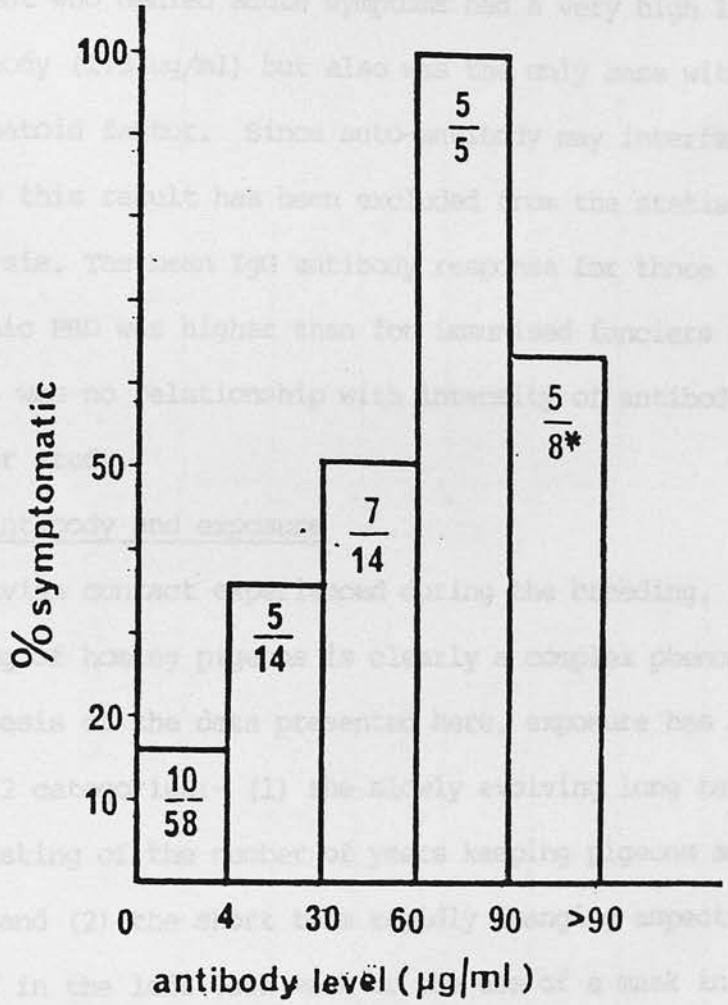



FIGURE 7 Histogram of proportion of pigeon fanciers with P B D grouped according to antibody response (1979/80). Chi-squared fourfold contingency table without Yate's continuity correction; 10 of 13 with >60ug/ml IgG had P B D versus 12 of 28 with 4-60ug/ml, $X^2 = 4.1$, $p < 0.05$

* Excludes result from rheumatoid factor positive subject.

showed a significant (chi-squared $p<0.05$) but less marked association between PBD and intense antibody response. One subject who denied acute symptoms had a very high level of antibody (173 ug/ml) but also was the only case with a positive rheumatoid factor. Since auto-antibody may interfere with the assay this result has been excluded from the statistical analysis. The mean IgG antibody response for those designated chronic PBD was higher than for immunised fanciers generally, but there was no relationship with intensity of antibody response in either study.

b) Antibody and exposure

Avian contact experienced during the breeding, rearing and racing of homing pigeons is clearly a complex phenomenon, but on the basis of the data presented here, exposure has been divided into 2 categories:- (1) the slowly evolving long term aspect consisting of the number of years keeping pigeons and the number kept and (2) the short term rapidly changing aspect such as time spent in the loft each week or the use of a mask in the loft environment. As already discussed the latter was employed more commonly among those with symptoms in the 1979/80 study, but this information was not available from the 1976/77 survey. In both studies there was a tendency for those with PBD to spend less hours per week in contact with their pigeons (Tables 8 and 11).

With regard to long term exposure the proportion of immunised fanciers at increasing levels of exposure in terms of years and number of pigeons is shown in Figure 8. There was a

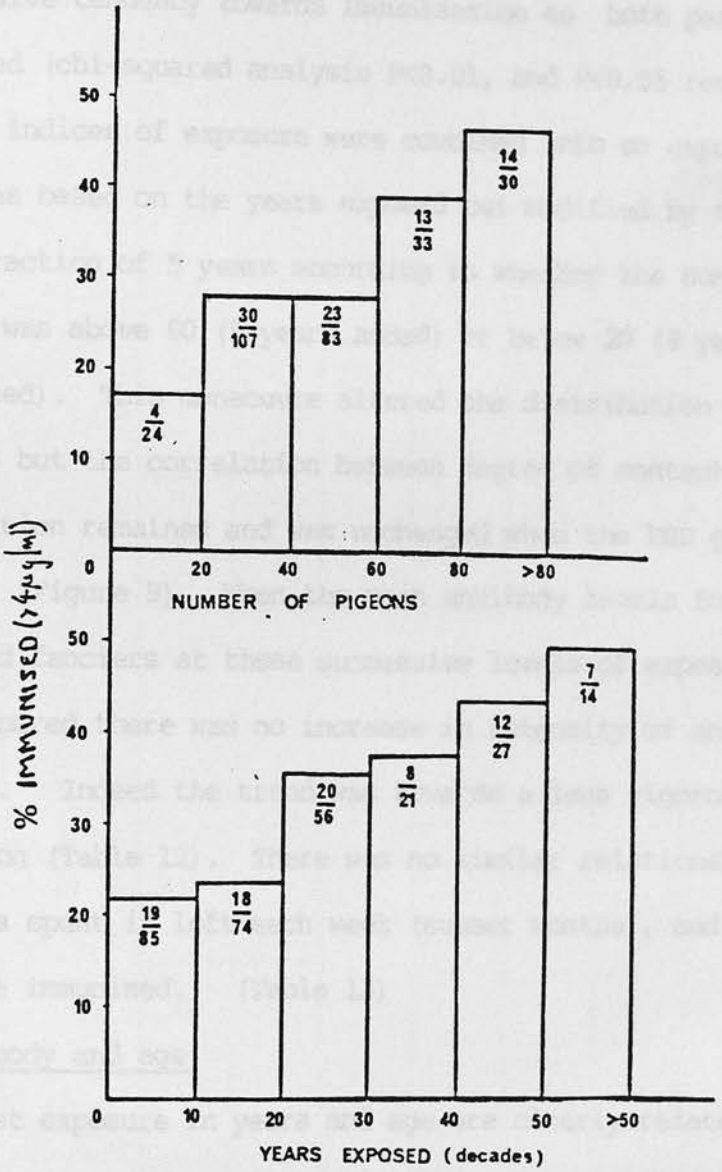



FIGURE 8

Histogram of the proportion of immunised pigeon fanciers in 1976/77 study according to increasing years exposed and number of pigeons kept.
Subjects divided into groups according to number of pigeons kept (increments of 20 pigeons), and duration of exposure (decades).
Number of pigeons kept: Chi-squared progression of proportions $X^2 = 6.6$, $p < 0.05$.
Years exposed: Chi-squared progression of proportions $X^2 = 9.8$, $p < 0.01$.

progressive tendency towards immunisation as both parameters increased (chi-squared analysis $P<0.01$, and $P<0.05$ respectively). These 2 indices of exposure were combined into an exposure "score" which was based on the years exposed but modified by the addition or subtraction of 5 years according to whether the number of pigeons was above 60 (5 years added) or below 20 (5 years subtracted). This maneouvre altered the distribution of pigeon fanciers but the correlation between degree of contact and immunisation remained and was unchanged when the PBD group were excluded (Figure 9). When the mean antibody levels for the immunised fanciers at these successive levels of exposure "score" were compared there was no increase in intensity of antibody response. Indeed the trend was towards a less vigorous antibody production (Table 12). There was no similar relationship between the hours spent in loft each week (summer months), and tendency to become immunised. (Table 13)

c) Antibody and age

Whilst exposure in years and age are clearly related, there was a much less obvious tendency towards immunisation when increasing age alone (decades) was plotted against proportion immunised to pigeon globulin (Figure 10).

d) Antibody and smoking

Data from the 1976/77 study formed part of an analysis of smoking and antibody previously reported (Boyd et al., 1977) and demonstrated a negative correlation between serum IgG against pigeon globulin and positive smoking history. This is in

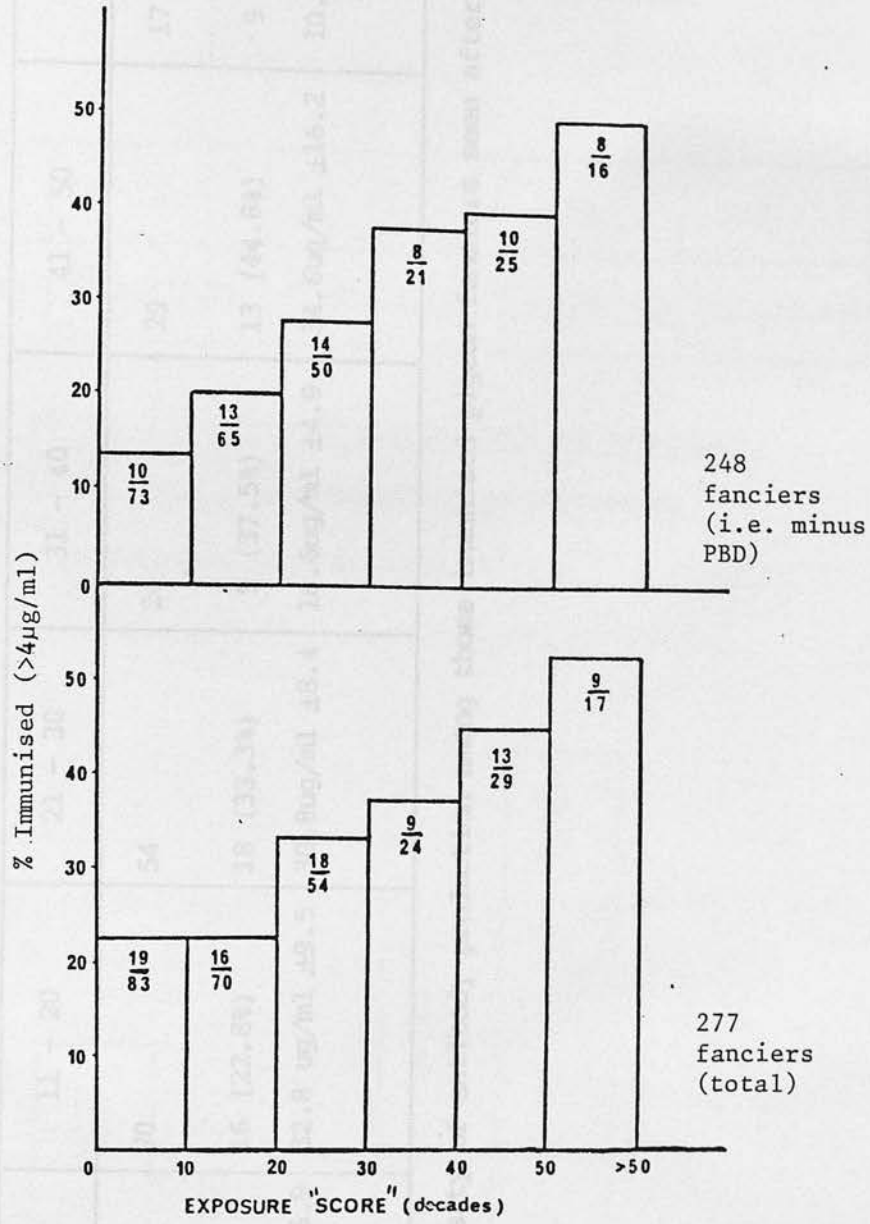



FIGURE 9 Histogram of the proportion of immunised pigeon fanciers in 1976/77 study according to an exposure "score".

Subjects divided into groups according to an exposure "score" (see text).
248 pigeon fanciers: Chi-squared progression of proportion $X^2 = 16.0$, $p < 0.005$
277 pigeon fanciers: Chi-squared progression of proportion $X^2 = 11.1$, $p < 0.001$.

TABLE 12 Qualitative and quantitative aspects of antibody in relation to exposure.

EXPOSURE "SCORES" (decades)

| | 0 - 10 | 11 - 20 | 21 - 30 | 31 - 40 | 41 - 50 | >50 |
|---|---|---|---|---|---|---|
| Number in group | 83 | 70 | 54 | 24 | 29 | 17 |
| Immunised (>4ug/ml) | 19 (22.9%) | 16 (22.8%) | 18 (33.3%) | 9 (37.5%) | 13 (44.8%) | 9 (52.9%) |
| IgG (immunised only, mean ± S.E.M.) | 58.9ug/ml ±16.9 | 32.8 ug/ml ±9.5 | 30.8ug/ml ±8.4 | 18.6ug/ml ±4.9 | 34.6ug/ml ±16.2 | 10.8ug/ml ±2.3 |

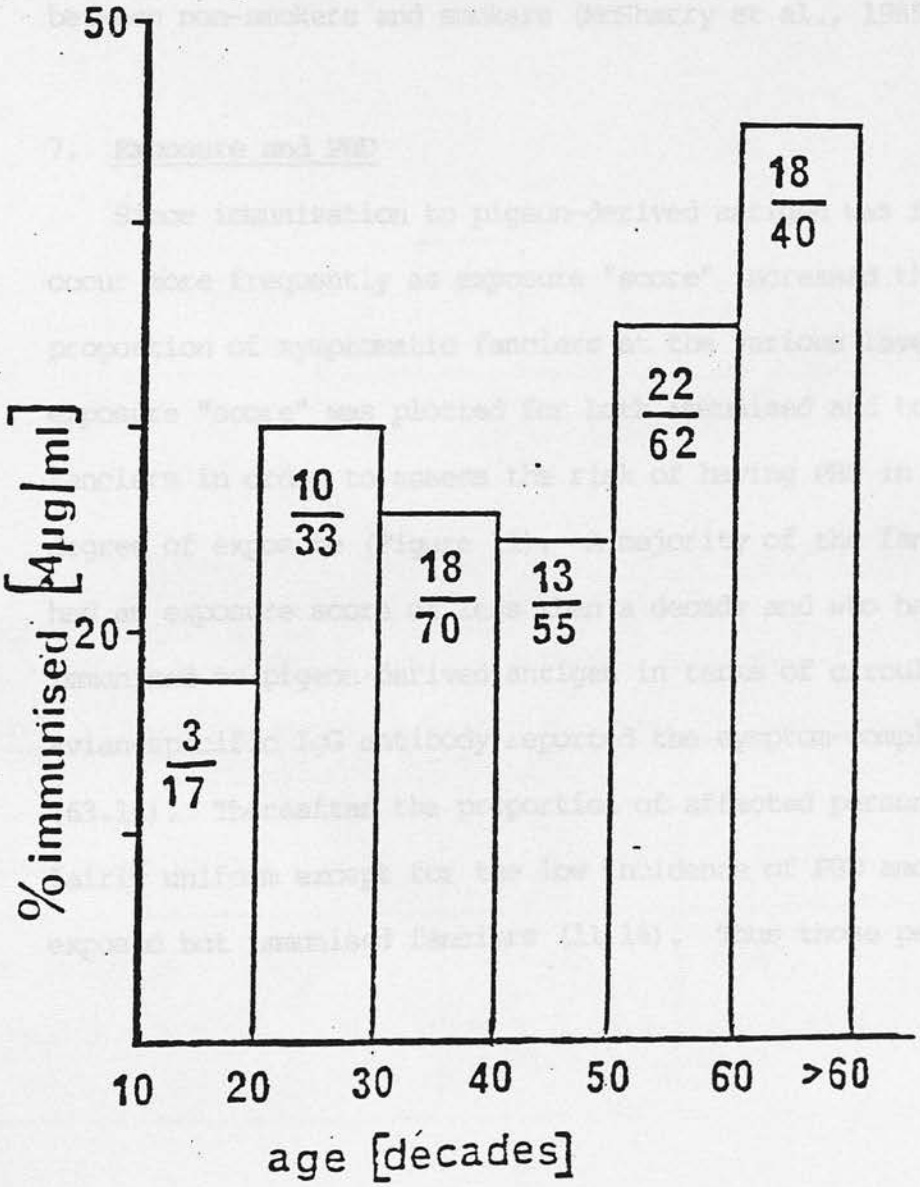
Trend towards **decreasing** intensity of antibody production among those immunised pigeon fanciers seen after longer exposure histories.

TABLE 13 Proportion of pigeon fanciers immunised according to increasing hours spent in loft/week (summer)

HOURS PER WEEK

| % | 0 - 15 | 16 - 30 | 31 - 45 | 46 - 60 | >60 |
|---|---|---|---|---|---|
| Immunised | $^{20}/_{72}$ (27.7%) | $^{24}/_{86}$ (27.9%) | $^{20}/_{58}$ (34.4%) | $^{15}/_{40}$ (37.5%) | $^{5}/_{21}$ (23.8%) |

FIGURE 10 Histogram of the proportion of pigeon fanciers immunised, according to increasing age.
Subjects divided into groups according to increasing age (decades).
Chi-squared progression of proportion, $X^2 = 5.3$, $p < 0.05$.



keeping with the finding of reduced antibody production amongst smokers exposed to antigens associated with EAA (Morgan et al., 1973). A reduced prevalence of EAA amongst smokers has been reported (Warren, 1977) and was the case for the 1976/77 data where 6 of 122 smokers reported PBD and 23 of 155 non-smokers (chi-squared analysis $P<0.05$). This was not the case for the 1979/80 study where although the tendency was for PBD to be less frequent among smokers the difference in distribution did not reach statistical significance. Nevertheless there was a highly significant difference in avian-specific antibody production between non-smokers and smokers (McSharry et al., 1985).

## 7. Exposure and PBD

Since immunisation to pigeon-derived antigen was found to occur more frequently as exposure "score" increased the proportion of symptomatic fanciers at the various levels of exposure "score" was plotted for both immunised and total pigeon fanciers in order to assess the risk of having PBD in relation to degree of exposure (Figure 11). A majority of the fanciers who had an exposure score of less than a decade and who had become immunised to pigeon-derived antigen in terms of circulating avian-specific IgG antibody reported the symptom-complex of PBD (63.1%). Thereafter the proportion of affected persons was fairly uniform except for the low incidence of PBD among the long exposed but immunised fanciers (11.1%). Thus those persons

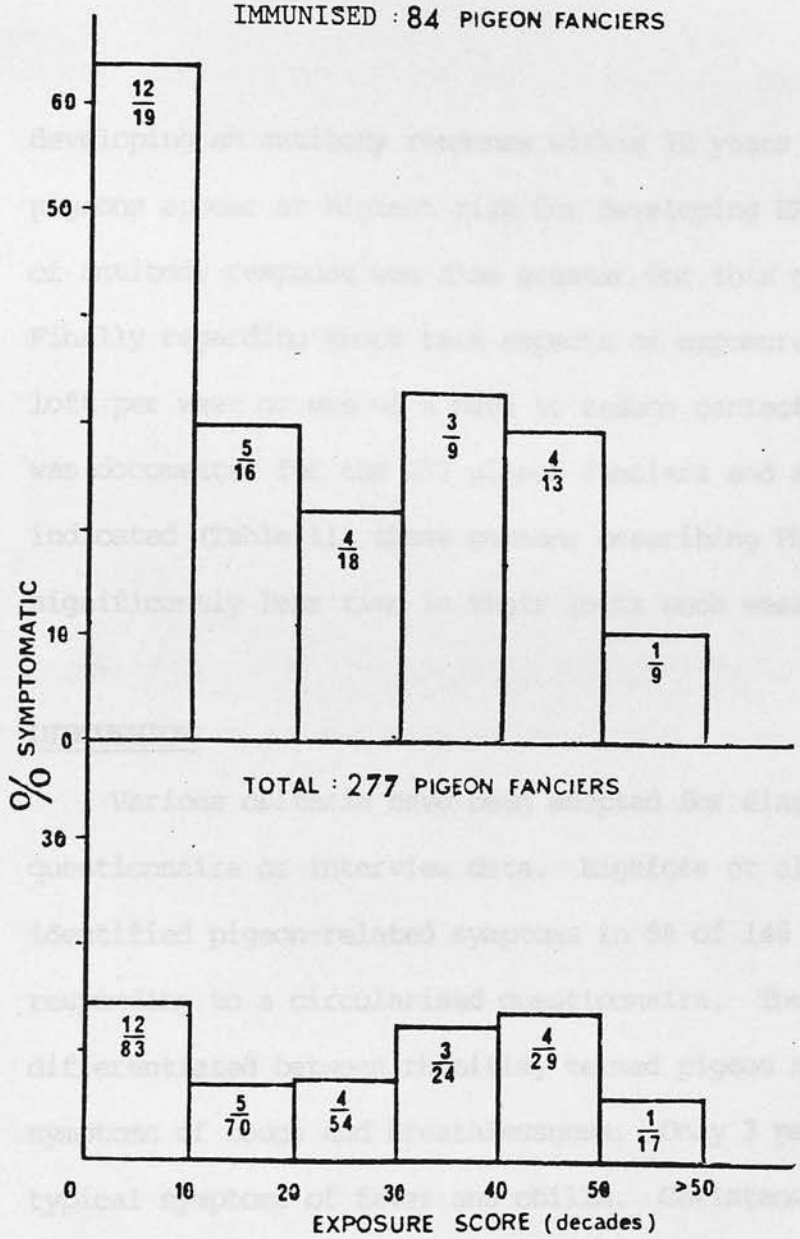



FIGURE 11 Histogram of proportion of affected pigeon fanciers arranged according to exposure score.

The highest proportion of persons with PBD is seen in the group with the lowest exposure score but a positive antibody response

TABLE 14 Antibody status according to length of exposure prior to symptoms for the 29 with PBD

| Exposure prior to PBD (years) | 0 - 10 | 11 - 20 | >20 |
|---|---|---|---|
| Number in Group | 12 | 8 | 9 |
| IgG (mean ± S.E.M.) | 82.5 ± 24.3 ug/ml | 42.5 ± 19.0 ug/ml | 60.7 ± 24.7 ug/ml |

developing an antibody response within 10 years exposure to pigeons appear at highest risk for developing EAA. The intensity of antibody response was also greater for this group (Table 14). Finally regarding short term aspects of exposure such as hours in loft per week or use of a mask to reduce contact only the former was documented for the 277 pigeon fanciers and as previously indicated (Table 11) those persons describing PBD spent significantly less time in their lofts each week.

## DISCUSSION

Various criteria have been adopted for diagnosing PBD from questionnaire or interview data. Elgefors et al. (1971) identified pigeon-related symptoms in 8% of 146 pigeon fanciers responding to a circularised questionnaire. These authors differentiated between rhinitis, termed pigeon nose, and lung symptoms of cough and breathlessness. Only 3 people reported the typical symptoms of fever and chills. Christensen et al. (1975) also divided pigeon-related symptoms into upper respiratory tract (URT) and lower respiratory tract (LRT) with systemic symptoms (SYS) separated. Symptomatic persons (22%) were those reporting either URT with LRT ± SYS or SYS with either LRT or URT. Several other large scale studies have produced data on the prevalence of symptoms but did not give full details of criteria applied (Jongerius, 1969; Maesen, 1972; Fink et al., 1972; Caldwell et al., 1973).

The lack of standardised criteria for the diagnosis of PBD

evident in reported surveys clearly imposes restrictions when comparing different series and a simple uniform approach would be helpful. The analysis of clinical data from the 1979/80 study has therefore been directed towards both identifying the range and character of pigeon-related syndromes and formulating simple criteria for diagnosis that might be applied in survey studies of "at risk" populations.

Although a large number of symptoms were recorded the criteria adopted for acute PBD identified a group (32 pigeon fanciers) who were significantly different in several respects from the other persons interviewed. This group more commonly reported the presence of chronic PBD, a medical suspicion of pigeon-related symptoms, and had serum IgG to pigeon globulin. When applied retrospectively to the 1976/77 self-completed questionnaire study 29 people fulfilled these criteria for PBD a prevalence of 10.4% which accords well with the 3 - 15% range of affected persons in other series (Chapter 1).

Immediate symptoms were commonly reported in the 1979/80 study but since 11 persons reported a single symptom with no other suggestion of pigeon-related problem it seems probable that on an individual basis such symptoms are susceptible to over-reporting. This would be consistent with the findings of Grant et al. 1972 from a survey of farm workers when it was noted that 35% of those interviewed recorded irritation of nose and throat during contact with hay which was apparently unrelated to farmers' lung. Similarly Boyer et al. (1974) also found a high

incidence (69%) of minor immediate symptoms in a survey of turkey farmers. In the present studies 24 persons reported at least 3 such symptoms, although the group did not correlate well with other aspects of immediate hypersensitivity. The 16 persons with a dual Immediate Response and PBD showed less of the typical immune responses associated with immediate hypersensitivity when compared to the Immediate Response "only" group or alveolitis when compared to PBD "only".

In conclusion immediate upper respiratory tract pigeon-related symptoms are commonly reported but on an individual basis appear of little consequence. Three or more such symptoms occurred in approximately 20% of both studies, mostly as part of a dual response (IR + PBD). This subgroup lacked a close relationship with other features of Type I allergy or alveolitis and appeared a "mixed bag" rather than a distinct entity. When multiple immediate symptoms predominated the picture was more in keeping with a Type I allergic disorder.

Symptoms of chronic PBD are especially difficult to assess in survey work because of a similarity to chronic bronchitis (Fink, 1983). The present studies attempted to assess chronic disease by excluding smokers and ex-smokers and in the 1979/80 study 21% came into this category. The association between non-smoking and presence of alveolitis reported previously by Warren (1977) might favour a correlation between PBD and a chronic symptom group that excludes smokers. However, there was only a weak association between non-smoking and PBD in the 1979/80

study, and similar findings were evident from the self-completed questionnaire study. Asthmatics were not excluded and might account for chronic symptoms in some of those with an Immediate Response, but even allowing for such contributing factors and a degree of over-reporting it appears that mild chronic respiratory symptoms arising from exposure are more common than is generally appreciated.

These investigations have resulted in simple sets of criteria which could form the basis for a standardised approach to the assessment of PBD. Once symptoms have developed several patterns of disease are discernable and the following modifications to the current classification are proposed:-

1) **Acute progressive disease** - accords with the classic case descriptions of severe acute disease, including fine reticular mottling on chest radiograph and responding well to antigen avoidance and steroids.

2) **Acute recurrent disease** - episodes of EAA without short or medium term clinical deterioration despite continued active participation in the hobby. Probably more common than acute progressive disease and includes most of the acute PBD subjects identified in surveys. Self-regulation of exposure may be important, and is discussed further.

3) **Immediate Response** - when the predominant type of symptom this represents a conventional Type I hypersensitivity response. Often part of a less distinct, dual response, group where more features are of alveolitis than Type I allergy.

4) **Chronic PBD** - a combination of present sub-acute and chronic

forms which are difficult to separate and where the essential feature is an insidious onset. Fanciers with recurrent episodes of alveolitis who have chronic symptoms should also be included.

A PBD grouping established solely from a questionnaire-interview is not intended to provide a precise individual diagnosis, and undoubtedly some persons will have been erroneously classified. Difficulties in distinguishing between definite and possible PBD particularly apply to the 1979/80 study which was orientated to a positive symptom status, and perhaps accounts for the clearer relationship between intensity of IgG response and presence of PBD evident in the "unselected" 1976/77 population. All the pigeon fanciers in the 1976/77 study with an antibody response above 90 ug/ml fulfilled the criteria for PBD and the high levels of antibody did not relate to increased exposure. In fact there was evidence of a reduction in certain types of exposure since the mean hours spent in the loft (summer) each week was significantly lower in the PBD group and this suggests an attempt at self-regulation of symptoms by those affected. Thus the various facets of exposure may be of different significance and any exposure index should take this into account (Banham et al., 1978).

There is little evidence to suggest exposure ordinarily has a major impact on the relative risk of developing PBD, although in 2 family studies the symptomatic individuals were considered to have been most intensely exposed (Purtillo et al., 1975; Schatz et al., 1976a). This contrasts with the present findings

where 40% of those with PBD in the 1976/77 survey had the lowest exposure "score" of less than 10 years. The mean antibody level for this subgroup of most rapidly affected persons was higher than for those with symptoms seen after a longer duration of contact. Furthermore the combination of a low exposure "score" and presence of circulating antibody constituted a group with very high risk for finding PBD thereby reaffirming a relationship between humoral response and clinical disease which is not explained in terms of intensity of exposure. These findings rather would suggest a differential susceptibility to PBD within the pigeon-keeping community which primarily relates to individual host susceptibility and that there is self-regulation of exposure once symptoms supervene.

When sampling a population at a given point in time it is not possible to be certain of any individual's subsequent progress but it seems possible that the group of most rapidly affected persons would be least likely to successfully modify their condition by reducing exposure and most likely to have acute progressive disease. Such persons will necessarily be under-represented in surveys of active pigeon fanciers. Accordingly the majority, who can continue participation despite episodes of alveolitis have recurrent but "non-progressive" disease.

The factors determining this "balance" are presently poorly understood but would seem to include self-regulation of exposure and the emerging picture of immunoregulatory events in EAA

(Chapter 1) could also be relevant. Nevertheless such individuals may be at risk of developing permanent respiratory symptoms since those with additional chronic symptoms had a longer mean duration of acute PBD than those without.

The correlation of an intense antibody response with PBD, rather than with exposure has been referred to already but the humoral response may also be considered simply with respect to the presence or absence of avian-specific antibody production. Previous work (Boyd 1975) has established that a level of >4ug/ml of IgG antibody to pigeon globulin can confidently be regarded as indicating an altered immune reactivity not seen in non-exposed persons. This situation where a significant but often minor avian-specific humoral response occurs is termed here immunisation in accordance with the terminology of Herbert & Wilkinson (1977) and need not imply any protective or pathological role for the antibody. Only 30.4% of pigeon fanciers surveyed had such a response, a figure somewhat lower than reported in other studies using less sensitive methods of antibody detection (Fink et al., 1972; Christensen et al., 1975). Possible reasons for the discrepancies between the studies were discussed in Chapter 1, but especially in the smaller surveys, the sample is likely to be unrepresentative.

For both years exposed and number of pigeons kept there was a progressive tendency towards immunisation with increasing levels of exposure, although the absolute levels of antibody in those becoming immunised did not rise. Only a slight tendency towards developing an antibody reponse was seen with advancing

age alone. Thus immunisation occurs in response to increasing exposure in keeping with the classic animal experiments of immune complex disease by Dixon et al. 1961,which also demonstrated low and high antibody responders. However, the further element of the relationship, that increasing intensity and duration of exposure results in a higher proportion with disease, is not of principal importance regarding PBD in humans both because the quantities of antigen concerned are much less, and because of active self-regulation and self-selection.

Among those regularly exposed immunisation can be considered to transfer a person from a situation of effectively "no risk" to that of "potential risk". It remains uncertain whether major changes in the humoral response occur subsequent to immunisation, but in any event a vigorous avian-specific antibody response is closely associated with a high risk of finding PBD compared to lesser degrees of response. These findings with respect to symptoms, antibody and exposure have been published (Banham et al., 1986a).

Schuyler et al. (1983) found the degree of pulmonary inflammation in a rabbit model of EAA due to M faeni directly proportional to the intensity of antibody response, and although the present findings do not prove a causal relationship between antibody and disease they do indicate that avian-specific antibody production relates chiefly to factors other than degree of exposure. Furthermore the humoral response may be involved via antibody capability other than immune-complex mediated tissue damage and this aspect is investigated in the following chapter (Chapter IV).

# CHAPTER IV

## Immediate hypersensitivity aspects of the immune response in PBD

### INTRODUCTION

Although pigeon-derived antigens have been found to induce a wide range of specific immune responses no single feature correlates precisely with the presence and progression of the clinical disease entity. The relevance and interaction of the various immune events in pathogenesis therefore remain uncertain. In recent years most attention has focused on cellular aspects of immunity such as the role of the alveolar macrophage and the ratio of helper to suppresser T-lymphocytes (Chapter 1), whilst the avian-specific antibody response has been dismissed as merely a reflection of the intensity of exposure (Burrell & Rylander 1981; Fink 1983). However the present studies suggest that this view is an inadequate explanation for the distinct differences between qualitative and quantitative aspects of the humoral immune response with regard to exposure and symptoms. The avian-specific humoral response therefore continues to merit investigation.

The intradermal skin test response to pigeon-derived

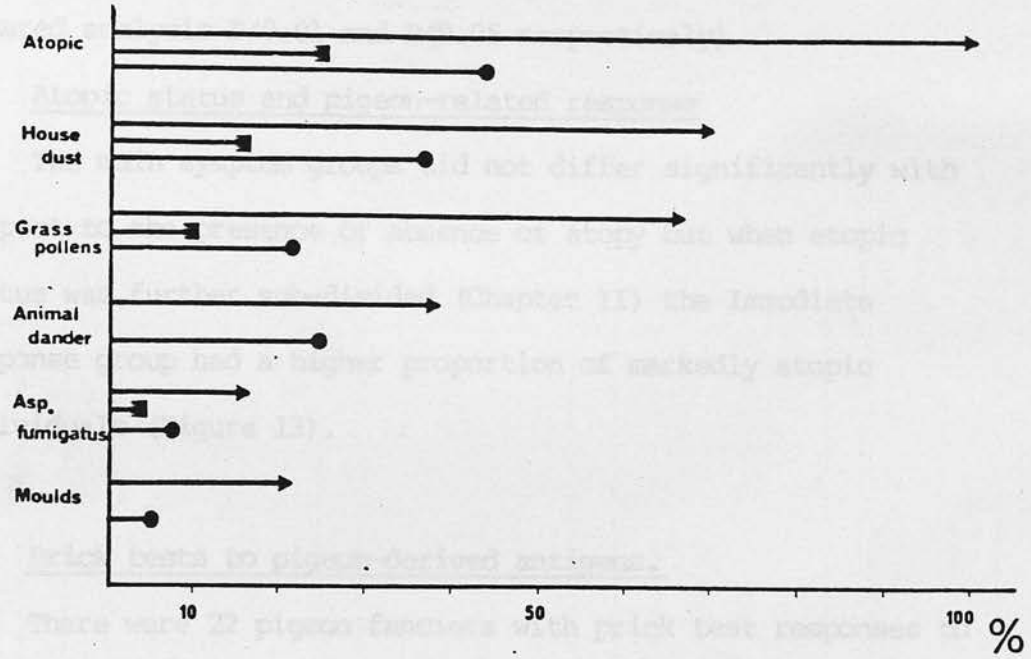
material was amongst the first evidence suggesting an immunological basis for PBD and indeed there is a close temporal relationship between the onset of the clinical syndrome 6 - 8 hours after exposure and the development of the late Arthus type component of the dual intradermal response. Despite these findings there have been no large scale studies into the profile of cutaneous reactions among pigeon fanciers and in this chapter a variety of skin test responses are examined in detail with respect to the symptom groups previously defined and with particular reference to immediate hypersensitivity. The full immunological data are detailed in Appendix 2, Section 2.

## RESULTS

### 1. Atopy

Forty five of the 100 pigeon fanciers were atopic (2mm weal, Chapter II) and 2 further subjects showed dermatographia with reactions to all allergens and the control test. The prevalence of atopy was compared to that published for an allergy clinic population and for unselected adults (Figure 12). The pigeon fanciers with symptoms following exposure reacted more commonly to all the allergens than did unselected adults but never as often as the allergy clinic subjects. Atopic status was examined with respect to other parameters of immediate hypersensitivity (Table 15) - (1) Elevated serum IgE (>200 u/ml), (2) Personal or family history of allergy, and (3) Presence of hay fever

FIGURE 12 Atopy in pigeon fanciers and other persons.
Data for allergy clinic subjects and unselected adults from Davies (1979)




Key:
- ▶ Allergy clinic population
- ■ Unselected adults
- ● Pigeon fanciers with symptoms

TABLE 15

Atopy and immediate hypersensitivity

| Skin test status | Total | >200U/ml IgE | History of allergy | Hay fever and/ or asthma |
|---|---|---|---|---|
| Atopic | 45 | 21 | 28 | 16 |
| Non-atopic | 55 | 7 | 21 | 10 |
| $X^2$ value, 2 x 2 table | | 9.6,p<0.01 | 5.9,p<0.01 | N.S. |

Chi-squared values calculated with Yate's continuity correction.

and/or asthma to non-avian stimuli. Of these elevated IgE and history of allergy were significantly associated with atopy (chi-squared analysis $P<0.01$ and $P<0.05$ respectively).

a) Atopic status and pigeon-related response

The main symptom groups did not differ significantly with respect to the presence or absence of atopy but when atopic status was further sub-divided (Chapter II) the Immediate Response group had a higher proportion of markedly atopic individuals (Figure 13).

## 2. Prick tests to pigeon-derived antigens.

There were 22 pigeon fanciers with prick test responses to pigeon serum (PS) or pigeon feathers (PF) including 6 persons who reacted to both antigens. As would be expected there was a relationship with atopy (chi-squared, $p<0.01$) but the association with other parameters of immediate IgE mediated hypersensitivity was less marked (Table 16). With regard to pigeon-related responses neither prick test correlated with any specific symptom group.

## 3. Intradermal skin tests to pigeon-derived antigens

At present only the immediate (15 min) responses are considered and the statistical comparisons refer to 90 pigeon fanciers where full intradermal skin test data were available. Responses to PS and PF antigens were examined with respect to the parameters previously described and there was no correlation with any of these indices of immediate hypersensitivity (Table 17).

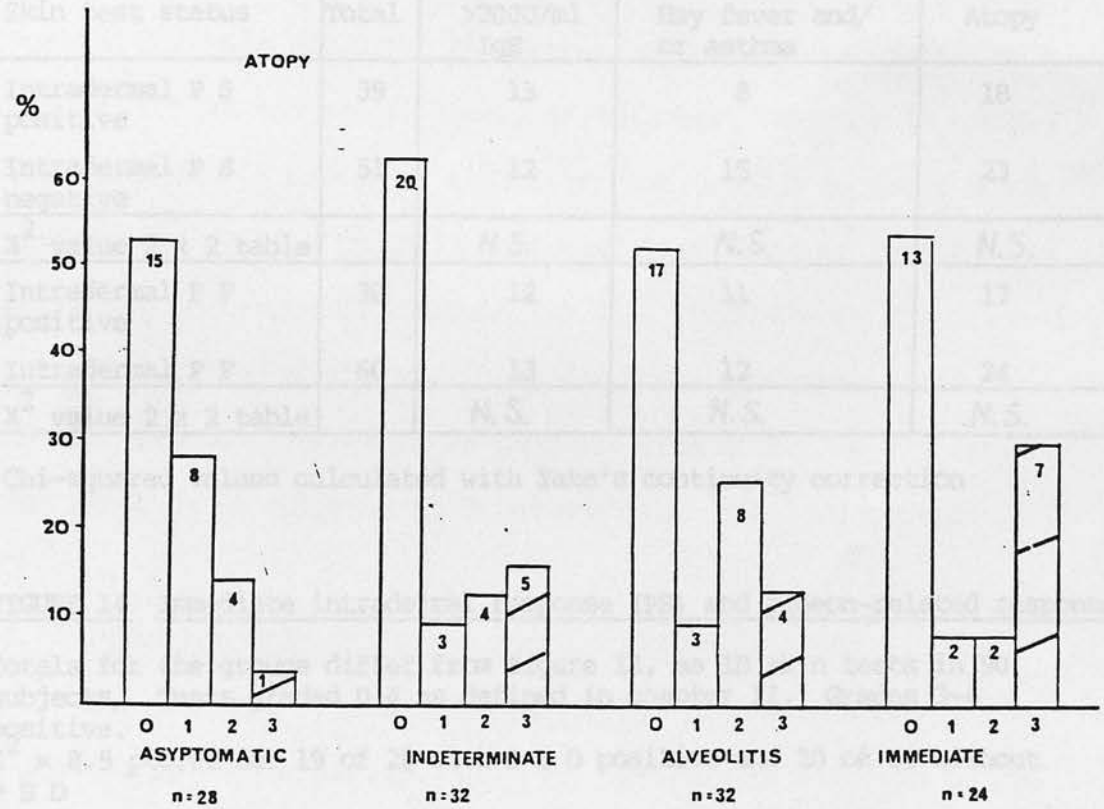



FIGURE 13 Atopy and pigeon-related responses
Some fanciers appear in both P B D and I R groups.
Atopy graded 0-3 as defined in Chapter II.

TABLE 16 Prick tests with pigeon-derived antigens and immediate hypersensitivity.

| Skin test status | Total | >200u/ml IgE | Hay fever and/ or asthma | Atopy |
|---|---|---|---|---|
| Prick P.S. pos. | 16 | 8 | 8 | 14 |
| Prick P.S. neg. | 84 | 8 | 18 | 3 |
| $x^2$ value, 2x2 table | | N.S. | 4.3 p<0.05 | 12.6, p<.001 |
| Prick PF pos. | 12 | 6 | 5 | 11 |
| Prick PF neg. | 88 | 6 | 21 | 34 |
| $x^2$ value, 2x2 table | | N.S. | 0.93 | 9.9, p<0.01 |

Chi-squared values calculated with Yate's continuity correction

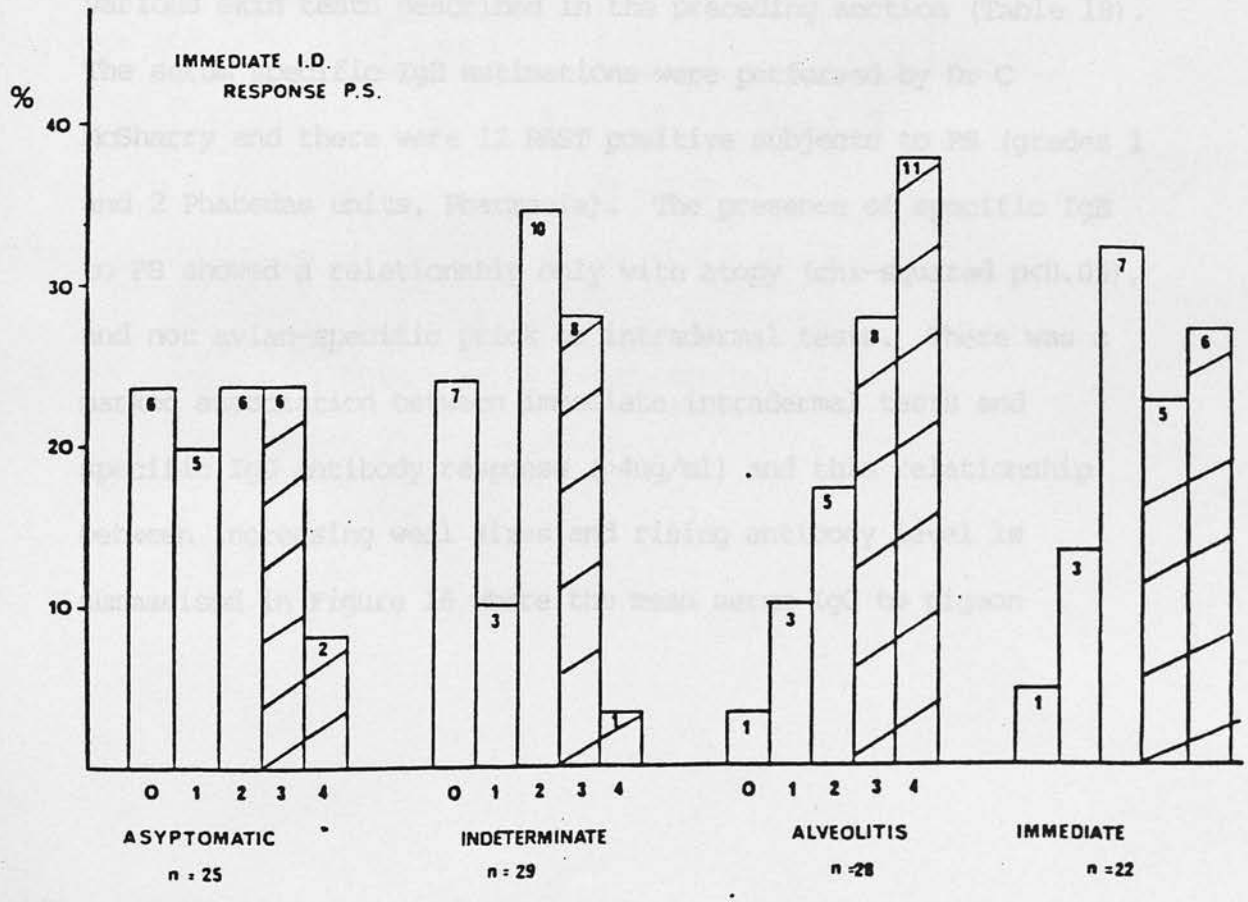
TABLE 17 Intradermal tests with pigeon-derived antigens and immediate hypersensitivity

| Skin test status | Total | >200U/ml IgE | Hay fever and/ or asthma | Atopy |
|---|---|---|---|---|
| Intradermal P S positive | 39 | 13 | 8 | 18 |
| Intradermal P S negative | 51 | 12 | 15 | 23 |
| $x^2$ value 2 x 2 table | | N.S. | N.S. | N.S. |
| Intradermal P F positive | 30 | 12 | 11 | 17 |
| Intradermal P F | 60 | 13 | 12 | 24 |
| $x^2$ value 2 x 2 table | | N.S. | N.S. | N.S. |

Chi-squared values calculated with Yate's continuity correction

FIGURE 14 Immediate intradermal response (PS) and pigeon-related response

Totals for the groups differ from figure 13, as ID skin tests in 90 subjects. Tests graded 0-4 as defined in chapter II. Grades 3-4 positive.
$X^2$ = 8.5 $p<0.01$ for 19 of 28 with P B D positive and 20 of 62 without P B D



a) Intradermal tests and pigeon-related response

The PS intradermal responses for the main symptom groups are shown in histogram form in Figure 14. There was a significant association between a positive (Grade 3 or 4) test and PBD (chi-squared analysis $P<0.01$). The proportion of affected fanciers (acute PBD) at successive levels of skin test response is shown in Figure 15. A change of intensity from Grade 3 to Grade 4 was accompanied by significantly increased likelihood of finding acute PBD (chi-squared analysis $P<0.05$). Furthermore the Grade 4 responses showed good specificity for acute PBD since 11 of the 15 such reactions occurred in fanciers with acute PBD (73.3%). The findings in relation to PF were similar but less marked.

## 4. Immediate skin tests and pigeon-related antibody

The presence of a serum specific IgG or IgE antibody response to pigeon gammaglobulin was examined in relation to the various skin tests described in the preceding section (Table 18). The serum specific IgE estimations were performed by Dr C McSharry and there were 12 RAST positive subjects to PS (grades 1 and 2 Phabedas units, Pharmacia). The presence of specific IgE to PS showed a relationship only with atopy (chi-squared $p<0.05$), and not avian-specific prick or intradermal tests. There was a marked association between immediate intradermal tests and specific IgG antibody response (>4ug/ml) and this relationship between increasing weal sizes and rising antibody level is summarised in Figure 16 where the mean serum IgG to pigeon

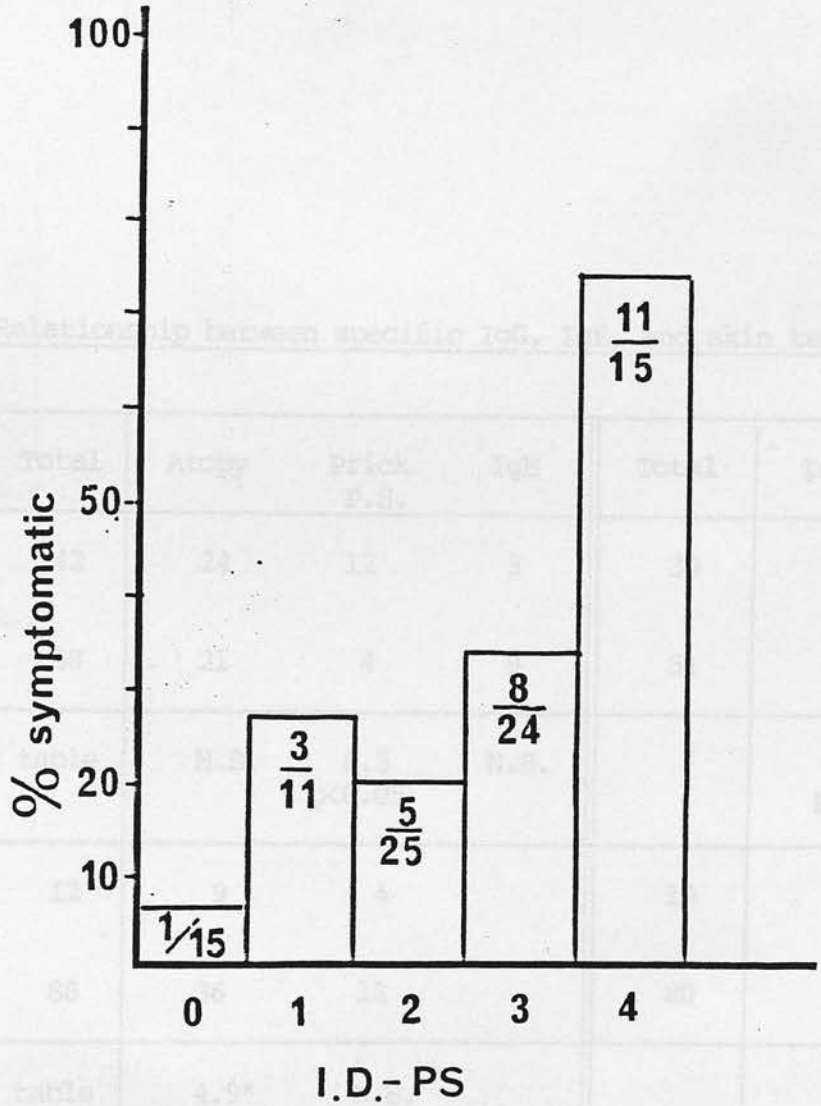



FIGURE 15 Histogram of proportion of subjects with P B D in groups of fanciers arranged by immediate intradermal skin test response to pigeon serum (Grades 0-4). Chi squared fourfold contingency table with Yate's continuity correction; 11 of 15 with Grade 4 had P B D versus 8 of 24 with Grade 3, $X^2 = 4.4$, $p<0.05$.

TABLE 18 Relationship between specific IgG, IgE, and skin tests

| Antibody status | Total | Atopy | Prick P.S. | IgE | Total | Intradermal P.S. |
|---|---|---|---|---|---|---|
| IgG pos (>4ug/ml) | 42 | 24 | 12 | 3 | 39 | 27 |
| IgG neg. | 58 | 21 | 4 | 9 | 51 | 12 |
| $X^2$ value, 2x2 table | | N.S. | 6.5 $p<0.05$ | N.S. | | 16.9 $p<0.001$ |
| IgE pos (Grade 1/2) | 12 | 9 | 4 | | 10 | 3 |
| IgE neg. | 88 | 36 | 12 | | 80 | 36 |
| $X^2$ value, 2x2 table | | 4.9* $p<0.05$ | N.S. | | | N.S. |

Chi-squared values calculated with Yate's continuity correction, except - IgE/atopy*.

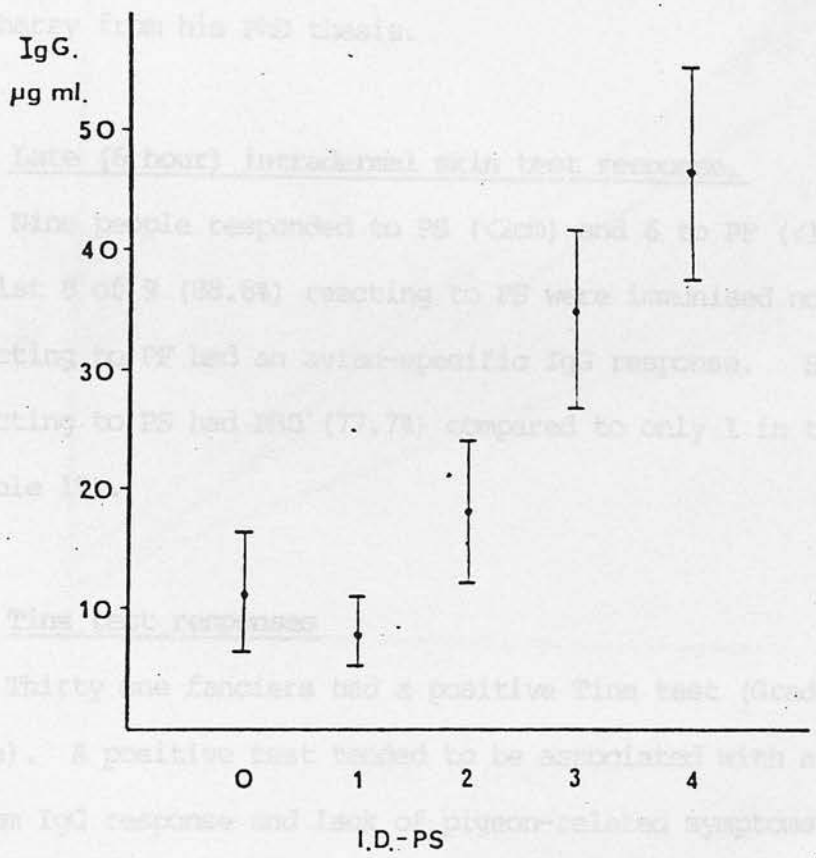



FIGURE 16. Graph of mean IgG to pigeon globulin for increasing degree of immediate intradermal response to P.S. (Grade 0 to 4).

Grade 0 - 11.0 ug/ml± 4.8 (s.e.m.)
Grade 1 - 7.6 ug/ml± 2.5
Grade 2 - 18.3 ug/ml± 6.2
Grade 3 - 34.9 ug/ml± 8.2 )significantly higher than
Grade 4 - 46.6 ug/ml± 9.3 )other groups, $p<0.01$, (t-test reduced data)

globulin (ug/ml) is shown for each grade of skin test response.

IgG subclass assays were performed by Dr McSharry and in subjects with detectable specific IgG there was evidence of a contribution from all 4 IgG subclasses. This is illustrated in Figure 17 which is reproduced with the kind permission of Dr McSharry from his PhD thesis.

5. **Late (6 hour) intradermal skin test response.**

Nine people responded to PS (<2cm) and 6 to PF (<1cm). Whilst 8 of 9 (88.8%) reacting to PS were immunised none of the 4 reacting to PF had an avian-specific IgG response. Seven reacting to PS had PBD (77.7%) compared to only 1 in the PF group (Table 19).

6. **Tine test responses**

Thirty one fanciers had a positive Tine test (Grade 2 or more). A positive test tended to be associated with absence of serum IgG response and lack of pigeon-related symptoms (PBD), although these relationships did not reach statistical significance except when the smaller group with Grades 3 or 4 reactions were examined. Then there was a statistically significant negative association, 2 Tine positive of 28 with PBD and 24 Tine positive of 62 without PBD. (chi-squared $p<0.05$).

7. **Relationships between immune response and PBD subgroups**

The avian-specific skin test reactions and antibody

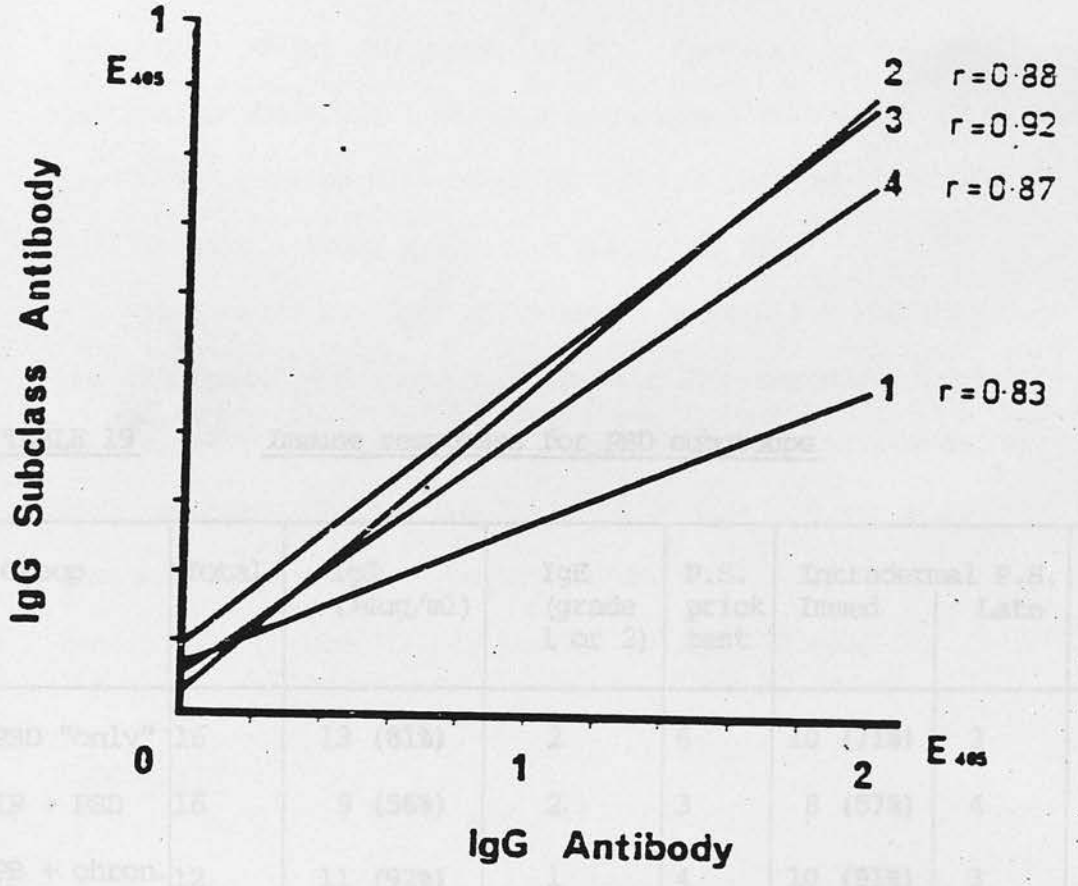



FIGURE 17 IgG subclasses in P B D
Correlations between the extent of the serum antibody activity, in each of the four IgG subclasses with the total IgG antibody activity against avian antigens.

TABLE 19 Immune responses for PBD subgroups

| Group | Total | IgG (>4ug/ml) | IgE (grade 1 or 2) | P.S. prick test | Intradermal P.S. Immed | Intradermal P.S. Late | Tine + ve |
|---|---|---|---|---|---|---|---|
| PBD "only" | 16 | 13 **(81%)** | 2 | 6 | 10 **(71%)** | 3 | 3 |
| IR + PBD | 16 | 9 **(56%)** | 2 | 3 | 8 **(57%)** | 4 | 5 |
| PB + chron. PBD | 12 | 11 **(92%)** | 1 | 4 | 10 **(91%)** | 3 | 3 |
| PBD | 32 | 22 **(69%)** | 4 | 9 | 19 **(68%)** | 7 | 7 |
| Chronic PBD | 21 | 16 **(76%)** | 3 | 9 | 12 **(60%)** | 4 | 3 |

IgG and IgE - avian-specific serum antibody responses

responses for the various sub-sets of PBD are shown in Table 19. The presence of serum IgG to pigeon globulin (>4ug/ml) identified 22 of those considered to have PBD (68.75%) and the positive immediate intradermal reaction to PS identified a similar proportion of the PBD group (67.8%). Considering the more intense antibody and immediate intradermal reactions, 10 of the 13 (76.9%) persons with >60ug/ml IgG had PBD and 11 of the 15 (73.3%) with a >10mm weal to PS described PBD.

There were no major differences between the PBD subgroups but PBD "only" and acute plus chronic PBD categories tended to be more closely associated with immunisation and intradermal skin test response. Neither the presence of specific serum IgE response or prick test reaction to PS showed appreciable sensitivity or specificity for PBD or any PBD subgroup.

## DISCUSSION

EAA has been considered essentially a condition of non-atopic persons (Pepys, 1969) but the present findings indicate that PBD is not restricted to such persons. The overall prevalence of atopy among persons admitting to acute symptoms following exposure was higher than reported for unselected adults. This association might have been attributed to the prevalence of immediate symptoms resulting from exposure, but atopy was more commonly present in the PBD "only" sub-group than those with an Immediate Response plus PBD. Some of the minor

atopic responses seen among PBD subjects may reflect a generalised B-cell stimulation that often accompanies EAA. When the more strongly atopic persons were considered there was a bias towards those reporting an Immediate Response and particularly the Immediate Response "only" group.

Although immediate skin test reactions to avian antigens have long been recognised their presence has been overshadowed by the subsequent 6 hour response which has been considered more informative regarding the aetiology of the disease. Moreover such immediate skin test reactions have not been widely reported in other alveolitis syndromes, perhaps because of the lack of suitable antigen preparations (Salvaggio & Karr, 1979). The immediate intradermal reactions to pigeon-derived antigens described in the present studies were not accounted for by IgE reagins. Neither the prick test responses to avian antigens nor the presence of an avian-specific IgE response correlated with the intradermal reactions. Furthermore,unlike the prick tests the immediate intradermal response did not correlate with other aspects of IgE mediated hypersensitivity. Instead there was a close relationship with the presence of avian-specific IgG antibody and with PBD (Banham, 1982; McSharry et al., 1983).

There have been recent reports of similar immediate intradermal skin test reactions that lack the characteristics of an IgE response relating to farmer's lung disease (Freedman et al., 1981) and humidifier fever (Edwards and Cockcroft, 1981). Freedman et al. performed a series of passive cutaneous transfer

tests and demonstrated that skin sensitisation was transferrable by an IgG rich serum fraction rather than an IgG depleted fraction. These authors also reported a correlation between positive specific IgG antibody response and both immediate and late components of the intradermal response, but particularly the latter. The present studies are in keeping with such findings and suggest a direct correlation between intensity of IgG response (ug/ml) and the intensity of immediate skin test reaction even though 6 hour reactions were rarely seen with the concentrations of antigen employed. Moreover an intense immediate skin test reaction correlated as well with the likelihood of finding PBD as did antibody.

As already discussed in recent years the humoral response typical of PBD has been considered to have only a minor, if any role, as regards immunopathogenesis. Attention has focused on the cell-mediated immune response and particularly T-lymphocyte sub-populations. There is now good evidence of altered helper to suppressor T-lymphocyte ratios in EAA and possibly an active and specific immunosuppression by antigen responsive T-cells inhibiting pulmonary inflammation in asymptomatic but antibody positive fanciers (Chapter 1). However, despite these advances there remains uncertainty as to the exact effector mechanisms involved in pathogenesis and some workers have postulated the importance of an early, inflammatory phase as an initiating event allowing increased antigen uptake and enhancing specific immune responses eventually resulting in chronic granulomatous

inflammation.

The traditional view of humoral-related inflammation in EAA via immune complex formation overlooks other antibody functions. Whilst the IgG reaginic activity evident in these studies might merely reflect a generalised B-cell stimulation they demonstrate a further potential for antibody involvement. It is possible that reaginic activity could play a part in any initiating inflammatory phase influencing antigen uptake and handling as well as enhancing subsequent immune responses essential to the development of clinical disease. Some support for this view can be found from a number of studies. Calvanico et al. (1980) reported an increase in the $IgG_4$ subclass levels found in BAL fluid from individuals with PBD. Both Calvanico et al. (1980) and Freedman et al. (1981) suggest a permissive role for specific IgG reagin in EAA. Recent BAL studies confirm the presence of mast cells within the lung parenchyma in a number of interstitial lung diseases including EAA (Kawanami 1981; Agius 1984). Of particular interest in this context is the report by Kawanami et al. (1983) that mast cells in open lung biopsies taken from 18 patients with chronic EAA had morphological alterations suggesting rapid release of chemical mediators. These authors also suggest a possible role for mast cells in EAA.

In summary there has been increasing recognition in recent years that the immunopathogenesis of EAA of which PBD remains the best model, is a compex multifactorial process involving various immunological and inflammatory mechanisms. The direct

involvement of a specific humoral response has been questioned because of the lack of other immunological or histological features typical of immune complex-mediated inflammation. The results presented here and supported by other studies indicate that the specific humoral response has a further functional capability again raising the question of active antibody participation in immunopathogenesis. Even small amounts of mediator release within the pulmonary compartment as a consequence of avian-specific reaginic antibody activity might have an important influence on the subsequent cell-mediated inflammatory process.

# CHAPTER V

## Pulmonary function

### INTRODUCTION

This chapter concerns pulmonary function tests performed to support and extend the clinical and immunological studies reported in the preceding chapters. Follow-up investigation of pigeon fanciers seen during the large scale questionnaire studies in 1976/77 and 1979/80 was restricted because of the wide area from which subjects were drawn. However a group from the 1976/77 study, who all lived in the same area, underwent a loft challenge and had follow-up pulmonary function assessment several years later. A further series of inhalation challenge tests were conducted under laboratory-controlled conditions and involved cases drawn from several sources including persons referred to hospital. Finally, lung permeability was investigated in a different group of symptomatic, but active, pigeon fanciers. The aims of the various investigations were:-

1) to validate the PBD criteria formulated in questionnaire work, and assess long term effects on pulmonary function.

2) to identify any early post-exposure, changes in lung function in view of the immediate symptoms and skin test reactivity prevalent in the clinical studies.

3) to assess the physiological integrity of affected and actively exposed pigeon fanciers.

RESULTS

1. **Loft Challenge**

Pulmonary function was monitored for 24 hours following a natural exposure consisting of an hour spent in the loft environment handling the birds and cleaning the loft. Two groups of pigeon fanciers living in the same vicinity and who had participated in the 1976/77 survey were studied. There was a group fulfilling the present criteria for PBD and another group with lesser symptoms, designated an indeterminate response. The PBD group included 2 local pigeon fanciers not seen in the original survey. All the subjects were immunised to pigeon globulin and were still connected with pigeon racing although some had substantially modified their contact after developing symptoms. The characteristics of the 2 groups are shown in Table 20. Details of the challenge procedures are described under methods (Chapter II).

Among the PBD group one person developed a clinical response with pyrexia and general malaise plus leukocytosis and a further 2 persons had a modest rise in peripheral white cell count. All subjects were monitored for at least 8 hours after entering the loft environment. Considerable individual variation was evident in the pattern of pulmonary function change seen during this

TABLE 20 Details of loft challenge; P B D and indeterminate response (ind. resp.)

| Subject | Age (years) | Duration of P B D (years) | Exposure+ Pattern | Immediate Response | Chronic P B D | Avian-specific IgG (ug/ml) | Response to challenge |
|---|---|---|---|---|---|---|---|
| H.M. | 29 | 3 | Active | | | 63 | "Alveolar" reaction |
| J.McK. | 55 | <1 | Active (M) | + | | 14 | None |
| A.W. | 63 | 7 | Less active | + | + | 113 | WBC 12.5 x $10^9$/L |
| W.O. | 39 | <1 | Active (M) | + | | 62 | None |
| W.K. | 49 | 3 | Active | + | + | 104 | None |
| R.S. | 30 | 3 | Active (M) | | + | 75 | WBC 13.2 x $10^9$/L |
| J.Br. | 56 | 2 | Less active | + | + | 48 | None |
| J.H. | 63 | >1 (ind.resp) | Little contact | | + | 36 | None |
| W.McC. | 46 | 2 " " | Active | | + | 18 | None |
| J.B. | 57 | 5 " " | Little contact | + | | 30 | None |
| A.B. | 60 | 7 " " | Less active | | + | 54 | None |
| P.B. | 67 | 2 " " | Active | + | | 21 | None |
| A.T. | 68 | 4 " " | Active | + | | 18 | None |
| A.McG. | 26 | <1 " " | Active | + | | 32 | None |
| T.C. | 32 | >1 " " | Active | | | 17 | None |
| J.M. | 52 | <1 " " | Active (M) | | | 9 | None |

Active - full participation
Less active - reduced weekly contact and/or wearing a mask (M)
Little contact - no pigeons but attends club

period but overall trends are summarised in Table 21. For the PBD group mean FVC decreased between 0and 4 hours post-challenge (paired t-test, $p<0.025$), remained reduced at 8 hours (compared to baseline) but had improved by 24 hours. A reduction in mean KCO was also evident between 0 and 8 hours (paired t-test, $p<0.05$) and had recovered by 24 hours. There was no significant change in mean $FEV_1/FVC$ (% predicted) for the group during the interval monitored but mean RV had risen at 8 hours (paired t-test, $p<0.025$) and returned to baseline level by 24 hours. Table 21 also shows the findings for those with an indeterminate response. None of this group had any clinical features to suggest a positive "alveolar" response (general malaise, pyrexia, leukocytosis) and the only significant change in pulmonary function was a reduction in mean FVC 8 hours after challenge (paired t-test, $p<0.02$). A separate analysis of mean $FEV_1/FVC$ (% predicted) was performed for those subjects reporting an Immediate Response (9 persons), irrespective of whether acute PBD, was present but no evidence of airways obstruction was found.

Since the change in lung function was small in relation to individual variation (S.E.M.), and occurred largely in the absence of definite clinical abnormality other factors which might influence these tests such as circadian rhythm, require consideration. A diurnal variation in airways resistance is well documented (Cotes 1979) with airflow obstruction most marked in the early morning and decreasing towards evening. The reduction

TABLE 21 Mean values (± S.E.M.) for F.V.C., $K_{CO}$, and R V during the 24 hours after loft challenge

| | | TIME (from commencing challenge) | | | |
|---|---|---|---|---|---|
| | | 0 | 4 | 8 | 24 hours |
| F.V.C. (litres) | PBD | 3.6 ± 0.33 | 3.45± 0.35 **(−0.15)** p<0.025 | 3.35 ± 0.35 **(−0.25)** p<0.05 | 3.6 ± 0.3 |
| | Ind. resp. | 3.7 ± 0.3 | 3.62 ± 0.31 | 3.57 ± 0.28 **(−0.13)** p<0.02 | 3.78 ± 0.32 |
| R.V. (litres) | PBD | 1.8 ± 0.16 | | 1.93 ± 0.11 **(+0.13)** p<0.025 | 1.85 ± 0.12 |
| | Ind. resp. | 1.75 ± 0.16 | | 1.72 ± 0.17 | 1.75 ± 0.17 |
| $K_{CO}$ (mmol/ min/ kPa/L) | PBD | 1.3 ± 0.11 | 1.5 ± 0.12 **(−0.13)** p<0.05 | 1.24 ± 0.17 | 1.4 ± 0.08 |
| | Ind. resp. | 1.52 ± 0.07 | 1.97 ± 0.1 | 1.45 ± 0.07 | 1.49 ± 0.08 |

Analysis by paired t-test, all values compared to time zero
Bold print - magnitude and direction of change
Ind. resp. - indeterminate response.

in FVC occurring around 1300 hours (4 hours post-challenge) in the absence of any change in $FEV_1/FVC$ would not therefore seem accounted for in this way.

Cinkatoi and Thomson (1966) reported a diurnal rhythm in $T_{L_{CO}}$ with a fall throughout the daytime and evening and therefore of potential importance in monitoring the effects of inhalational challenge in EAA. This was investigated by performing a series of sequential single breath $T_{L_{CO}}$ measurements on normal healthy adults between 9am and 5pm. The individual results are shown in Figure 18 and although considerable variation is evident in the pattern of change over the 8 hour period there was no discernable downward trend. When the KCO measurements of the volunteers were examined there was an initial decrease (0 - 2 hours paired t-test, $p<0.02$) but thereafter no appreciable change (Figure 19). The initial KCO readings may have been inappropriately high due to the effect of apprehension at the first set of measurements but in any event no general trend in $T_{L_{CO}}$ or KCO was seen during the period (1pm - 5 pm) relevant to monitoring post-exposure events in EAA (4 - 8 hours).

## 2. Chronic PBD

There were 7 subjects with chronic PBD (Table 20) and the baseline mean FVC (% predicted) was 68.2% ± 4.0, significantly less than the 82.2% ± 3.39 value for the other 9 persons challenged (paired t-test, $p<0.05$). The mean KCO was also lower for the group with chronic PBD (1.32 versus 1.55), but the

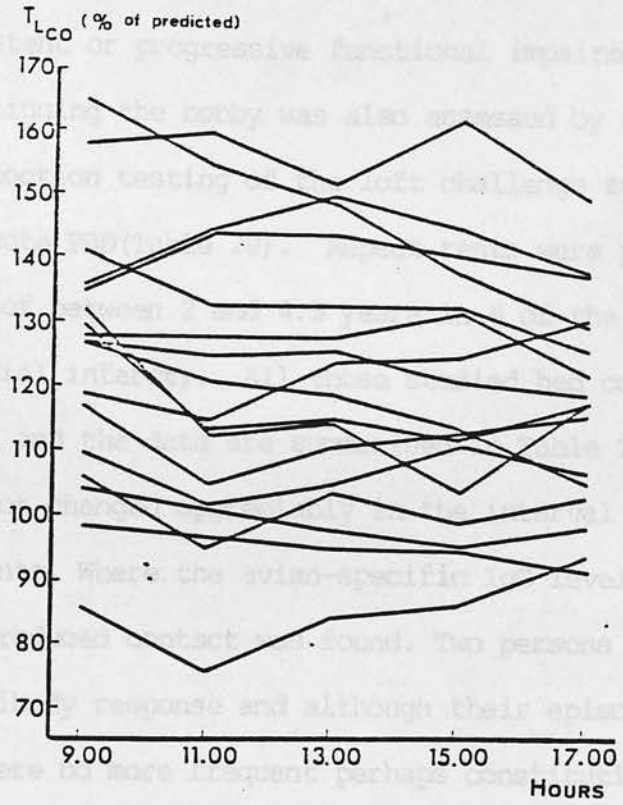



FIGURE 18 Individual measurements of $T_{L_{CO}}$ in normal subjects (9am–5pm)

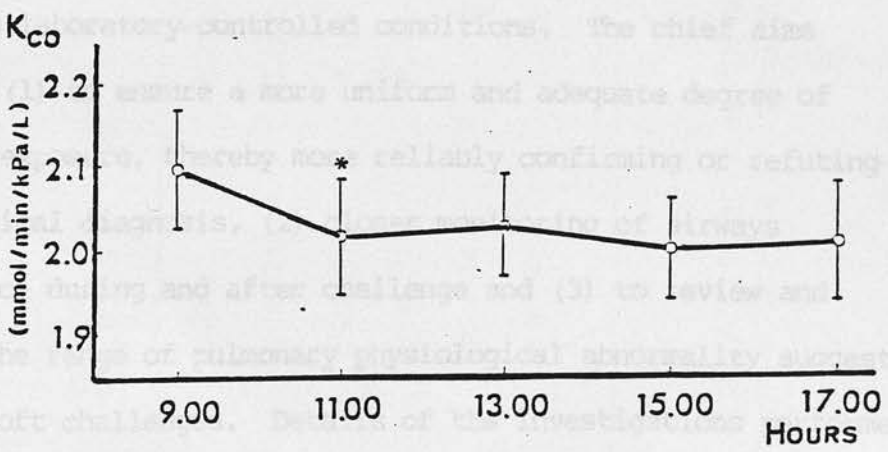



FIGURE 19 $K_{CO}$ measured between 9am–5pm in normal subjects (mean ± S.E.M.)

* difference from 9.00 hours (t-test, $p<0.02$)

difference did not reach statistical significance.

Persistent or progressive functional impairment in affected persons continuing the hobby was also assessed by follow up pulmonary function testing of the loft challenge subjects who described acute PBD(Table 20). Repeat tests were performed after an interval of between 2 and 4.5 years in 6 of the 7 (AW deceased from myocardial infarct). All those studied had continued to keep pigeons and the data are summarised in Table 22. Clinical status had not changed appreciably in the interval between the two assessments. Where the avian-specific IgG level had fallen evidence of reduced contact was found. Two persons had a more vigorous antibody response and although their episodes of alveolitis were no more frequent perhaps constitutional symptoms were more severe. Neither showed a deterioration in FVC or KCO and nor did the group overall.

### 3. Laboratory challenge

A further 10 challenge tests were conducted under more rigorous laboratory-controlled conditions. The chief aims being:- (1) to ensure a more uniform and adequate degree of antigen exposure, thereby more reliably confirming or refuting the clinical diagnosis, (2) closer monitoring of airways resistance during and after challenge and (3) to review and extend the range of pulmonary physiological abnormality suggested by the loft challenges. Details of the investigations performed are again described under methods (Chapter II).

TABLE 22 Follow-up lung function tests, (P B D)

| Subject | Interval between tests | F.V C | | K$_{CO}$ | | Avian-specific IgG (ug/ml) | |
|---|---|---|---|---|---|---|---|
| | | 1st value | Recent value | 1st value | Recent value | 1st value | Recent value |
| H.M. | 2yrs | 5.35L | 5.28L | 1.76L | 1.63 | 63 | 95 |
| W.O. | 3yrs 5mo | 3.93L | 3.4 L | 1.60 | 1.76 | 62 | 32 |
| R.S. | 4yrs 5mo | 3.17L | 3.05L | 1.56 | 1.85 | 75 | 152 |
| J.McK. | 4yrs 5mo | 3.27L | 3.3 L | 1.26 | 1.01 | 14 | 18 |
| J.Br. | 4yrs 6mo | 3.6 L | 3.15L | 0.96 | 1.03 | 48 | 55 |
| W.K. | 4yrs 4mo | 3.5 L | 3.65L | 1.44 | 1.51 | 104 | 64 |

Loss of lung volume in some cases (W.O./J.Br.) but no consistent trend in pulmonary function evident (paired t-test)

The general characteristics, immunological features, and the clinical findings following inhalational challenge are detailed in Table 23. Five subjects had previously undergone loft challenge with 2 (HM and RS) showing a response. Both again responded but with more definite features of an acute episode including pyrexia and general malaise. A further person (J McK) responded to this second challenge and in all 7 of the 10 persons developed features of an "alveolar" reaction.

The PEFR was recorded frequently during the inhalational challenge with $FEV_1/FVC$ and $^{Vmax\ 25}/Vmax75$ recordings also performed at 30 minute intervals for 2 hours after challenge, but in no case did airflow obstruction develop. Fluctuations in lung function parameters are summarised for the 7 responding persons in Table 24. There was a significant decrease in mean FVC by 8 hours compared to baseline (paired t-test $P<0.02$) but the KCO had risen.

Five persons agreed to insertion of an arterial line for sequential measurements of A-a02 gradient. A separate analysis was performed for this group which included 3 subjects lacking an "alveolar" response. No significant abnormality developed in any of the routine parameters but the A-a02 gradient increased in all subjects (Figure 20) commencing around 3 hours after challenge, prior to the onset of symptoms.

TABLE 23 Details of laboratory-controlled challenge group.

| Subject | Age (years) | Duration of P B D (years) | Immediate Response | Chronic P B D | Avian-specific IgG(ug/ml) | Atopy (Grade) | Immediate intradermal skin tests (P S) (Grade) | Response to challenge |
|---|---|---|---|---|---|---|---|---|
| J.S. | 45 | 3 | + | | 173 | 1 | 4 | "Alveolar" reaction |
| R.S. | 33 | 8 | | + | 152 | 2 | 3 | " |
| T.P. | 50 | >1 | | + | 122 | | 4 | " |
| J.P. | 25 | <1 | | + | 35 | 2 | 2 | " |
| R.D. | 34 | 5 | | | 89 | | 4 | " |
| H.M. | 33 | 7 | | | 95 | 2 | 3 | " |
| J.McK | 59 | 5 | + | | 18 | 3 | 3 | " |
| D.S. | 32 | <1 | | + | 121 | | 3 | None |
| W.O. | 43 | 5 | + | | 32 | 2 | 2 | " |
| J.Br. | 60 | 6 | + | + | 55 | | 2 | " |

TABLE 24 Mean values (±S.E.M.) for F.V.C., $K_{CO}$ and $^{Vmax25}/_{Vmax75}$ for 7 persons responding to laboratory challenge

| | TIME (from commencing challenge) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 8 hours |
| F.V.C. (litres) | 4.1 ± 0.37 | | 4.0 ± 0.42 | **3.73 ± 0.37 (−0.37) p<0.02** |
| $^{Vmax25}/_{Vmax75}$ | 0.18 ± 0.04 | 0.2 ± 0.04 | 0.2 ± 0.04 | 0.17 ± 0.03 |
| $K_{CO}$ (mmol/min/kPa/L) | 1.58 ± 0.07 | | 1.57 ± 0.08 | **1.61 ±0.08 (+0.13) p<0.01** |

Analysis by paired t-test, all values compared to time zero
Bold print - magnitude and direction of change

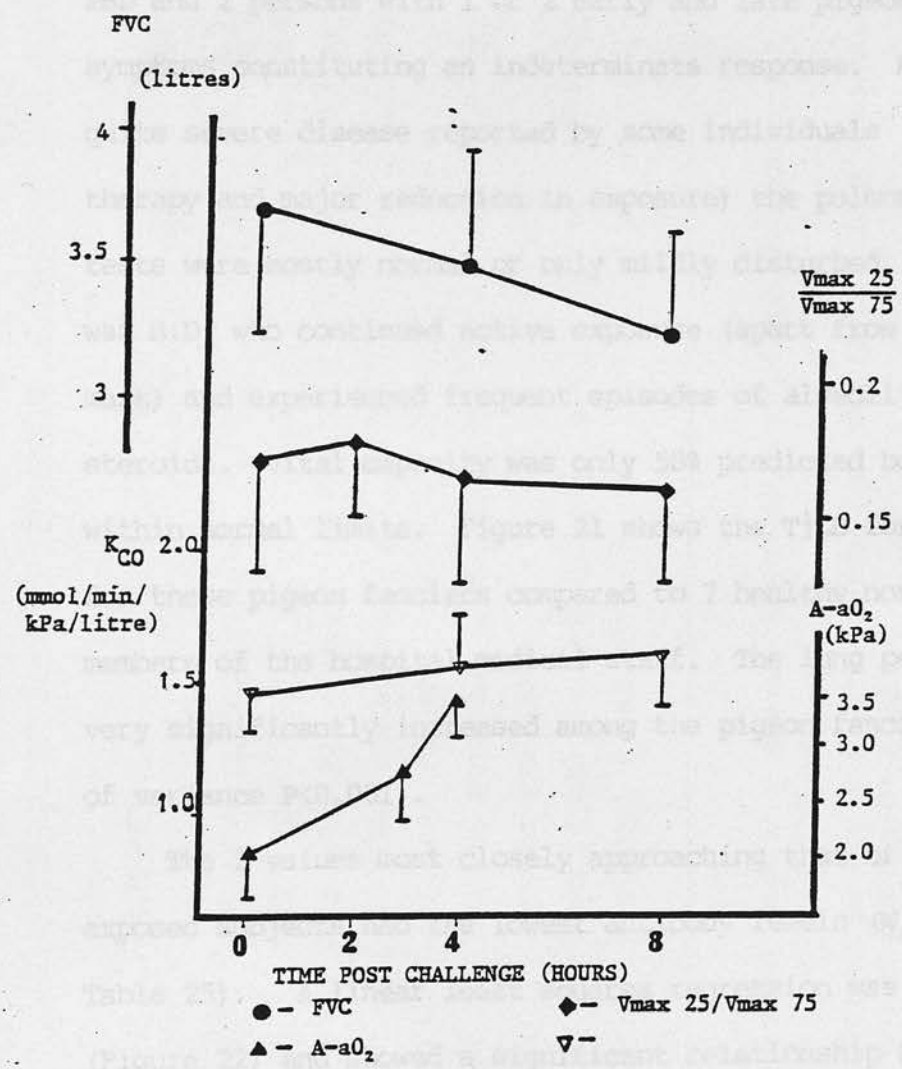



FIGURE 20 A-a$O_2$ gradients and associated pulmonary function parameters following laboratory-controlled challenge

4. **Lung permeability**

The details of the 9 persons who underwent a lung permeability study (Chapter II) are shown in Table 25. Subjects were selected from pigeon fanciers who had more recently (1983/84) come to attention due to suspected symptoms, were non smokers and mostly continuing to keep pigeons. They were a varied group with 4 describing acute PBD, 3 describing an insidious onset of cough and breathlessness suggesting chronic PBD and 2 persons with 1 or 2 early and late pigeon-related symptoms constituting an indeterminate response. Despite the quite severe disease reported by some individuals (ie steroid therapy and major reduction in exposure) the pulmonary function tests were mostly normal or only mildly disturbed. The exception was B.D. who continued active exposure (apart from wearing a mask) and experienced frequent episodes of alveolitis even on steroids. Vital capacity was only 58% predicted but $K_{CO}$ remained within normal limits. Figure 21 shows the $T\frac{1}{2}LB$ for 99m-TcDTPA for these pigeon fanciers compared to 7 healthy non-smoking male members of the hospital medical staff. The lung permeability was very significantly increased among the pigeon fanciers (analysis of variance $P<0.001$).

The 2 values most closely approaching that of the non-exposed subjects had the lowest antibody levels (W.K. and D.O. Table 25). A linear least squares regression was performed (Figure 22) and showed a significant relationship between increasing intensity of IgG antibody response (ug/ml) and increasing lung permeability (falling $T\frac{1}{2}LB$) (correlation coefficient 0.72, $p<0.01$).

TABLE 25 Details of pigeon fanciers undergoing lung permeability study

| Subject | Age (years) | Symptoms | Duration (years) | IgG ug/ml | PFTs | $T\frac{1}{2}LB$ (mins) |
|---|---|---|---|---|---|---|
| W.W. | 28 | PBD - had predn,giving up | 2 | 90 | N | 29.0 |
| D.O. | 31 | PBD - mask | 1 | 12 | V.C. 75% | 63.0 |
| P.McM. | 32 | I.R. plus PBD - mask | 2 | 72 | $FEV1/FVC$ 65% | 27.1 |
| B.D. | 38 | PBD -10mg predn, mask | 2 | 180 | V.C. 58% | 14.2 |
| W.K. | 47 | ind.resp. - mask | 1 | 4 | N | 43.6 |
| J.McK. | 52 | chronic PBD - had predn, mask | 1 | 90 | V.C. 70% | 8.7 |
| J.McK. | 55 | chronic PBD - mask | 1 | 36 | N | 23.0 |
| D.A. | 65 | chronic PBD - giving up hobby | 1.5 | 94 | $FEV1/FVC$ 40% | 18.8 |
| A.C. | 67 | ind.resp. - mask | 1 | 90 | N | 15.8 |

Subjects had never regularly smoked cigarettes, except D.A. who was an ex-smoker of 15 years standing

N - denotes normal pulmonary function tests (helium dilution lung volumes, spirometry and single breath $T_{L_{CO}}$).

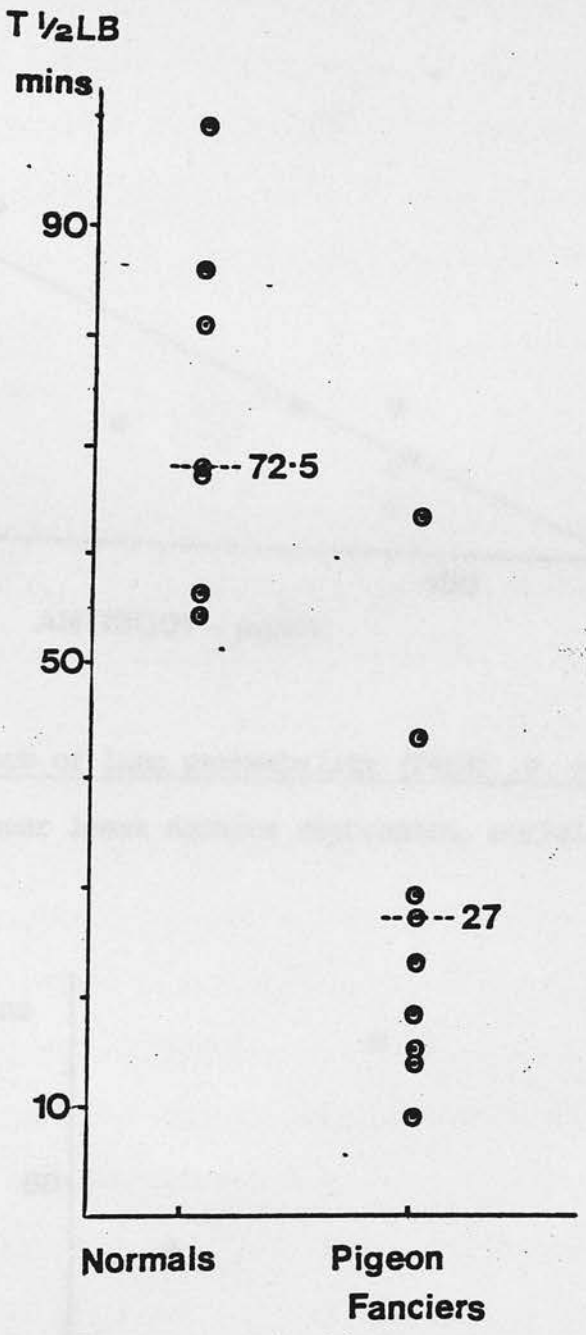



FIGURE 21 Lung permeability ($T_{\frac{1}{2}}LB$) in pigeon fanciers and non-exposed volunteers

normals - healthy non-smoking male members of medical staff (mean age 32.4; range 28 - 40 years)

pigeon fanciers - as detailed in Table 25, (mean age 48.2; range 31 - 66 years)

analysis of variance, $p<0.001$

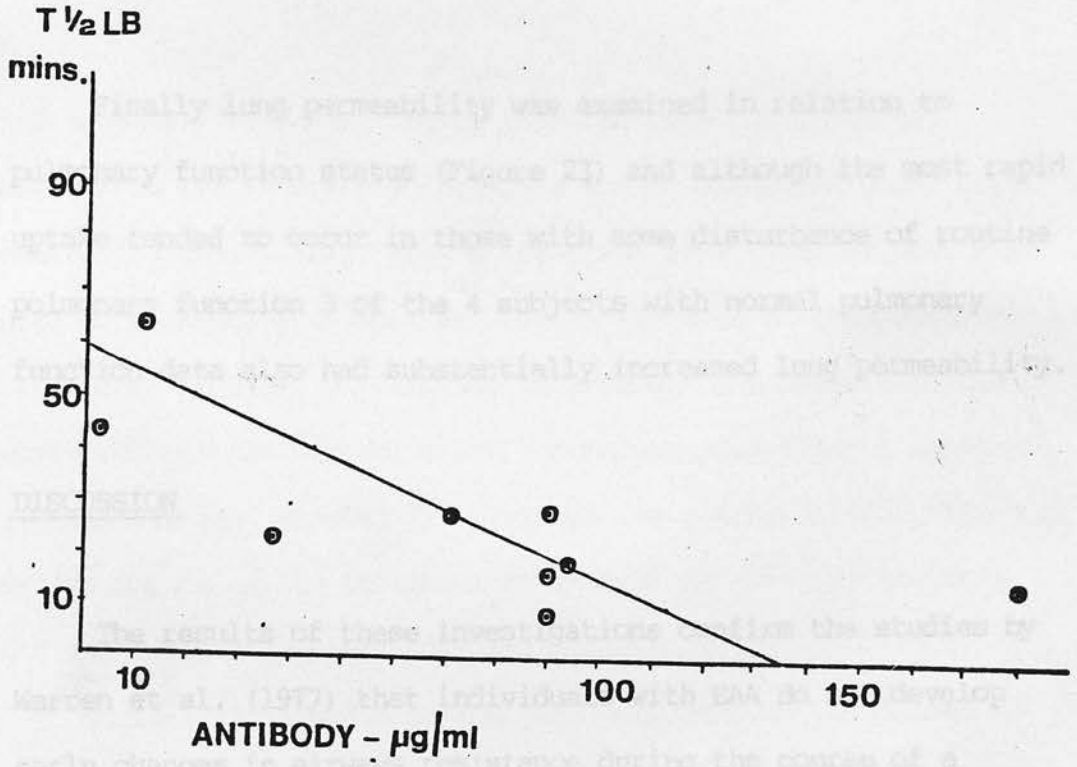



FIGURE 22 Graph of lung permeability ($T\frac{1}{2}LB$) .v. avian-specific IgG (ug/ml)

Linear least squares regression, correlation coeff. 0.72, $p<0.01$.

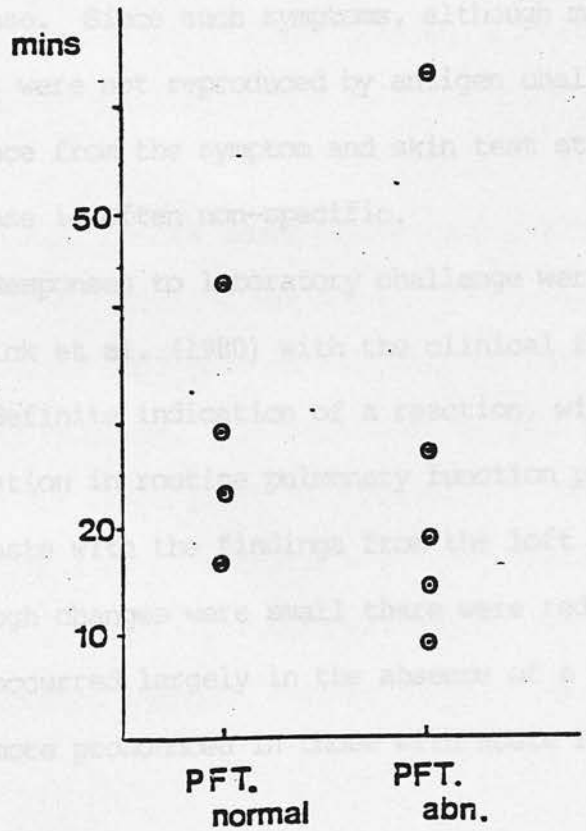



FIGURE 23 Lung permeability ($T\frac{1}{2}LB$) and pulmonary function (PFT)

PFT normal and abnormal are as shown in Table 25

Finally lung permeability was examined in relation to pulmonary function status (Figure 23) and although the most rapid uptake tended to occur in those with some disturbance of routine pulmonary function 3 of the 4 subjects with normal pulmonary function data also had substantially increased lung permeability.

## DISCUSSION

The results of these investigations confirm the studies by Warren et al. (1977) that individuals with EAA do not develop early changes in airways resistance during the course of a positive inhalational challenge test despite having immediate skin test reactivity. Furthermore neither immediate symptoms nor early post-exposure airways obstruction were evident after antigen challenge in fanciers who had reported an Immediate Response. Since such symptoms, although mostly upper respiratory tract, were not reproduced by antigen challenge this supports the evidence from the symptom and skin test studies that such a response is often non-specific.

Responses to laboratory challenge were similar to those of Hendrick et al. (1980) with the clinical features providing the most definite indication of a reaction, without a consistent alteration in routine pulmonary function parameters. This contrasts with the findings from the loft challenge where although changes were small there were reductions in FVC and $K_{CO}$ that occurred largely in the absence of a clinical response and were more pronounced in those with acute PBD compared to an

indeterminate response. These findings were not confirmed however in the laboratory challenge series conducted under more controlled conditions. Only a reduction in FVC was evident with diffusing capacity unimpaired despite the more obvious clinical responses. No clear explanation is apparent but these findings again reflect the disparity and inconsistencies evident in other reported series (Chapter I). Perhaps the patchy distribution and varied nature of the inflammatory process in some way accounts for these differing physiological abnormalities.

Overall the results of the challenge studies would seem to support the criteria for response and classification formulated in the clinical studies including the concept of acute recurrent disease since medium term follow up of a small group with acute PBD showed no appreciable deterioration. Nevertheless it is possible that the persistent symptoms of chronic PBD could become more evident after a longer period, especially in view of the evidence of altered physiology found using more "sensitive" tests.

In all 5 cases where measurements were performed an increase in the $A\text{-}a0_2$ gradient was found. Interestingly this abnormality was observed in 3 persons with PBD who did not develop a frank clinical response. In both cases who did respond the alteration in gradient began prior to the onset of clinical features suggesting that some disturbance of pulmonary physiology does occur along with the evolving clinical features and underlying immunological events. Further support for the view that

physiological impairment occurs despite apparently normal pulmonary function is derived from the results of the lung permeability studies. Whilst the exact significance of this relatively new technique is still debated increased permeability has been reported in a number of interstitial lung diseases (Rinderknecht et al., 1980; Pantin et al., 1984).

In the present series, which represents one of the first in relation to EAA there was a much more rapid uptake from lungs to blood of 99m-TcDPTA in pigeon fanciers compared to normals. Although the groups were not matched values obtained in normal subjects were similar to those obtained in other studies (Jones et al., 1980; Gellert et al., 1985). Moreover the 2 lung permeability values approaching normal occurred in subjects with a minimal humoral response and indeed linear regression suggests a relationship between increasing intensity of antibody response and increasing permeability. These findings have been published in preliminary form (Banham et al., 1986b).

Schuyler et al. (1983) have shown rapid handling and transit of antigen specific inhaled particulate material in an animal model of EAA, and suggest that increased permeability at the air-blood barrier is a contributing factor. The present findings would also be consistent with a degree of alveolar epithelial damage. These interesting findings require confirmation in a larger controlled series and should form the basis for future investigations in EAA.

In summary the routine pulmonary function data are generally

in keeping with the results of previous studies and provide some further support with respect to the criteria for response and classification adopted in the present work, including the recognition of an apparently stable, although symptomatic phase. However whilst routine pulmonary function parameters remain satisfactory there is evidence of altered pulmonary physiology with respect to A-a$0_2$ gradient and lung permeability. Whether such findings are relevant regarding the long term clinical outcome is at present speculative but merits further investigation.

## RESUME OF MAIN FINDINGS

1. Considerable difficulties are encountered when comparing cases of PBD from different studies due to the various definitions of disease that have been employed. Evaluation of detailed clinical information obtained in the present investigations enabled simple criteria to be constructed that gave a satisfactory diagnostic index and could provide a standardised approach to survey studies in EAA.

2. The spectrum of PBD evident among active pigeon fanciers was found to differ in some respects from that seen in routine clinical practice. Immediate symptoms were common but generally occurred in conjunction with delayed symptoms and did not usually suggest conventional IgE mediated hypersensitivity. Acute PBD appeared "milder", in that affected persons were continuing the hobby without rapid deterioration in their health. However, a modified pattern of exposure has been identified (wearing mask, reducing weekly contact) indicating a self-regulation of symptoms that is an integral part of their overall condition.

There are many causes of chronic breathlessness and accordingly there has been little indication as to the prevalence of chronic pigeon-related respiratory symptoms. However, in these studies unexplained persistent respiratory symptoms

occurred in 20% of non-smokers and were often associated with acute PBD. Permanent sequelae may therefore result from repeated episodes of alveolitis as well as developing insidiously, although pulmonary function data over a 2 - 5 year time span suggests stability rather than deterioration in the few selected cases that could be studied.

3. As a result of these clinical findings it is proposed that the current classification be modified to encompass the following categories which need not be mutually exclusive.

a) **Acute progressive disease** - typical hospital-referred case where exposure usually ceases.

b) **Acute recurrent disease** - "milder" variety encountered in survey studies with a prevalence of 10% in the present work. Self-regulation of exposure is an important aspect.

c) **Immediate Response** - common but appear most subject to non-specific over-reporting. Mostly reported with acute recurrent PBD constituting an indistinct group.

d) **Chronic PBD** - perhaps the commonest type overall affecting possibly 20% or more. Consists of both insidious onset of breathlessness alone and permanent symptoms in those with acute recurrent disease continuing exposure.

4. Serum avian-specific IgG estimated by a quantitative radioimmunoassay showed differences in the significance of the

humoral response depending on the intensity. Immunisation was progressively more probable as exposure increased (years, number of pigeons) but only intense responses (>60 ug/ml), were closely associated with PBD and these tended to occur in persons with short exposure histories. A vigorous humoral response is thus closely linked to individual susceptibility for the disease essentially independent of the degree of exposure.

5. The presence of atopy and/or elevated serum IgE did not appear to actively influence the likelihood for finding PBD. However, skin testing and serological investigations showed a close relationship between immediate intradermal reaction (PS) and IgG response to pigeon globulin. This skin reactivity was not associated with IgE (total or specific) or Type I clinical allergy but did correlate with the presence of PBD. Moreover this relationship was evident irrespective of whether late 6 hour reactions developed. The avian-specific IgG response therefore includes reaginic activity and this correlated as well with the presence of PBD (Grade 4 immediate intradermal reaction to PS) as did the overall IgG response (>60ug/ml)

6. Routine pulmonary function monitoring after antigen challenge failed to identify consistent abnormalities with clinical features providing the most reliable evidence of a response. A reduction in FVC is the most frequently observed

change although a decrease in FVC and $K_{CO}$ were found even in some cases without accompanying clinical features. The A-$aO_2$ gradient increased in all 5 cases where this was measured beginning prior to the development of any clinical response and occurring even in the absence of such a response. Immediate or delayed airways obstruction was not a feature in any of the challenge studies even when numerous immediate symptoms (IR) were reported. The variable patterns and degree of pulmonary function impairment probably reflect the nature of the pulmonary inflammation which is neither uniformly distributed, affecting variably the gas exchanging area and small airways, nor of the same character, ranging from pleomorphic cellular lesions to organising fibrosis.

7. Lung permeability was greatly increased among a group of pigeon fanciers with varying degrees of clinical and humoral response. The increased permeability showed a relationship to vigorous antibody response and was less associated with abnormality of routine pulmonary function tests. Further investigation and confirmation of this potentially sensitive and early indicator of altered physiological integrity is required.

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## APPENDIX 1

Questionnaire used in the 1979/80 survey study. Forms completed by direct interview supervised by myself. Data coded and transferred to PDP-11 computer.

G.R.I. - C.R.I. : PBD/SWB Winter 1979

Name: Survey No.

Address: Unit No.

G.P.'s Name and Address: Date

Date of Birth

Instructions: Please tick the appropriate box, or write in the space provided when indicated.

If in doubt answer 'NO'

Pigeon Data:

1. Do you keep pigeons at present?

| | |
|---|---|
| YES | |
| NO | |

if NOT when did you stop and why? _____

_____

2. Have you kept pigeons continuously? ☐ or periodically? ☐

give details of any 12 months, or longer interruptions _____

_____

3. How long in total have you kept pigeons ? _____YRS _____MTHS

4. How many pigeons do you generally keep? _____

5. Have you ever been in regular contact with other birds?

| | |
|---|---|
| YES | |
| NO | |

if yes, please give details _____

_____

6. How many hours, on average, do you spend in contact with the pigeons each week during the season?

_____

7. Do you ever wear a mask when in contact with pigeons?

| | |
|---|---|
| YES | |
| NO | |

Has this helped your chest? _____

8. When in contact with your pigeons have you EVER noticed:

| | Yes | No |
|---|---|---|
| a. shortness of breath | ☐ | ☐ |
| b. wheezing in your chest | ☐ | ☐ |
| c. a dry cough | ☐ | ☐ |
| d. sneezing, blocked or running nose | ☐ | ☐ |
| e. itchy/running eyes | ☐ | ☐ |
| f. tightness in chest | ☐ | ☐ |

9. Have the above symptoms EVER been severe enough to make you come away from the pigeons?

| | |
|---|---|
| YES | ☐ |
| NO | ☐ |

10. How long after coming into contact with pigeons do these symptoms occur?

| | |
|---|---|
| less than ten minutes | ☐ |
| ten to thirty minutes | ☐ |
| more than thirty minutes | ☐ |

11. Do these symptoms occur:

| | |
|---|---|
| occasionally (once per 3mths) | ☐ |
| sometimes (once per 2 wks) | ☐ |
| usually (at least twice per wk) | ☐ |

12. How long have you had them? _____

13. Several hours (4 to 8) AFTER being in contact with pigeons have you EVER experienced attacks of, or persistent:

| | Yes | No |
|---|---|---|
| a. shortness of breath | ☐ | ☐ |
| b. wheeze | ☐ | ☐ |
| c. shivering and/or feeling cold | ☐ | ☐ |
| d. sweating | ☐ | ☐ |
| e. fever or temperature | ☐ | ☐ |
| f. dry cough | ☐ | ☐ |

| | YES | NO |
|---|---|---|
| g. tiredness, aching muscles or headache | ☐ | ☐ |
| h. tightness in chest | ☐ | ☐ |

14. How long is it before you feel well again? _____

15. Have you ever noticed "flu-like" feelings in the evening and at night which have gone by the following morning?

| | |
|---|---|
| YES | |
| NO | |

16. How often do these symptoms occur?

occasionally (once per 3 mths) ☐

sometimes (once per 2 wks) ☐

usually (at least twice per wk) ☐

17. How long have you had them? _____

18. Are these symptoms different -

a. at the weekend

| | |
|---|---|
| WORSE | |
| BETTER | |
| SAME | |

b. after attending a pigeon show

| | |
|---|---|
| WORSE | |
| BETTER | |
| SAME | |

c. after a holiday away from home

| | |
|---|---|
| WORSE | |
| SAME | |
| BETTER | |

d. after cleaning out the loft

| | |
|---|---|
| WORSE | |
| SAME | |
| BETTER | |

19. Has any doctor suggested that you may have chest trouble related to keeping pigeons

| | |
|---|---|
| YES | |
| NO | |

please give details of any tests/treatment _____

RESPIRATORY DATA

20. Do you USUALLY cough first thing in the morning in winter?

| | |
|---|---|
| YES | |
| NO | |

21. Do you USUALLY cough during the day in the winter?

| | |
|---|---|
| YES | |
| NO | |

22. Do you cough like this on most days for as much as three months each year?

| | |
|---|---|
| YES | |
| NO | |

23. Do you USUALLY bring up any spit or phlegm from your chest first thing in the morning in the winter?

| | |
|---|---|
| YES | |
| NO | |

24. Do you USUALLY bring up any spit or phelgm from your chest during the day in the winter?

| | |
|---|---|
| YES | |
| NO | |

25. Do you bring up spit like this on most days for as much as three months each year?

| | |
|---|---|
| YES | |
| NO | |

26. Do you ever get short of breath when walking with others of your own age, on level ground?

| | |
|---|---|
| YES | |
| NO | |

27. During the past three years have you ever had any chest illnesses which have kept you from your usual activities for more than a week?

| | |
|---|---|
| YES | |
| NO | |

28. Have you ever had –

a. heart trouble ☐

b. Bronchitis ☐

c. Pneumonia/Pleurisy ☐

d. Tuberculosis ☐

e. Asthma ☐

f. Other chest illnesses ☐

29. Have you ever had any illness requiring a period in hospital?

| | |
|---|---|
| YES | |
| NO | |

Give details _____

_____

30. Do you require regular medicines for any condition?

| | |
|---|---|
| YES | |
| NO | |

a. What is the condition? _____

b. What are the medicines? _____

31. Have you ever worked in

| | | |
|---|---|---|
| a. | a coal mine? | YES / NO |
| b. | any other mine? | YES / NO |
| c. | a quarry? | YES / NO |
| d. | a foundry? | YES / NO |
| e. | a pottery? | YES / NO |
| f. | a cotton, flax or hemp mill? | YES / NO |
| g. | with asbestos? | YES / NO |

32. Do you smoke? YES / NO

33. How many cigarettes/cigars do you smoke per day _____

34. How long have you smoked? _____

35. If you have ever smoked,

a. How long did you smoke for? _____ yrs

b. How many did you smoke per day _____

c. When did you stop? _____

36. Allergies.

Have you ever suffered from

| | | |
|---|---|---|
| a. | Hay fever | YES / NO |
| b. | Asthma | YES / NO |

c. Eczema/Dermatitis

| | |
|---|---|
| YES | |
| NO | |

d. Were you "chesty" as a child?

| | |
|---|---|
| YES | |
| NO | |

37. What do you suspect causes the/these condition/s in your case?

_____

38. Does any relative have any of the conditions listed above?

_____

C.R.I. - C.R.I. : PBD/SWB Winter 1979

Survey No.

Date

Data

| | | | |
|---|---|---|---|
| Questionnaire completed | ☐ | Intradermal pigeon serum performed | ☐ |
| 20 mls of venous blood obtained | ☐ | Intradermal pigeon feather performed | ☐ |
| Tine test performed | ☐ | Prick skin tests performed | ☐ |

Skin test results

| | |
|---|---|
| mixed feathers | ☐ |
| pigeon serum | ☐ |
| pigeon feathers | ☐ |
| intra dermal P.S. | ☐ |
| intradermal P.F. | ☐ |
| late reaction P.S. | ☐ |
| late reaction P.F. | ☐ |
| Tine test | ☐ |
| H. Dust | ☐ |
| Grass pollen | ☐ |
| Cat Fur | ☐ |
| Aspergillus fumigatus | ☐ |
| Sporobolo myces | ☐ |
| Control | ☐ |
| **Control** (I.D.) | ☐ |

## APPENDIX 2

Section 1: Clinical information for the 100 pigeon fanciers interviewed in 1979/80.

Section 2: Skin test responses and serological data for the 100 pigeon fanciers in the 1979/80 study.

APPENDIX 2. Section 1. General characteristics and symptoms reported for subjects with P.B.D. "only".

| Subjects | Age (years) | Exposure Data: Years exposed | Exposure Data: Other | Mask | Chronic P.B.D. | Immediate Symptoms | Delayed Symptoms | Allergic History |
|---|---|---|---|---|---|---|---|---|
| 029 | 56 | 25 | | | | 8cd | 13ef | 36c |
| 102 | 42 | 30 | | + | + | | 13acdegl5 | 36b |
| 101 | 44 | 33 | | + | | | 13acefgl5 | 36a |
| 080 | 63 | 46 | | | + | 8d | 13fgl5 | 36c |
| 030 | 36 | 16 | steroids | | + | 8df | 13dhl5 | |
| 054 | 64 | 25 | | | + | 8c | 13cdefgl5 | 36a |
| 065 | 63 | 54 | | | | | 13afl5 | |
| 072 | 70 | 20 | | | | | 13afg | |
| 095 | 44 | 30 | | | | | 13acdefhl5 | |
| 010 | 37 | 20 | | + | | | 13agh | 36ab |
| 024 | 27 | 20 | | | | 8a | 13bl5 | |
| 078 | 46 | 8 | | | | | 13al5 | 36b |
| 017 | 56 | 50 | | + | + | | 13ag | |
| 026 | 46 | 7 | | | | 8a | 13acdegl5 | |
| 056 | 46 | 15 | | | | | 13edgl5 | F |
| 001 | 48 | 30 | Stopped now | + | + | | 13cdegl5 | |

F - family history of allergic disease

APPENDIX 2. Section 1. General characteristics and symptoms reported for subjects with P.B.D. plus Immediate Response or Immediate Response "only".

| Subjects | Age (years) | Exposure Data: Years exposed | Exposure Data: Other | Mask | Chronic P.B.D. | Immediate Symptoms | Delayed Symptoms | Allergic History |
|---|---|---|---|---|---|---|---|---|
| 013 | 38 | 28 | | + | + | 8abcf | 13abfgh15 | 36b |
| 048 | 39 | 23 | | | | 8abcef | 13abcdefgh 15 | 36c |
| 005 | 54 | 40 | | | + | 8abef | 13afg | 36c |
| 100 | 55 | 45 | | | + | 8abcef | 13abcdefgh 15 | 36a |
| 086 | 40 | 30 | | | | 8bcf | 13dfh | |
| 083 | 43 | 28 | Stopped now | + | | 8acd | 13acdef15 | |
| 041 | 44 | 32 | | | | 8acd | 13cf15 | |
| 082 | 58 | 23 | | | | 8abcdf | 13dfg15 | 36d |
| 097 | 47 | 17 | | | + | 8abc | 13acdefgh15 | 36cd |
| 002 | 56 | 15 | Steroids | + | + | 8abcf | 13cdfh15 | 36b |
| 025 | 68 | 60 | | | | 8abf | 13ade | |
| 015 | 42 | 30 | | + | | 8acdf | 13f 15 | 36c |
| 040 | 48 | 35 | | | | 8adf | 13gh | |
| 086 | 45 | 20 | | + | + | 8abcf | 13abcdegh 15 | 36ab |
| 031 | 45 | 16 | | | | 8abc | 13cdeg15 | |
| 039 | 29 | 22 | | + | | 8abcdef | 13cdeg15 | |
| 028 | 24 | 17 | | | + | 8abd | 13abh | 36a |
| 088 | 45 | 12 | | + | | 8abcdef | 15 | 36ab |
| 052 | 63 | 43 | | | + | 8acde | 15 | 36a |
| 068 | 28 | 21 | | | | 8bcf | 13h | |
| 023 | 56 | 41 | | + | + | 8acdef | 13f | 36ac |
| 033 | 38 | 6 | | | | 8abf | 15a | 36ac |
| 093 | 31 | 20 | | | | 8def | 15 | 36a |
| 053 | 46 | 40 | | | | 8abd | 13cd | |

APPENDIX 2. Section 1. General characteristics and symptoms reported for subjects with an indeterminate response.

| Subjects | Age (years) | Exposure Data: Years exposed | Other | Mask | Chronic P.B.D. | Immediate Symptoms | Delayed Symptoms | Allergic History |
|---|---|---|---|---|---|---|---|---|
| 027 | 62 | 20 | | | | | 13g 15 | 36c |
| 089 | 49 | 41 | | | + | 8c | | |
| 014 | 44 | 30 | | + | | 8be | | |
| 020 | 55 | 25 | | | | 8a | | 36a |
| 016 | 26 | 8 | | | + | 8de | | 36ab |
| 044 | 35 | 12 | | + | | 8de | | |
| 037 | 59 | 40 | | + | | 8bc | | 36d |
| 042 | 33 | 25 | | | | 8d | | |
| 059 | 41 | 30 | | | | 8e | | |
| 073 | 67 | 20 | | | | 8f | | |
| 049 | 44 | 10 | | + | + | 8d | | 36c |
| 046 | 60 | 30 | | | | 8c | | |
| 021 | 46 | 20 | | + | | 8d | | |
| 047 | 61 | 51 | | | | 8f | | |
| 035 | 77 | 68 | | | | 8de | | |
| 063 | 56 | 47 | | | | 8e | | |

APPENDIX 2. Section 1. General characteristics and symptoms reported for subjects with an indeterminate response.

| Subjects | Age (years) | Exposure Data: Years exposed | Other | Mask | Chronic P.B.D. | Immediate Symptoms | Delayed Symptoms | Allergic History |
|---|---|---|---|---|---|---|---|---|
| 099 | 40 | 20 | | | | 8d | 15 | 36a |
| 012 | 63 | 50 | | + | | 8ac | 13f | 36b |
| 022 | 28 | 8 | | | | 8ab | 13h | |
| 096 | 17 | 5 | | | | 8de | 13e15 | 36c |
| 092 | 46 | 23 | | + | | 8af | 13ah | |
| 051 | 72 | 25 | | + | | 8a | 13bf | 36a |
| 064 | 54 | 47 | | + | | 8c | 13af | |
| 061 | 21 | 11 | | | | 8d | 13g15 | 36a |
| 009 | 42 | 1 | | | | 8cd | 15 | |
| 090 | 42 | 10 | | | | 8c | 13f | |
| 032 | 41 | 11 | | | | 8c | 13f | |
| 081 | 54 | 40 | | | | 8e | 15 | |
| 066 | 29 | 20 | | | | 8d | 13h | |
| 034 | 42 | 26 | | | | | 13f | |
| 058 | 44 | 20 | | + | | | 15 | 36a |
| 091 | 45 | 14 | | | | | 15 | |

APPENDIX 2. Section 1 General characteristics for subjects with no acute response.

| Subject | Age (Years) | Years Exposed | Mask | Chronic PBD | Allergic History |
|---|---|---|---|---|---|
| 074 | 75 | 65 | | | F |
| 045 | 53 | 10 | + | + | 36d |
| 050 | 53 | 50 | | | 36abF |
| 004 | 66 | 25 | | | |
| 069 | 57 | 49 | | | F |
| 003 | 70 | 60 | | + | |
| 043 | 33 | 25 | | | |
| 070 | 55 | 22 | | | |
| 019 | 52 | 25 | | | |
| 029 | 69 | 19 | | | 36c |
| 062 | 14 | 2 | + | | F |
| 071 | 41 | 5 | | | |
| 067 | 34 | 12 | | | |
| 077 | 36 | 22 | | | |
| 076 | 40 | 6 | | | 36aF |
| 007 | 43 | 25 | | | 36aF |
| 098 | 64 | 50 | + | | |
| 008 | 45 | 25 | | | |
| 038 | 61 | 40 | | | |
| 006 | 53 | 45 | | | |
| 087 | 46 | 18 | | | |
| 060 | 67 | 59 | | | |
| 075 | 46 | 30 | | | |
| 084 | 20 | 19 | | + | 36ad |
| 085 | 36 | 7 | | | |
| 079 | 69 | 4 | | | 36b |
| 078 | 54 | 40 | | | |
| 011 | 52 | 45 | | | |

F - family history of allergic disease

APPENDIX 2. Section 2. Immune data for subjects with P.B.D. "only".

| Study Number | IgG µg/ml | IgE U/ml | Grade Atopy | Prick Test (mm) PS | PF | Intradermal PS(mm) Immed. | Late | Intradermal PF(mm) Immed. | Late | Tine Grade |
|---|---|---|---|---|---|---|---|---|---|---|
| 029 | 22 | < 50 | | | | 7 | | 6 | | 1 |
| 102 | 150 | 140 | 2 | 3 | 2 | 6 | | | | |
| 101 | 47 | 100 | | | | 5 | | 3 | | |
| 080 | 65 | < 50 | 2 | | | 9 | | 6 | | |
| 030 | 109 | < 50 | 1 | | | 10 | | | | |
| 054 | 66 | 130 | | | | 16 | 1 | 7 | 1 | |
| 065 | <4 | 60 | | | | | | 3 | | |
| 072 | 50 | 170 | | | | 14 | | 4 | | |
| 095 | < 4 | 200 | | | | 8 | | 4 | | 1 |
| 010 | 58 | 600 | 3 | 5 | 3 | | | | | |
| 024 | 99 | < 50 | 2 | 2 | | 12 | 2 | 10 | | 4 |
| 018 | 72 | 240 | 1 | | | 10 | 1 | 10 | | 2 |
| 017 | 18 | < 50 | 2 | 2 | | 10 | | 10 | | |
| 026 | 43 | 130 | 2 | 2 | | | | | | |
| 056 | < 4 | 500 | | | | 3 | | 6 | | 2 |
| 001 | 37 | 210 | 2 | 3 | 4 | 7 | | 3 | | |

APPENDIX 2. Section 2. Immune data for subjects with P.B.D. plus Immediate Response or Immediate Response "only".

| Study Number | IgG µg/ml | IgE U/ml | Grade Atopy | Prick Test (mm) PS | PF | Intradermal PS(mm) Immed. | Late | Intradermal PF (mm) Immed. | Late | Tine Grade |
|---|---|---|---|---|---|---|---|---|---|---|
| 013 | 49 | 115 | 3 | | | 8 | 1 | 4 | | |
| 048 | 75 | 210 | 2 | | | 10 | 1 | 10 | 1 | 2 |
| 005 | 19 | 145 | 1 | | | 7 | | 8 | | 2 |
| 100 | 16 | <50 | | | | 12 | | 9 | | 1 |
| 086 | <4 | 190 | 2 | | | 3 | | 5 | | |
| 083 | <4 | 70 | | | | | | | | |
| 041 | <4 | <50 | | | | 4 | | 3 | | |
| 082 | <4 | 150 | | | | 2 | | | | 1 |
| 097 | 93 | <50 | | | | 8 | 2 | 5 | | 2 |
| 002 | 97 | 80 | | 2 | | | | | | |
| 025 | <4 | 250 | | | | 3 | | 3 | 1 | 1 |
| 015 | 67 | 250 | 3 | | | 14 | 1 | 1 | | |
| 040 | <4 | <50 | | | | 10 | | | | |
| 036 | <4 | 330 | 3 | 3 | 3 | 4 | | 8 | | 4 |
| 031 | 54 | 200 | | | | 14 | | 10 | | |
| 039 | 28 | <50 | | 2 | | 6 | | 5 | | 2 |
| 028 | <4 | 240 | 3 | 2 | | 8 | | 7 | | |
| 088 | <4 | 800 | 3 | | | 6 | | 9 | | 3 |
| 032 | 14 | 150 | | | | 6 | | | | 1 |
| 068 | <4 | 220 | | | | 5 | 1 | | | 2 |
| 023 | 33 | 140 | 3 | 2 | 2 | 5 | | 6 | | 1 |
| 033 | <4 | 104 | | | | | | 4 | | 3 |
| 093 | <4 | <50 | 1 | | | 8 | | | | 3 |
| 053 | <4 | 900 | 3 | | 5 | 11 | | 3 | | 3 |

APPENDIX 2. Section 2. Immune data for subjects with indeterminate symptoms.

| Study Number | IgG µg/ml | IgE U/ml | Grade Atopy | Prick Test (mm) PS | Prick Test (mm) PF | Intradermal PS(mm) Immed. | Intradermal PS(mm) Late | Intradermal PF(mm) Immed. | Intradermal PF(mm) Late | Tine Grade |
|---|---|---|---|---|---|---|---|---|---|---|
| 099 | < 4 | 150 | 1 | | | 8 | | 5 | | 3 |
| 012 | 57 | 380 | 2 | 4 | 3 | | | 10 | | 1 |
| 022 | 21 | 250 | 3 | | | 8 | | 9 | | 3 |
| 096 | 17 | 600 | 2 | | 2 | 8 | | 15 | | 1 |
| 092 | < 4 | < 50 | | | | | | | | |
| 051 | < 4 | < 50 | | | | | | 8 | | 3 |
| 064 | < 4 | < 50 | | | | 12 | | 5 | | |
| 061 | < 4 | < 50 | | | | 6 | | 7 | | 1 |
| 009 | < 4 | 100 | | | | | | 5 | | 1 |
| 090 | 107 | < 50 | | | | 7 | | 8 | | 1 |
| 032 | < 4 | < 50 | | | | 5 | | 3 | | |
| 081 | 21 | 70 | | | | 2 | | 10 | | 1 |
| 066 | < 4 | 130 | | | | 5 | | 6 | | 2 |
| 034 | < 4 | 220 | 1 | | | | | 4 | | 3 |
| 058 | < 4 | 100 | | | | | | | | |
| 091 | < 4 | < 50 | | | | 4 | | 3 | | 4 |
| 027 | < 4 | < 50 | | | | | | 2 | | |
| 089 | 12 | < 50 | | | | 8 | | 12 | | |
| 014 | 107 | < 50 | | | | 9 | | 10 | | |
| 020 | < 4 | 120 | | | | 3 | | 5 | | 2 |
| 016 | < 4 | 360 | 3 | | | 6 | | 7 | | 1 |
| 044 | 50 | < 50 | 3 | | 2 | 4 | | 6 | | 3 |
| 037 | < 4 | 165 | | | | 8 | | | | 1 |
| 042 | 12 | 200 | 1 | | | 6 | | 4 | | |
| 059 | < 4 | 165 | 3 | 3 | | 5 | | 12 | | |
| 073 | < 4 | 150 | 2 | | | 6 | | 7 | | |
| 049 | < 4 | < 50 | 2 | | 3 | | | | | 1 |
| 046 | 42 | < 50 | 2 | | 2 | 5 | | 6 | | 4 |
| 021 | < 4 | < 50 | | | | 3 | | | | 2 |
| 047 | < 4 | < 50 | | | | | | | | |
| 035 | < 4 | 130 | | | | | | 3 | | 1 |
| 063 | < 4 | 72 | | | | 7 | | | | 3 |

APPENDIX 2. Section 2. Immune data for subjects with no acute response

| Study Number | IgG ug/ml | IgE U/ml | Grade Atopy | Prick Test (mm) PS | Prick Test (mm) PF | Intradermal PS (mm) Immed. | Intradermal PS (mm) Late | Intradermal PF (mm) Immed. | Intradermal PF (mm) Late | Tine Grade |
|---|---|---|---|---|---|---|---|---|---|---|
| 074 | <4 | 90 | | | | 5 | | 6 | | |
| 045 | 173 | 110 | | | | 9 | | 4 | | |
| 050 | <4 | <50 | | | | | | | | |
| 004 | <4 | <50 | | | | | | 3 | | |
| 069 | <4 | 100 | | | | 10 | | | | |
| 003 | 30 | 370 | 2 | 2 | | 3 | | 6 | | |
| 043 | <4 | 135 | 1 | | | 6 | | 6 | | |
| 070 | <4 | 210 | 1 | | | 8 | | 5 | | 1 |
| 019 | <4 | 190 | | | 2 | 5 | | 8 | 1 | 2 |
| 029 | 118 | 200 | 3 | 2 | | 8 | | 10 | | |
| 062 | <4 | <50 | | | | | | | | |
| 071 | <4 | <50 | 1 | | | 3 | | 3 | | 3 |
| 067 | <4 | 200 | | | | 9 | | 5 | | 2 |
| 077 | <4 | <50 | 1 | | | | | 7 | | 1 |
| 007 | <4 | 270 | 1 | | | | | | | |
| 076 | 56 | 200 | 2 | 2 | | | | 8 | | |
| 098 | 23 | 135 | | | | 8 | 1 | 7 | | 3 |
| 008 | <4 | 250 | | | | 5 | | 5 | 1 | 2 |
| 038 | <4 | <50 | | | | 3 | 3 | | | |
| 006 | 17 | 150 | 1 | | | 4 | | 6 | | 2 |
| 087 | 58 | 150 | | Dermatographia | | 14 | | 9 | | 1 |
| 060 | <4 | <50 | | Dermatographia | | 3 | | 8 | | |
| 075 | <4 | 70 | 1 | | | 3 | | 2 | | |
| 084 | <4 | 120 | | | | | | | | |
| 085 | <4 | 100 | 2 | | | 6 | | 4 | | |
| 079 | <4 | <50 | | | | | | 4 | | 2 |
| 078 | 47 | 100 | 1 | | | 7 | | 6 | | |
| 011 | 210 | 210 | 2 | | | | | | | |

## APPENDIX 3

Oral presentations at scientific meetings and published papers relating to the studies comprising this MD thesis.

**Banham S W, Lynch P P & Boyd G (1978)**
Environmental and constitutional factors determining hypersensitivity to avian antigens in pigeon fanciers
Thorax, 33: 674
**Oral presentation to Thoracic Society (Summer 1978)**

**Banham S W, Lynch P P, McKenzie H, & Boyd G (1979)**
Pulmonary function abnormalities following exposure to antigen in a group of asymptomatic pigeon fanciers.
Scottish Medical Journal, 24: 180.
**Oral presentation to Scottish Thoracic Society (Winter 1978)**

**Banham S W, Lynch P P, Boyd G (1980)**
Pigeon Breeder's Disease. Constitutional and environmental influences on the hypersensitivity reaction.
Clinical Allergy, 10: 350 - 351
**Oral presentation to British Society for Allergy and Clinical Immunology (Summer 1980)**

**Banham S W (1981)**
Immediate and delayed skin reaction in Pigeon Breeder's Disease
Proceedings of the IV Charles Blackley Symposium.
**Oral presentation to the IV Charles Blackley Symposium (Summer 1981)**

**Banham S W (1982)**
Immediate and delayed skin reactions in pigeon fanciers
Proceedings of the XI International Congress of Allergology and Clinical Immunology. Abstract no. 266
**Oral presentation to the XI ICACI**

**Banham S W, McKillop J, Carlyle D & Boyd G (1986)**
Lung permeability in pigeon fanciers
Thorax, 41: 227
**Oral presentation to the British Thoracic Society (Winter 1985)**

**McSharry C, Banham S W, Lynch P P, & Boyd G (1983)**
Skin testing and extrinsic allergic alveolitis
Clinical and Experimental Immunology 54: 282 - 288 **see attached**

**Banham S W, McSharry, Lynch P P, & Boyd G (1986)**
Relationships between avian exposure, humoral immune response, and pigeon breeders disease among Scottish pigeon fanciers
Thorax, 41: 274 - 278 **see attached**

*Clin. exp. Immunol.* (1983) **54**, 282–288.

# Skin testing and extrinsic allergic alveolitis

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## SUMMARY

Skin testing with six common allergens, tuberculin and a sterile avian antigen preparation from pigeon serum was performed on 102 pigeon fanciers. The incidence of positive prick tests to common allergens was no different for subjects with extrinsic allergic alveolitis, EAA, caused by avian exposure than the whole group. Positive immediate weal and flare reactions following skin prick testing with avian antigen occurred in 22 subjects and was closely correlated with atopy. However, when the same antigen was administered intradermally, 69 subjects developed an immediate (15 min) weal and flare reaction which did not correlate with atopy, instead, the weal diameter correlated significantly with the serum IgG antibody titre against pigeon serum gamma-globulin antigen, and furthermore, the higher grades of reaction were highly selective for subjects with EAA. Ten subjects, all with strong early intradermal skin reactions, developed a late (4–6 h) skin reaction; this was again highly selective for EAA. The subjects with cutaneous anergy to tuberculin had markedly higher IgG antibody titres to avian antigens, and these included the majority of the subjects with alveolitis.

**Keywords** extrinsic allergic alveolitis anaphylactic IgG

## INTRODUCTION

Extrinsic allergic alveolitis (EAA) is a pulmonary hypersensitivity disease characterized in the acute phase following antigen inhalation by a late (4–8 h) reaction, involving a decrease in lung function, pyrexia and leucocytosis with symptoms of fever, muscle pains and general malaise along with shortness of breath, all of which usually resolve within 24 h (Roberts & Moore, 1977). An immune complex, type III hypersensitivity has been suggested to mediate disease (Pepys, 1969) based, in part, on the close temporal relationship between this lung reaction and the late (4–8 h) Arthus type skin reaction following intradermal skin testing of patients with the same alveolitis inducing antigens (Fink, Bárboriak & Sosman, 1967). It has been noted that this reaction may be preceded by an immediate weal and flare skin reaction (Hargreave & Pepys, 1972) and, although this is of considerable diagnostic value in type I hypersensitivity diseases such as asthma and hay fever (Bryant, Burns & Lazarus, 1975) the significance of this early reaction in alveolitis has received little attention. This study focussed on the prevalence of the immediate and late skin reactions to a pigeon serum antigen preparation in a population of pigeon fanciers; some with identifiable Pigeon breeders disease a model example of EAA (Reed, Sosman & Barbee, 1965; Boyd, 1978). The subjects were also skin tested with tuberculin and six common allergens. The incidence of these skin

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reactions together with the atopic status, total IgE and circulating avian antigen specific IgG and IgE antibody were assessed for their usefulness to the clinician investigating respiratory symptoms amongst pigeon fanciers and were discussed in the light of current views on disease mechanism.

## MATERIALS AND METHODS

*Subjects.* A research team of medical and scientific staff attended several large pigeon shows in Central Scotland. A hundred and two active pigeon fanciers were extensively interviewed by an experienced medical observer and clinical information was obtained regarding the nature, frequency and severity of immediate (less than 30 min) and late (4–8 h) respiratory and systemic symptoms related to avian exposure. Further relevant background features such as other respiratory illnesses in accordance with the MRC questionnaire on chronic bronchitis, smoking habit and the degree of avian exposure were noted. A clinical diagnosis of pigeon breeders disease on the observer's judgement required the occurrence of at least one respiratory symptom such as shortness of breath or dry cough, with at least one systemic symptom such as fever or general malaise, occurring together 4–8 h after avian contact on at least three occasions. Immediate hypersensitivity was considered significant if the subject described the frequent occurrence of at least three symptoms such as wheeze, rhinitis and conjunctivitis within 30 min of avian contact.

*Serology.* Pigeon serum gamma-globulin, the specific antigen in pigeon breeders disease (Faux, Wells & Pepys, 1971) was prepared by 40% ammonium sulphate precipitation of fresh pigeon serum and passing the reconstituted and dialysed precipitate through Sephadex G-200 (Pharmacia UK Ltd). The second protein peak eluted contained the gamma-globulin antigen and the fractions were pooled and concentrated to 20 mg/ml for use. This soluble protein was bound to cyanogen bromide activated sepharose (Pharmacia UK Ltd) according to the manufacturers instructions.

Each pigeon breeder agreed to donate a 10 ml blood sample and the serum IgG antibody to this solid phase antigen was measured by radioimmunoassay (Neilsen *et al.*, 1974) using sheep anti-human IgG, $\gamma$-chain specific, supplied by the Scottish Antibody Production Unit (SAPU, Law Hospital, Carluke). Control sera for this assay were from subjects with no avian exposure including 52 normal sera and pathological sera from 40 asthmatics and 52 subjects with hypergammaglobulinaemia. These sera were uniformly negative.

Total IgE was measured by PRIST (Pharmacia UK Ltd), and specific IgE antibody to pigeon antigens was measured by RAST using commercially available pigeon dropping extract (e7 discs, Pharmacia, UK Ltd) and the solid phase pigeon serum antigen coupled to Sepharose. Using this latter preparation required chequerboard titration of the patients serum and the anti-IgE (Pharmacia, UK Ltd) for estimating optimal proportions, and it was found that only when the antigen was in large excess could IgE antibody activity be measured. This suggested that the high titres of serum IgG antibody directed against the same antigen blocked the IgE binding.

*Skin testing.* Skin prick testing reagents for *Dermatophagoides pteronyssinus*, grass pollen, cat fur, mixed feathers, Sporobolomyces and *Aspergillus fumigatus* allergens were obtained from Bencard and used according to the manufacturers instructions, using the appropriate prick test control solution. The tuberculin Tine (Lederley Labs) and PPD (Evans Medical Ltd) antigens were injected intracutaneously on the flexor aspect of the forearm, by an experienced operator, and were read after 72 h by each subject, who indicated the size, if any, of the reaction on an illustrated returnable postcard. The results for the Tine test were graded 0 for no reaction, grades 1 and 2 for increasingly positive reactions before the four individual points coalesce, grade 3 when the four reactions coalesce and grade 4 for any larger reaction. The mantoux was positive if the reaction was 5 mm or greater.

Sterile pigeon serum was used neat for prick testing as above and was diluted 1 in 5 with sterile saline for intracutaneous administration (0·01 ml, approximately 50 $\mu$g of protein). The reactions were measured at 15 min and 6 h. The weal diameter was measured in millimetres for the prick test, and was graded for the immediate intradermal reaction as follows. Grade 0 was no reaction or reaction equal to the control intradermal test, grade 1 was 1–3 mm greater than control, grade 2 was 3–6 mm, grade 3 was 7–9 mm, and grade 4 was 10 mm or greater. The antigen preparation was

non-irritant when administered by this route and produced only grade 0 reactions in control subjects with no avian exposure.

## RESULTS

*Skin testing with common allergens*

Fifty of the 100 subjects skin prick tested with six common allergens had one or more positive immediate weal and flare reaction, however, 15 of these had only modest skin reactions (1 mm or 2 mm reaction to only one allergen or 1 mm to 2 allergens) and were considered as a separate group on Table 1. From this table the skin test reactivity was seen to correlate with total IgE levels and with a history of symptoms of atopic diseases. However, the incidence of hypersensitivity symptoms related to avian exposure was only marginally greater in the skin test positive group, and the percentage of positive skin test responses to each of the common allergens in the alveolitis group was almost identical to those of the whole group. Therefore, atopy, as judged by skin testing with common allergens, was not a feature of extrinsic allergic alveolitis due to avian exposure, however, the extent of the IgG antibody response to avian antigens was lower in the skin test negative group ($t = 3{\cdot}1$, $P < 0{\cdot}005$).

**Table 1.** Skin prick test response to common allergens compared with the total IgE (iu/ml) and the personal history of atopic diseases. Also with the mean specific IgG level (μg/ml) and IgE (RAST positive) for avian antigens, and the incidence of immediate and late symptoms following avian exposure.

| | Immediate reactions (15 min weal) | | |
|---|---|---|---|
| | | Positive | |
| | Negative | Marginal | Definite |
| Number | 50 | 15 | 35 |
| IgE (mean+s.d.) | 75·5 (66·9) | 129·3 (128·0) | 225·9 (215·4) |
| Atopic history | 20% | 27% | 54% |
| Avian hypersensitivity symptoms: | | | |
| Immediate—asthmatic | 3 (6%) | 5 (10%) | |
| Late—alveolitis | 16 (32%) | 17 (34%) | |
| Avian antigen specific: | | | |
| IgG (mean+s.d.) | 13·3 (28·4) | 35·4 (38·7) | |
| IgE RAST positive | 2 (4%) | 12 (24%) | |

**Table 2.** The IgG (μg/ml) and IgE (RAST positive) antibody titres to pigeon antigens and the atopic status of 22 subjects prick test positive to pigeon serum

| | Test population ($n = 102$) | Skin test positive to pigeon serum ($n = 22$) |
|---|---|---|
| IgG antibody (mean+s.d.) | 24·0 (36·1) | 52·5 (43·0) |
| IgE RAST positive | 14 (13·7%) | 8 (36·4%) |
| Total IgE (mean+s.d.) | 135·5 (159·0) | 215·4 (210·2) |
| Atopic history | 33% | 50% |
| Positive prick test to common allergens | 35% | 82% |

*Skin testing with pigeon serum antigens*

*Prick test.* Twenty-two of the 102 subjects had an immediate, 15 min, weal and flare reaction to neat pigeon serum. This group had a significantly higher mean IgG antibody level against pigeon serum antigens than the whole group (Table 2), was associated with a higher total IgE level ($t = 3{\cdot}9$, $P < 0{\cdot}001$) and occurred predominantly in subjects with positive skin tests to common allergens.

This immediate skin reactivity was correlated with serum IgE antibody by RAST measurements against pigeon droppings extract (PDE) and pigeon serum antigens. Of the 102 subjects, four had a low level (RAST grade 1) of IgE antibody against PDE, and 12 were positive against pigeon serum, nine grade 1 and three grade 2 (moderate level of IgE antibody). Two subjects were positive for both antigens. Of this group of 14 RAST positive subjects, there were eight with a positive prick test to pigeon serum (Chi-squared = 12·1, $p < 0{\cdot}001$).

*Intradermal testing: immediate, 15 min, skin reaction.* Ninety-eight subjects were skin tested intradermally with pigeon serum antigen and 69 (70%) had an immediate weal and flare reaction. The extent of this skin reaction as measured by the weal diameter was quantitatively related to the serum IgG antibody level against pigeon serum gamma-globulin antigen (Fig. 1) and not to total IgE. There was no correlation between this skin reaction and immediate skin reactivity to common allergens nor with an allergic history.

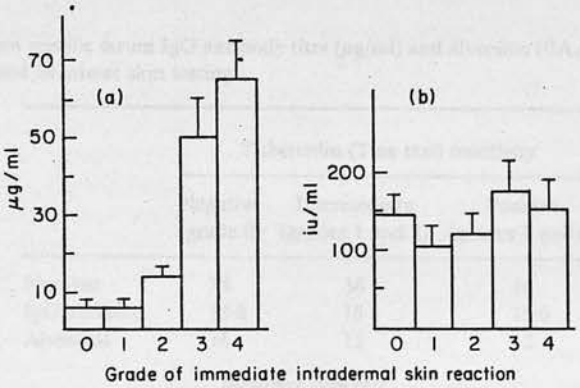


**Fig. 1.** The mean plus standard error of (a) serum IgG antibody levels specific for avian antigen and (b) total IgE within each skin reaction grade against intradermally applied pigeon serum antigen.

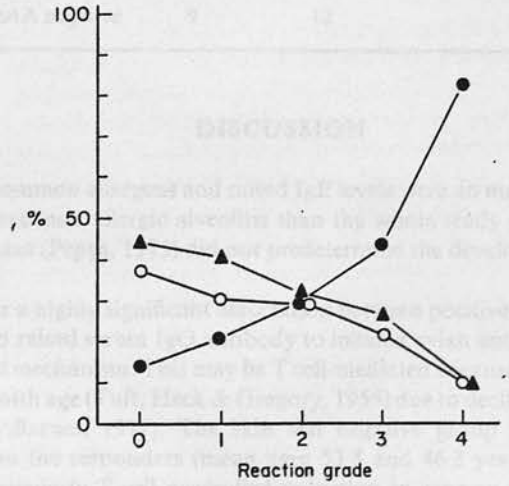


**Fig. 2.** The percentage of subjects in each of the immediate i.d. skin reaction grades with alveolitis (●——●), with indeterminate symptoms (▲——▲) and with no symptoms (○——○), related to avian exposure.

The stronger positive skin reactions were progressively more selective for the subjects with avian induced extrinsic allergic alveolitis (Fig. 2). Thirteen per cent of all subjects with no skin reactivity had alveolitis symptoms and this increased to 82% of the grade 4 reactors.

*Intradermal testing: late 4–6, skin reaction.* Of the 98 subjects skin tested with pigeon serum 10 reported a late skin reaction, maximal between 4 and 8 h. All of these subjects had had strong early reactions and could be considered as 'dual' skin reactors. This late reaction was a feature of subjects with particularly high IgG antibody titres (mean = 66·1 μg/ml). One subject however had insignificant IgG antibody to avian antigens but described prominent avian related asthmatic symptoms and eight of the remaining nine had symptoms of avian related extrinsic allergic alveolitis.

*Delayed, 48–72 h, tuberculin skin reactions.* There was a 89% return of the tuberculin Tine test score cards, and the grades of the reactions were listed on Table 3. There was a markedly lower mean avian–antigen specific IgG antibody response in tuberculin positive subjects and this was associated with a lower incidence of alveolitis. However, due to reports of occasional false negative tuberculin skin reactions using the Tine test, the association between antibody responsiveness, disease incidence and tuberculin sensitivity was followed up using a 1:1,000 Mantoux test on a further 57 pigeon fanciers (Table 3). The lower mean antibody levels in the tuberculin positive subjects was confirmed ($0{\cdot}05 < P < 0{\cdot}1$) and there was a significant correlation between alveolitis and cutaneous anergy to tuberculin (chi-squared = 5·9, $P < 0{\cdot}02$).

**Table 3.** Avian antigen specific serum IgG antibody titre (μg/ml) and alveolitis (EAA) in relation to tuberculin sensitivity, by Tine and Mantoux skin testing

| | Tuberculin (Tine test) reactivity | | |
|---|---|---|---|
| | Negative (grade 0) | Intermediate (grades 1 and 2) | Positive (grades 3 and 4) |
| Number | 39 | 36 | 16 |
| IgG antibody | 33·8 | 18·8 | 16·6 |
| Alveolitis | 16 | 12 | 2 |
| | *Mantoux skin test* | | |
| | *Negative* | *Positive* | |
| Number | 36 | 31 | |
| IgG antibody | 44·5 | 24·0 | |
| EAA positive | 27 | 9 | |
| EAA negative | 9 | 12 | |

## DISCUSSION

Positive skin tests to common allergens and raised IgE levels were no more prevalent among the 32 pigeon fanciers with extrinsic allergic alveolitis than the whole study group, suggesting that the individuals' atopic status (Pepys, 1975) did not predetermine the development of alveolitis caused by avian exposure.

There was however a highly significant association between positive skin test responsiveness to common allergens and raised serum IgG antibody to inhaled avian antigens suggesting that there was a common control mechanism. This may be T cell-mediated because skin test responsiveness is known to deteriorate with age (Tuft, Heck & Gregory, 1955) due to decline in T helper cell function (Allison, Denman & Barnes, 1971). The skin test negative group in the present study was significantly older than the responders (mean ages 53·8 and 46·3 years, respectively) and these subjects may have a similarly T cell controlled reduction in capacity to mount or maintain an antibody response to inhaled antigens (Heidrick & Makinodaw, 1973).

A further involvement of T cell function was suggested by the higher IgG antibody titres against inhaled avian antigens in subjects with cutaneous anergy to tuberculin. An early report (Fuller, 1962), published when positive tuberculin responses were more prevalent in the general population, had suggested that extrinsic allergic alveolitis among farmers (farmers lung) would be unlikely if subjects had a positive mantoux. This view was confirmed by our results which significantly correlated EAA with cutaneous anergy to tuberculin. This would tend to support the view that both arms of the immune response are involved in the pathogenesis of EAA (Roberts & Moore, 1977).

An immediate skin prick test to pigeon serum in this study was a feature of atopic subjects with high IgG antibody titres, and serum IgE antibody to avian antigens measured by RAST. This is in agreement with Faux *et al.* (1971) who described IgE antibody and positive immediate prick tests in subjects who developed immediate asthmatic symptoms only, when challenged with avian antigens.

In the present study, however, there was very little evidence of IgE responsiveness among all the pigeon fanciers despite a high incidence of atopy and extensive avian contact (mean years exposure = 22 years). Other workers have been unable to detect specific IgE among pigeon fanciers (Patterson *et al.*, 1976; Cohen, Yunginger & Fink, 1979) however this may be explained by the high titre serum IgG antibody blocking the RAST system. This potential of IgG to block the RAST test has been discussed by Aalberse, Reerink-Brongers & Vermeulen (1972), and has been demonstrated in patients allergic to Aspergillus and budgerigar proteins by Pepys *et al.* (1979), who showed that short term sensitizing antibody (IgG S-TS) could interfere with the specific IgE activity by blocking RAST tests and baboon PCA tests.

The late (4–8 h), type III skin reaction against intracutaneously administered avian antigens has received considerable attention in the study of extrinsic allergic alveolitis because it has a similar time course to the acute pulmonary and systemic reactions which follow inhalation challenges with these same antigens, (Hargreave & Pepys, 1972). These authors demonstrated that the majority of subjects in their study with late respiratory symptoms after an inhalation challenge with avian antigens had late skin test reactions (85%) and just over half had preceding immediate reactions (58%); whereas all those with dual, immediate and late, respiratory reactions had dual skin tests.

Of the 10 subjects who reported a late skin reaction to intradermal pigeon serum antigen in the present study, eight had evidence of avian related extrinsic alveolitis, and this skin reactivity appeared to be associated with a particularly high specific IgG titre to pigeon serum gamma-globulin antigen. One subject who had insignificant IgG against avian antigen but with a positive RAST had a similar late skin reaction. He described significant immediate and late asthma symptoms related to avian exposure, and it was possible that this late skin reaction was mediated therefore by IgE antibody, as has been described in other atopic conditions (Dolovitch *et al.*, 1973).

A high percentage (70%) of the study population developed an immediate weal and flare reaction following intracutaneous administration of diluted pigeon serum. This skin reactivity was not associated with atopy as judged by total IgE levels, allergic history or skin reactivity to common allergens, but was quantitatively associated with the titre of IgG antibody to pigeon serum antigens. Anaphylactic IgG was first described by Parish (1970), and an IgG associated skin sensitizing factor has been reported in extrinsic allergic alveolitis among farmers (Freedman *et al.*, 1981; Edwards & Davies, 1981). The present work with subjects exposed to definable avian antigens which can be purified, and in whom the IgG antibody response can be quantified, offers an opportunity to isolate the class and subclass of this anaphylactic antibody activity, and these studies are underway.

A role for a possible IgG anaphylactic antibody in the pathogenesis of extrinsic allergic alveolitis should now be considered, perhaps in combination with the traditionally postulated immune complex aetiology (Pepys, 1969). Certainly, Fink *et al.* (1978) found a clear difference in the PCA antibody titre between pigeon breeders with and without alveolitis, and Morell-Brotad *et al.* (1981) found that positive immediate intradermal skin tests using appropriate antigens were highly selective for farmers lung patients, more so than other diagnostic tests including lymphocyte transformation using the same antigens. Similarly, in the present study, strong positive immediate intradermal skin tests using pigeon serum as an antigen was highly selective for patients with alveolitis caused by avian exposure, and this simple and safe technique may prove a useful adjunct in the diagnosis and management of alveolitis.

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# Relationships between avian exposure, humoral immune response, and pigeon breeders' disease among Scottish pigeon fanciers

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ABSTRACT In a large scale clinical survey of Scottish pigeon fanciers, 277 people completed a detailed questionnaire and provided a venous blood sample. There were 29 (10.4%) who fulfilled the clinical criteria for pigeon breeders' disease used in the study and 84 (30.3%) who showed a significant serum IgG antibody response to pigeon gammaglobulin (4 μg/ml) and were considered to have been sensitised. Increasing exposure was associated with a progressive tendency towards sensitisation, but the intensity of the antibody response was related to the presence of symptoms and not the degree of exposure. Thirteen out of 15 subjects in the survey with a serum concentration of antipigeon IgG greater than 60 μg/ml fulfilled the clinical criteria for pigeon breeders' disease, and those affected within 10 years of starting the hobby had the highest mean antibody response. Accurate quantitation of antibody response is therefore helpful in the investigation of pigeon breeders' disease. The radioimmunoassay provides a quantitative method for determining antibody response that can be used in the routine screening of pigeon fanciers and in serial monitoring of their response.

An association between pigeon breeders' disease and serum IgG antibodies to antigens derived from pigeon has been recognised since the first description of this type of hypersensitivity pneumonitis in 1965.[1] The relationship between exposure, circulating IgG antibody against pigeon protein antigens, and the development of pigeon breeders' disease remains uncertain,[2] however, and this is reflected in the results of clinical studies. Patients reported in detail,[3] usually with an acute, severe illness, typically have an intense antibody response and are rarely antibody negative[4]; whereas surveys conducted among the general population of pigeon fanciers regularly identify people who are apparently affected but who lack serum antibodies.[5 6] Furthermore, these studies have found an appreciable proportion of healthy fanciers who do have circulating antibody against pigeon proteins, and it has been suggested that the humoral response largely reflects the intensity of exposure.[7] We have used a radioimmunoassay technique to investigate the humoral antibody response and the factors which influence this and the development of pigeon breeders' disease in a large group of pigeon fanciers.

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## Methods

Pigeon fanciers in Central Scotland were approached through meetings organised at their local pigeon racing clubs and any person regularly exposed to pigeons, whether primarily engaged in the hobby or helping a relative with the pigeon husbandry, was asked to complete a questionnaire and provide a 20 ml venous blood sample. The questionnaire was divided into several sections, the questions generally requiring a simple "yes/no" reply. One section related to the circumstances and degree of exposure, another concerned the presence of any immediate or delayed symptoms after contact with the pigeons, and a further section recorded the details of any other illnesses. The completed questionnaires were coded and the data transferred to the disc storage of a PDP11/45 computer. Criteria for extrinsic allergic alveolitis were derived from Christensen *et al*,[6] requiring a delayed (6–12 hours after exposure) respiratory symptom,

Table 1 *Characteristics of all pigeon fanciers studied and of those with pigeon breeders' disease (PBD) (values are means with standard errors in parentheses)*

| | *All 277* | *29 with PBD* |
|---|---|---|
| Age (y) | 43.3 (0.86) | 43.8 ((2.9) |
| Smoking habit (< 5 pack y) | 122 [44%] | 6 [20.6%] |
| Asthma or hay fever* | 44 [15.8%] | 5 [17.2%] |
| Duration of exposure (y) | 20.4 (0.89) | 21.4 (4.97) |
| No of pigeons kept | 46.6 (1.48) | 49.2 (4.97) |
| Weekly contact (h): | | |
| Peak summer exposure | 29.6 (1.13) | 18.7 (2.34)‡ |
| Winter exposure | 10.6 (0.55) | 9.1 (1.26) |
| Number sensitised† | 84 [30.3%] | 21 [72.4%] |
| Specific IgG (μg/ml) | 10.9 (1.86) | 64.6 (13.5)§ |

*Personal history of asthma or hay fever not confined or relating to contact with pigeons.
†Serum concentration of ⩾ 4μg/ml of IgG antibody to pigeon gammaglobulin.
‡$p < 0.005$.
§$p < 0.001$.

such as cough or breathlessness, and a systemic symptom, such as fever or arthralgia. Only when this had occurred at least three times, however, was the diagnosis of pigeon breeders' disease accepted. There were 32 people who completed their questionnaires incorrectly with respect to some of the questions about symptoms, but in only 15 cases were the answers uninterpretable, and in no instance did this affect the diagnosis of pigeon breeders' disease.

## ANTIGEN

Pigeon serum was obtained by cardiac puncture of pigeons anaesthetised with ether. The blood was allowed to clot and the serum aspirated. The gammaglobulin fraction was then extracted by precipitation, with saturated ammonium sulphate added dropwise at 4°C until 40% v/v. After an hour the precipitate was sedimented by centrifugation at 2000 rev/min for 15 minutes, and after being washed with 40% aqueous ammonium sulphate solution and further centrifugation the precipitate was redissolved and dialysed extensively against saline. This antigen preparation was stored at −20°C until use.

## RADIOIMMUNOASSAY

Pigeon antigen specific IgG was measured by radioimmunoassay as developed by Nielson *et al.*[8] Pigeon gammaglobulin was bound to cyanogen bromide activated sepharose 4B (Pharmacia) to provide the solid phase antigen. The radiolabelled antiserum was an antihuman IgG (Scottish Antibody Production Unit, Law Hospital, Carluke) conjugated to $I^{125}$ by the method of McConahey and Dixon.[9] After the optimal proportions for the reagents had been established, 40 μl of the patient's serum was incubated with the antigen for at least 30 minutes at room temperature. The tubes were washed three times to remove unbound protein, and the radiolabelled antiserum was added. After 30 minutes' incubation and extensive washing to remove unbound radioactivity the tubes were counted in a gamma counter (Gamma Set). The IgG values were obtained by interpolating the counts into a standard curve of counts from serial dilutions of a standard serum previously measured for specific IgG by quantitative precipitation. Control sera for the assay included 58 normal sera from non-exposed persons and sera from 40 patients with unrelated respiratory disorders; serum concentrations in control subjects were always less than 4 μg/ml.

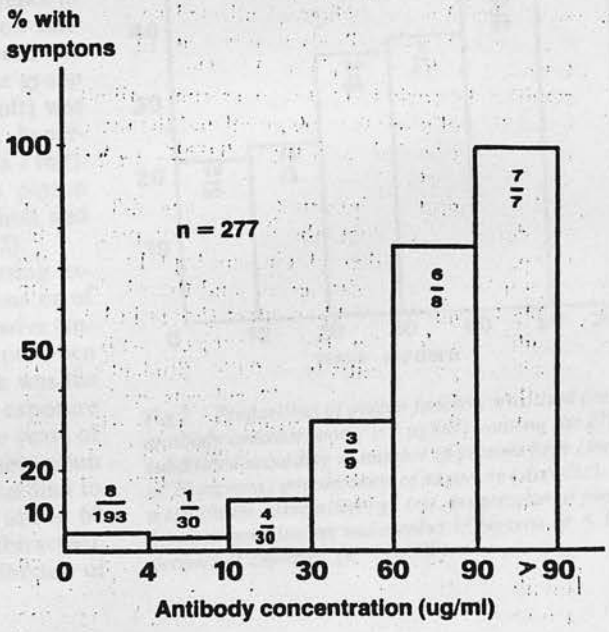


Fig 1 *Proportions of subjects with pigeon breeders' disease among fanciers subdivided according to serum concentration of IgG antibody to pigeon gammaglobulin (μg/ml). There is a highly significant association between an antibody concentration of > 60 μg/ml and symptoms of pigeon breeders' disease ($\chi^2$ test with Yates's correction, < 0.0005).*

Table 2 *Mean (SE) serum antibody concentrations in 29 individuals with pigeon breeders' disease according to length of exposure before development of symptoms*

| *Exposure (y) before PBD* | *0–10* | *11–20* | *>20* |
|---|---|---|---|
| Number | 12 | 8 | 9 |
| Specific IgG (μg/ml) | 82.5 (24.3) | 42.5 (19.0) | 60.7 (24.7) |

## Results

Two hundred and seventy seven individuals were studied and most reported at least one symptom that they related to avian exposure. Where multiple symptoms were recorded many different subgroups of symptoms were evident, but there were only 29 people who fulfilled the criteria for pigeon breeders' disease. Table 1 summarises the results for the 277 pigeon fanciers studied, and compares the findings with those from the 29 with definite symptoms. Eighty four (30.3%) of those surveyed were sensitised to pigeon gammaglobulin, but a much higher percentage (72.4%) of the pigeon breeders' disease group had detectable antibody. An association between intensity of antibody response and pigeon breeders' disease is seen when the percentage of affected persons at increasing levels of antibody response are plotted in the form of a histogram (fig 1). There is a highly significant association between a serum antibody concentration of more than 60 μg/ml and the presence of pigeon breeders' disease ($p < 0.0005$, $\chi^2$ test with Yates's correction).

With respect to exposure there was no difference in the degree of contact, the duration of pigeon fancying, or the number of pigeons kept between the pigeon breeders' disease group and the whole group (table 1), but the weekly contact (hours in loft) was significantly less for the 29 subjects with hypersensitivity pneumonitis ($p < 0.005$, Student's $t$ test). Furthermore, among the 29 subjects with pigeon breeders' disease the subgroup affected earliest had the most vigorous antibody response (table 2).

For the overall study population, increasing exposure (in terms either of number of pigeons or of years exposed) was associated with a progressive tendency towards sensitisation (fig 2)—except when number of hours spent in the loft each week was the parameter of exposure (data not shown). An exposure "score" was constructed on the basis of the years of contact but modified, by the addition or subtraction of an increment of five years' exposure, according to whether the number of pigeons kept was above 60 (five years added) or below 20 (five years subtracted). Although this manoeuvre altered the distribution of fanciers over the "decades" of exposure, the relationship between degree of contact and sensitisation remains (fig 3). Furthermore, this relationship is unchanged when the pigeon breeders' disease group is excluded. An increasing exposure score was not, however, associated with an increasingly vigorous antibody response (table 3).

## Discussion

The diagnosis of pigeon breeders' disease is most sat-

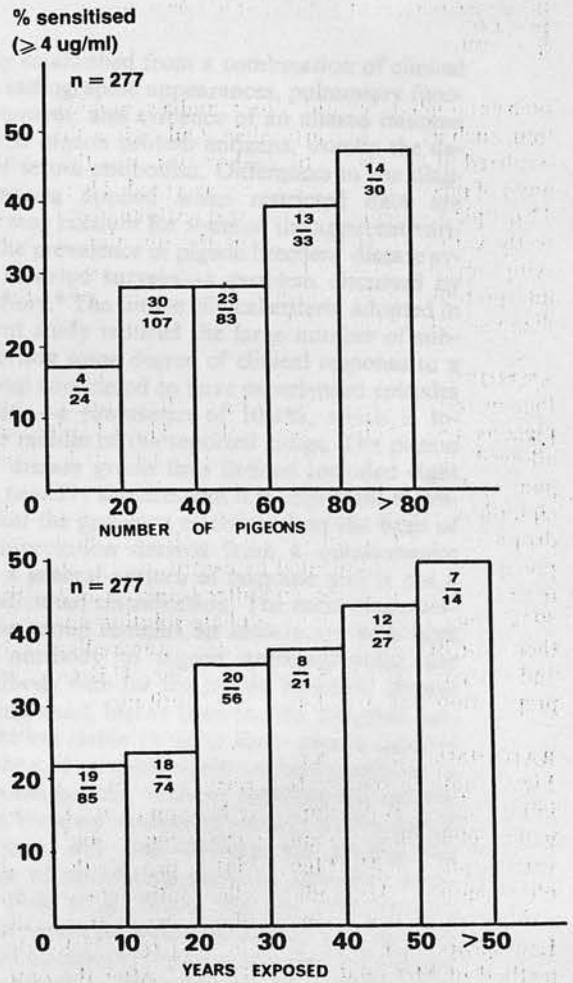


Fig 2 *Proportions of pigeon fanciers sensitised (serum antibody concentration (≥4 μg/ml)) among the 277 subjects subdivided according to number of pigeons kept (increments of 20 pigeons) and duration of exposure (decades). There is a significant association ($\chi^2$ test, progression of proportions) between sensitisation and number of pigeons ($p < 0.01$) and decades of exposure ($p < 0.005$).*

Table 3 *Distribution of antibody to pigeon gammaglobulin and serum concentrations according to exposure score*

| *Exposure score** | *0–10* | *11–20* | *21–30* | *31–40* | *41–50* | *>50* |
|---|---|---|---|---|---|---|
| Number in group | 83 | 70 | 54 | 24 | 29 | 17 |
| Number sensitised† | 19 | 16 | 18 | 9 | 13 | 9 |
| Mean (SE) specific IgG (μg/ml) in those sensitised | 58.9 (16.9) | 32.8 (9.5) | 30.8 (8.4) | 18.6 (4.9) | 34.6 (16.2) | 10.8 (2.3) |
| Number with PBD | 12 | 5 | 4 | 3 | 4 | 1 |

*See under "Results."
†Serum concentration of ⩾4 μg/ml of IgG antibody to pigeon gammaglobulin.

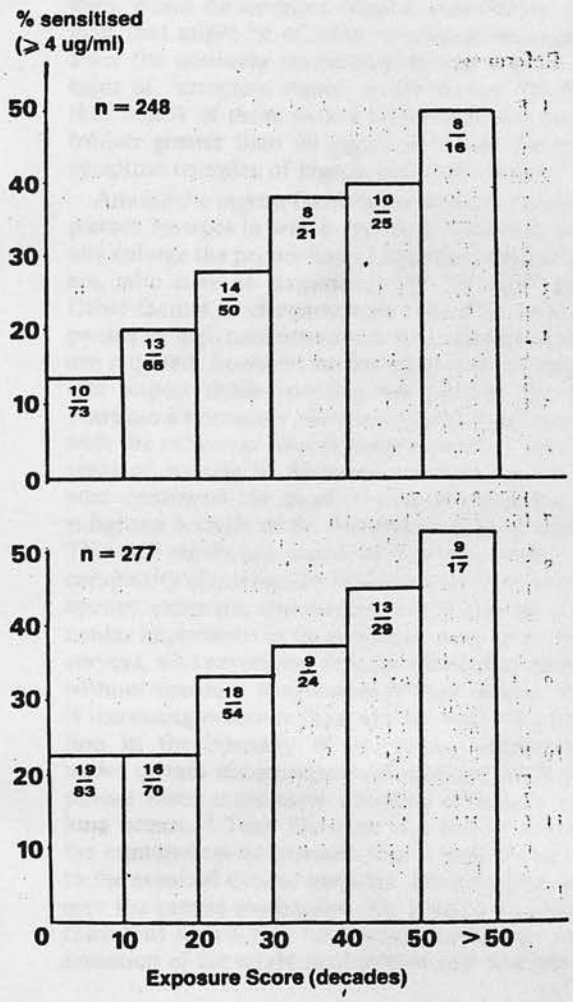


Fig 3 *Proportions of pigeon fanciers sensitised (serum antibody concentration (⩾4 μg/ml)) in the survey population—both including (277) and excluding (248) those with pigeon breeders' disease—subdivided according to exposure score (see under "Results"). There is a significant association ($\chi^2$ progression of proportions) between sensitisation and exposure score ($p < 0.005$ for the 248 fanciers and $< 0.001$ for the 277 fanciers).*

isfactorily established from a combination of clinical findings, radiographic appearances, pulmonary function assessment, and evidence of an altered immune response to pigeon protein antigens, usually the detection of serum antibodies. Differences in the diagnostic criteria applied when restricted data are available may account for some of the apparent variation in the prevalence of pigeon breeders' disease evident in reported surveys—a problem discussed by other authors.[6] The simple clinical criteria adopted in the present study reduced the large number of subjects reporting some degree of clinical response to a small group considered to have experienced episodes of alveolitis—a prevalence of 10.4%, which is towards the middle of the reported range. The pigeon breeders' disease group thus defined included eight antibody negative subjects, but it is important to emphasise that the grouping established on the basis of clinical information derived from a questionnaire identifies a general pattern of response and is not a precise individual classification. The main characteristic of the group remains an association with high levels of antibody to pigeon gammaglobulin, the mean antibody titre for the pigeon breeders' disease group being much higher than for the complete survey population (table 1). Sixty three pigeon fanciers (22.7%) showed an appreciable antibody response to pigeon gammglobulin without fulfilling the criteria for pigeon breeders' disease. Since only a minority of such subjects will ever develop the disease the importance of circulating antibody has been questioned.[10] Fink *et al*[7] found that the presence and intensity of serum precipitins to pigeon proteins correlated with an exposure index rather than with symptoms in a survey of 200 pigeon fanciers where none described typical pigeon breeders' disease. Our findings confirm and extend the relationship between exposure and presence of specific IgG antibody. There was a progressive tendency towards sensitisation as several indices of exposure increased, although after more than 50 years' contact with pigeons half had no detectable antibody. We did not, however, find a correlation between the intensity of antibody response and increasing exposure. Rather, a vigorous antibody response was closely associated with pigeon breeders' disease and did not depend on

intense exposure. Indeed, a subgroup consisting of the most rapidly affected fanciers (that is, the most susceptible) had the highest antibody concentration of all.

Some studies in which precipitin reactions have been graded as weak, moderate, or strong have suggested a generally more pronounced antibody response in fanciers with symptoms than in symptomless fanciers.[11] This relationship is now firmly established by the present radioimmunoassay data, which furthermore suggest quantitative guidelines that might be of value in clinical management since the antibody concentration was a good indicator of "symptom status" in the survey. We found that 86.6% of those with a serum antibody concentration greater than 60 $\mu$g/ml described the typical symptom complex of pigeon breeders' disease.

Among the pigeon fanciers' community therefore a picture emerges in which exposure alone will gradually enlarge the proportion of sensitised pigeon fanciers, who may be considered the "at risk" group. Other factors or circumstances related to or accompanied by high concentrations of circulating antibody are required, however, before symptoms develop. In this respect those pursuing the pastime for many years are a rigorously self selected group and contrast with the subgroup who develop symptoms within 10 years of starting it. Although we found individuals who continued the pastime despite symptoms this subgroup is likely to be underrepresented in surveys. This self regulatory aspect of exposure adds to the complexity of interaction between specific immune response, exposure, and disease, which may be of particular importance in those people, most prevalent in surveys, who experience definite episodes of alveolitis without apparent progression of their disease. There is increasing evidence from animal work of a reduction in the intensity of pulmonary inflammation under certain circumstances of repeated antigen exposure where more rapid handling of antigen in the lung occurs.[12] Thus tolerance is a further aspect of the immunological response that is possibly relevant to the eventual clinical outcome. Nevertheless, whatever the precise mechanism, the present findings are consistent with a role for immune complexes in the evolution of the established disease and reaffirm that the humoral aspect of extrinsic allergic alveolitis continues to merit attention. The radioimmunoassay provides a quantitative estimation of serum IgG antibody to pigeon gammaglobulin for identifying and monitoring "at risk" members of the pigeon keeping community.

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