

Allergens causing bird fancier's asthma

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The study investigates to what extent bird feathers contain relevant allergens/antigens involved in bird fancier's asthma. The study group consisted of two budgerigar fanciers, two parrot fanciers and one canary fancier. All subjects complained of asthmatic symptoms, caused by contact with their birds, and they showed a significant bronchial hyperreactivity to acetylcholine. Positive IgE antibody reactions to bird sera as well as to extracts of feathers were observed in RAST. Well-defined major allergenic bands could be detected and identified in the IgE immunoblots with feather extracts as well as with serum proteins of budgerigar, parrot, pigeon, canary, and hen (mol. mass 20–30 kDa and 67 kDa). The most pronounced bands appeared with the extracts of species to which an exposure had taken place. Weaker IgG-binding patterns were also observed. Our results show that inhalable feather dust contains several allergenic components which cross-react with serum allergens/antigens of the same as well as of other bird species. This emphasizes the significance of bird feathers for immediate-type allergic reactions.

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The number of domestic birds is currently estimated at 25 million in the USA and more than 8 million in German households. The most common bird species are budgerigars, parrots, and canaries. In Germany, an additional 11 million pigeons are kept by more than 140 000 registered breeders of racing and fancy pigeons. This number does not include the breeding of fattened pigeons for the table (12).

These birds may cause disease by releasing antigens from feathers, epithelial cells, or droppings. Such materials are known to cause allergic alveolitis and bronchial asthma (1, 3, 8, 14, 15, 17, 19, 21, 24). IgE antibodies against pigeon serum and droppings were found by the radioallergosorbent test (RAST) (6, 10). However, direct sensitization through blood and serum constituents of birds is unlikely because bird fanciers do not come into contact with blood. On the other hand, the dust of droppings could be inhaled, especially during cleaning of cotes and cages (4, 9). In addition to droppings, cotes are contaminated by ubiquitous fungi, mucopolysaccharides, and bacteria (7). Our bird fanciers complained of asthmatic symptoms not only when cleaning the cotes, but also when attending bird exhibitions.

In the present work, we investigated to what extent bird feathers are sources of allergens/antigens, what is the relationship of these allergens to serum con-

stituents, and what is their clinical importance in the pathogenesis of bird fancier's asthma.

Material and methods

Two parrot fanciers, two budgerigar fanciers, and one canary fancier, all women, attended our outpatient department for asthmatic symptoms from contact with their birds (Table 1).

Allergen extracts

To obtain the feathers, we killed the birds with ether. We took five birds of every species. All feathers were carefully plucked except in the cloacal region, tail, and wing. Special care was taken in plucking the feathers, especially the pulviplumes. According to the species, we received about 10–60 g of usable feather material. The feather antigens were then extracted by 0.05 M phosphate buffer containing 0.05% NaN₃ and dialyzed against distilled water. The obtained extract was analyzed by SDS-PAGE. Afterwards the dialysate was lyophilized. The amount of lyophilized extract was 15–70 mg; it was stored at –20°C.

Bird blood was collected from wing veins (vena ulnaris and vena ulnaris superficialis). Blood samples were centrifuged at 4000 rpm, and the serum samples

Table 1. Case histories and exposure data of five patients with bird fancier's asthma

	Subjects				
	M.C.	S.E.	O.R.	G.H.	S.H.
Age	39	47	48	46	39
Sex	F	F	F	F	F
Contact with ¹	p	p	b	b	c
Duration of exposure (years)	3	14	14	5	6
Duration of symptoms (years)	2	13	5	3	3
Asthmatic symptoms	Yes	Yes	Yes	Yes	Yes
Therapy (bronchodilator)	Yes	Yes	Yes	Yes	Yes
FEV ₁ % vital capacity	73	74	63	75	67
R _{aw} ²	0.2	0.2	0.3	0.2	0.3
R _{aw} ² (acetylcholine)	0.9	0.6	0.7	0.8	0.5
IVC (% of normal) ³	95	66	96	98	84
Total IgG (g/l) ⁴	11.2	10.9	10.4	15.2	11.1
Total IgE (U/ml) ⁵	566	660	754	312	675

1. p=parrot; b=budgerigar; c=canary.

2. Airway resistance in kPa $\times$ l⁻¹ s measured by body plethysmograph (Masterlab Jaeger, Würzburg, Germany) (normal range <0.3 kPa $\times$ l⁻¹ s).

3. By regression equation published by Quanjer et al. (20).

4. Normal range of total IgG 7–17 g/l.

5. Normal range of total IgE <150 U/l.

of each bird species were dialyzed and stored at -20°C after lyophilization (2).

Estimation of specific IgE antibodies

Cellulose disks were used as solid phase for allergens. They had been punched out from filter paper (Munktell 00) and activated by cyanogen-bromide (Merck, Darmstadt, Germany) by the method of Ceska & Lundkvist (5). An amount of 2 mg lyophilized serum or feather extract was dissolved in 10 ml 0.1 M NaHCO₃ and incubated with 125 mg CNBr-activated cellulose disks for 5 h. The supernatant was aspirated and the disks were washed (0.5 M NaHCO₃, pH 8.4). The remaining active groups were blocked with ethanolamin 0.1 M overnight at room temperature. After several washes, incubation with Na-acetate/H-acetate buffer, pH 4, followed. The disks were stored at 4°C in 0.15 M phosphate buffer, pH 7.4, containing 0.2% Tween and 0.5% sodium acid in 0.1 M EDTA.

IgE-RAST

Cellulose disks coupled with different allergens were incubated with 50 μ l patient serum for 3 h. After repeated washing with saline-PBS, the specifically bound IgE was detected with 50 μ l of ¹²⁵I-labeled rabbit anti-IgE (Pharmacia, Freiburg, Germany). Bound radioactivity was measured in a gamma-counter (type BF 5300, Berthold, Wildbad, Germany) for 2 min.

All assays were done in duplicate. The coefficient of variation was below 10%. The results were ex-

pressed in U/ml by means of a reference standard curve (Phadebas RAST reference system; Pharmacia, Freiburg, Germany), and were classified from 0 to 4 according to the RAST scale. RAST values <0.35 PRU/ml were regarded as negative.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

The components of serum and feather extracts of the various bird species were analyzed for mol. mass by SDS-PAGE according to Laemmli (13). The stacking gel was 3%, and separation took place in 10% polyacrylamide gel. The allergens/antigens were reduced by mercaptoethanol after treatment with SDS. The amount of extract per PAGE was 10 mg. The mol. mass of single components was estimated with a set of standards (Pharmacia, Uppsala, Sweden). Protein was stained with Coomassie brilliant blue.

Immunoblot and autoradiography

After electrophoretic separation of the extracts, immunoblot with sera was done according to Towbin (25). The fractions of the bird extracts were transferred from the polyacrylamide gel to a nitrocellulose sheet. This sheet was incubated for 1 h in saline-PBS buffer (0.1% bovine serum albumin (BSA, Sigma), 0.1% gelatin-hydrolysate, and 0.1% Tween 20 in NaCl-phosphate, pH 7.4); after washing (0.5% Tween, NaCl), it was cut into 4-mm strips. Each strip was incubated with 200 μ l bird fancier's serum. The antibodies were detected after incubation with ¹²⁵I-marked anti-IgE for 4–6 weeks or anti-IgG for 3–7 days on Kodak RP-film at -20°C .

Results

Both budgerigar and parrot fanciers as well as the canary fancier had been complaining of asthmatic symptoms in the home for years (Table 1). All patients also complained of repeated wheezing, two of rhinitis, and one of conjunctivitis. None had had fever, chills, or malaise. The initiation of asthmatic symptoms from the birds was not related to duration of exposure. Two patients (O.R., S.H.) had mild bronchial obstruction at rest, and all patients showed a significant bronchial hyperreactivity in the bronchial challenge test with acetylcholine (airway resistance: R_{aw} $\geq$ 0.5 kPa $\times$ l⁻¹ s). Vital capacity was reduced in one subject (S.E.). However, there were no abnormalities on her chest radiograph or of those of the other patients. Total IgE was elevated in all cases, but total IgG was always normal. Skin prick tests with a panel of 20 common allergens, including those of mites, molds, pollen, and domestic animals other than birds, were negative in all five patients.

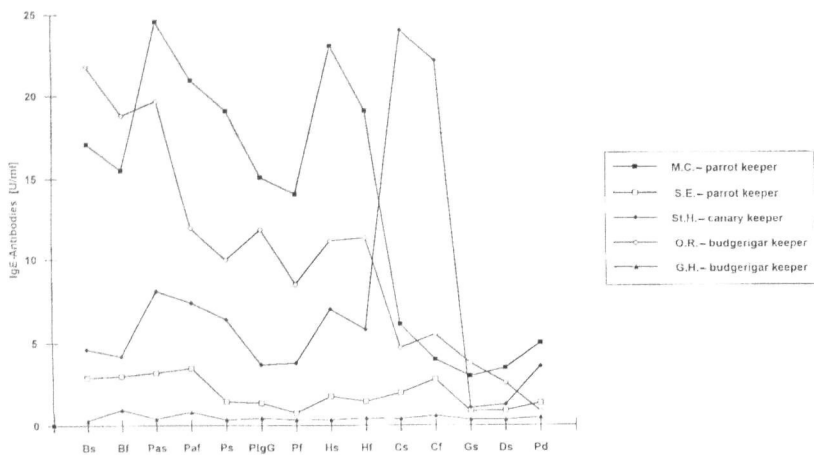


Fig. 1. Graph of specific IgE antibodies of patients with bird fancier's asthma. Allergens: Bs – budgerigar serum; Bf – budgerigar feathers; Pas – parrot serum; Paf – parrot feathers; Ps – pigeon serum; PlgG – pigeon IgG; Pf – pigeon feathers; Hs – hen serum; Hf – hen feathers; Cs – canary serum; Cf – canary feathers; Gf – goose feathers; Df – duck feathers; Pd – pigeon droppings.

Feathers and sera of seven bird species (budgerigar, pigeon, parrot, canary, hen, goose, and duck), as well as pigeon droppings, were tested for binding of IgE antibodies. In addition to the corresponding bird sera, the feathers of the bird species with which the bird fanciers had contact showed the highest values (Fig. 1). Lower but significant values were obtained with extracts of those bird species to which none of the patients had been exposed. Common allergen sources including mites, molds, pollen, and animals other than birds showed negative values. The significant IgE titer against bird species without contact suggests cross-reactivity between phylogenetically related bird species. For example, high RAST values were obtained with the antibodies of one parrot and one budgerigar fancier (M.C., O.R.) for all budgerigar, parrot, pigeon, and hen sera and

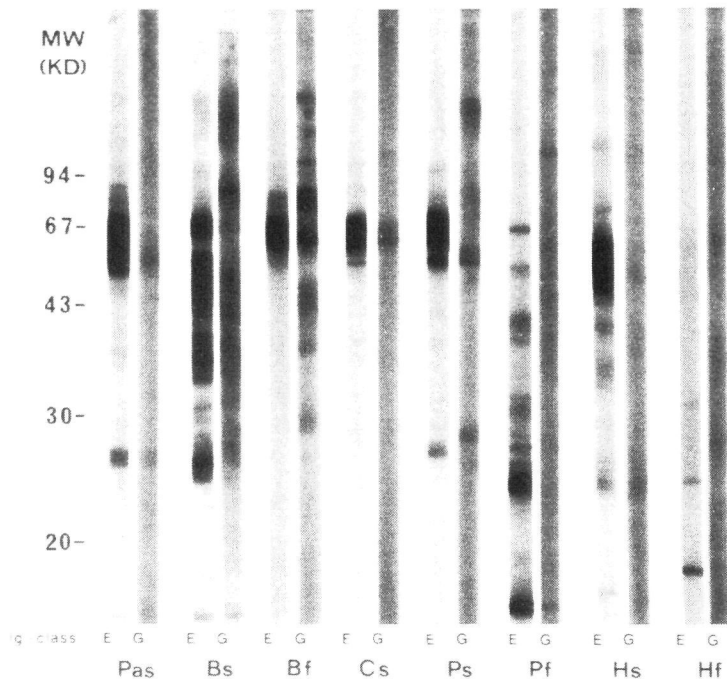


Fig. 3A. Immunoblot with the serum of parrot keeper (M.C.). Allergen abbreviations defined in Fig. 1 legend.

feather extracts. Lower IgE binding took place with extracts of canary, goose, and duck feathers and pigeon droppings. Antibodies of two other patients (S.E., G.H.) showed a similar pattern of reactivity but with generally low values. The canary fancier (S.H.) reacted strongly against canary antigens.

SDS-PAGE (Fig. 2) followed by immunoblotting with patients' sera showed major IgE-binding components in the mol. mass range 20–30 kDa and at 67 kDa (Figs. 3a–e). It is interesting that the corresponding IgG-immunoblot investigations also gave positive results for all patients. IgG binding was mostly much weaker than that of IgE; approximately half of the bands were identical in both immunoblot

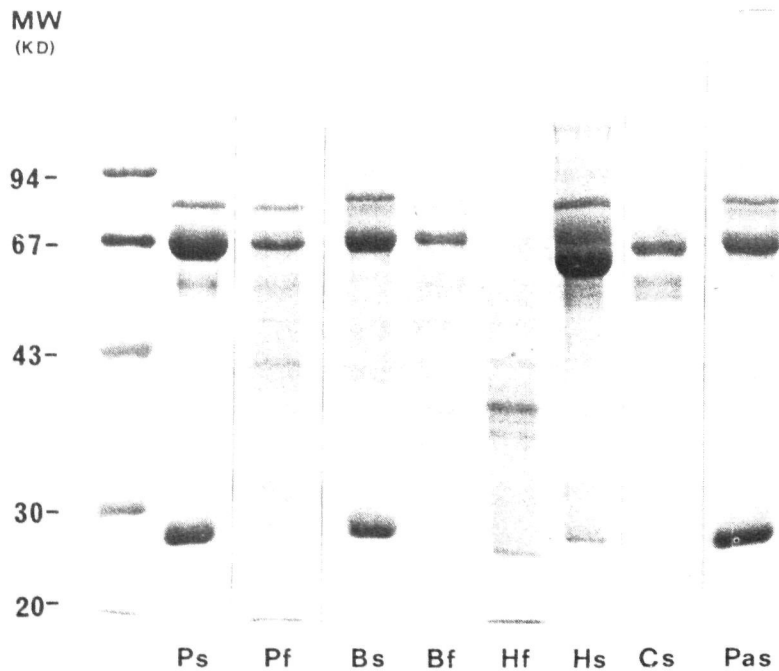


Fig. 2. Polyacrylamide gel electrophoresis with bird allergens. For details, see text. Fig. 1 legend defines abbreviations of allergens.

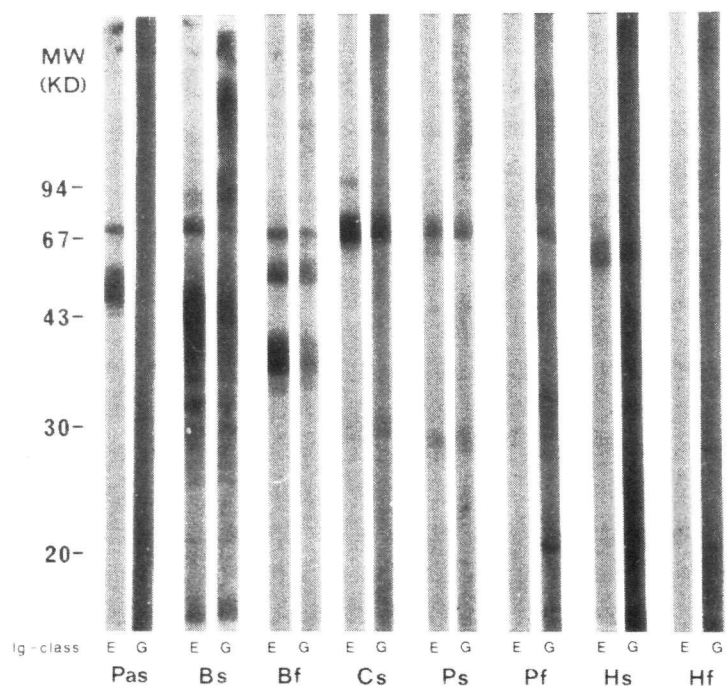


Fig. 3B. Immunoblot with the serum of a parrot keeper (S.E.).

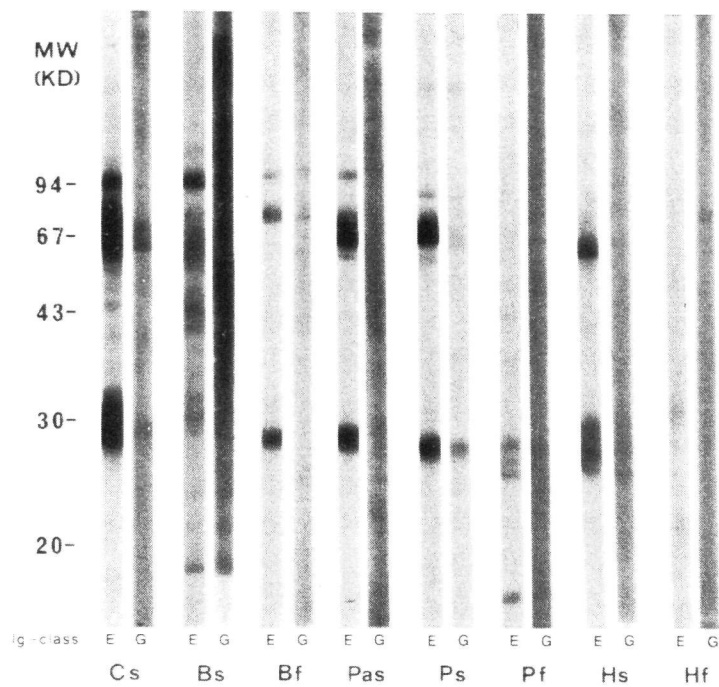


Fig. 3C. Immunoblot with the serum of a canary keeper (St.H.).

studies. Corresponding cross-reacting components in extracts of bird species without contact were easily recognizable with IgE as well as IgG antibodies. Moreover, cross-reactivities between serum and feather components of the tested bird species already obtained with RAST results were confirmed. Individual patients' sera showed a different pattern of reactivity, especially for weaker bands. However, a pool serum obtained from four unexposed controls showed neither IgE (Fig. 4) nor IgG binding (not shown). Protein staining of SDS-PAGE-separated extracts suggests that the main components in the range 28–70 kDa are obviously identical with major allergens/antigens; however, several allergenic pro-

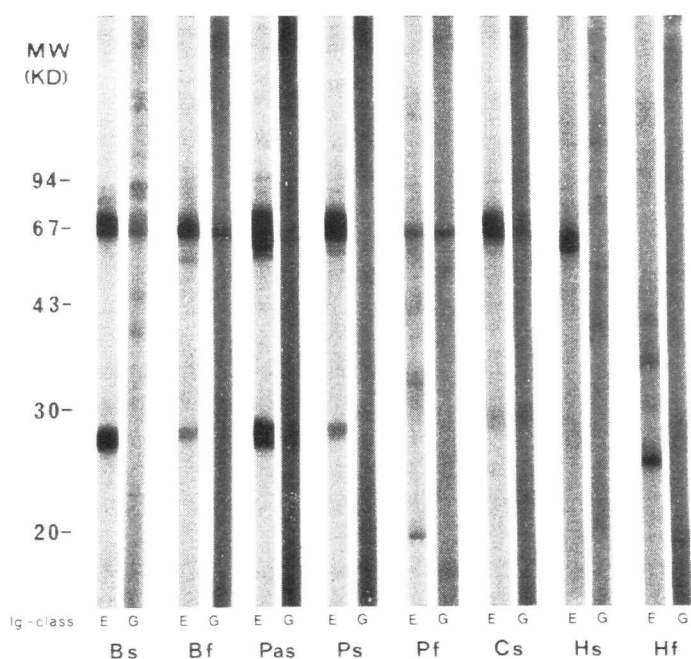


Fig. 3D. Immunoblot with the serum of a budgerigar keeper (O.R.).

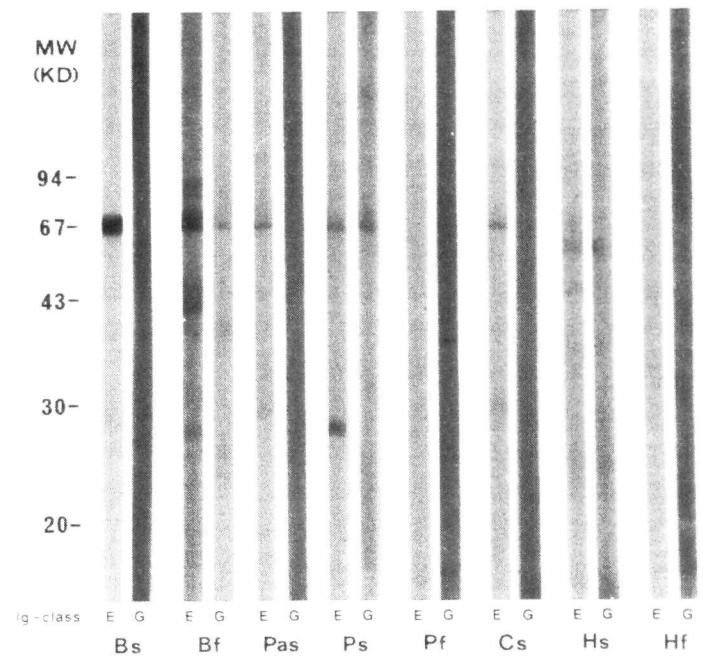


Fig. 3E. Immunoblot with the serum of a budgerigar keeper (G.H.).

teins strongly recognized by some patients were only weakly visible after Coomassie brilliant blue staining (Fig. 2).

Discussion

Our study clearly shows that bird feathers contain components which cross-react with serum constituents. Allergen/antigen levels in the tested feather extracts were shown to correspond to those of the respective bird sera. This could be demonstrated by

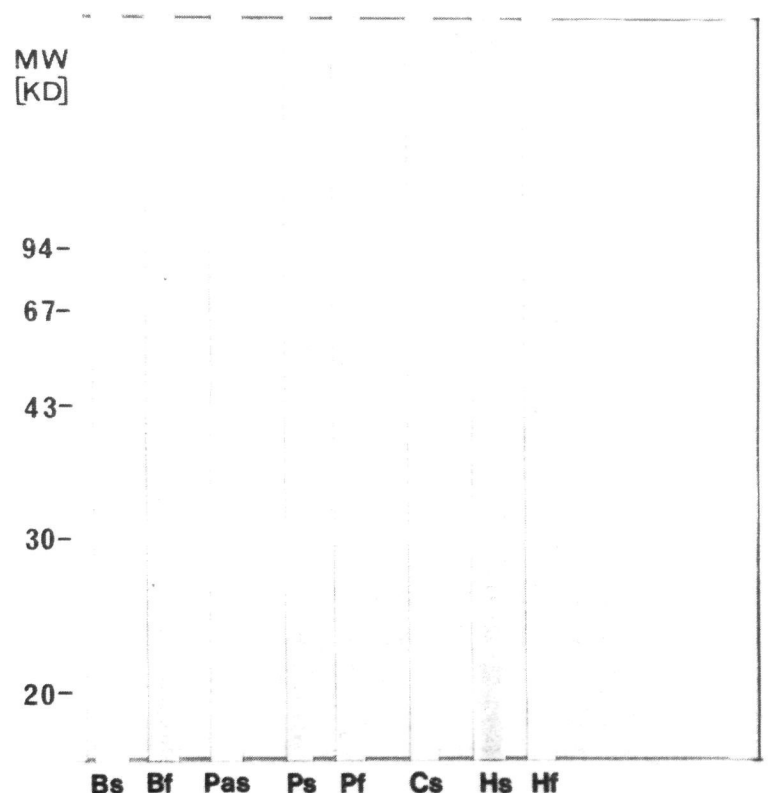


Fig. 4. Representative IgE immunoblot with control sera. Allergen abbreviations defined in Fig. 1 legend.

RAST and immunoblot procedure with IgE and IgG antibodies of the five subjects suffering from bird fancier's asthma.

Clinically, there was no sign of allergic alveolitis in our patients, although the immunoblot studies clearly showed the presence of IgG antibodies against bird antigens. On the other hand, 11 subjects from our outpatient department suffering from bird fancier's lung showed no IgE binding but very high IgG binding in the immunoblot (22, 23).

According to the immunoblot studies, the major bird allergens/antigens of sera and feathers have mol. mass of about 20–30 kDa and 67 kDa. Obviously, some of these components are present only in sera and others only in feather extracts.

van Toorenbergen et al. (24) reported that extracts of canary and budgerigar feathers contain allergens/antigens that are not found in corresponding bird sera and droppings. Banham et al. (1), investigating sera of 79 pigeon fanciers, found a higher prevalence of precipitins against pigeon bloom extracts (60%) than pigeon serum (38%). Corresponding results were found by Longbottom (15), who performed quantitative immunoelectrophoresis and identified 29 antigenic components in pigeon bloom but only 10 in pigeon serum, one of them being pigeon IgA.

Since antibodies of each of our patients also recognized allergens/antigens of birds with which they were not in contact, and in view of the close relationship among the different avian species, immunologic cross-reactivity is suggested. This cross-reactivity between individual species reflects the degree of the phylogenetic relationship. Thus, antibodies of a budgerigar fancier detect not only the major allergenic components in the parrot feather extract of the same mol. mass, but also a number of weaker bands. On the other hand, these antibodies detect only one major band in the extracts of canary and hen feathers but no other components.

Hen feathers show a weak binding pattern. This is not unexpected, since there are no chicken breeders represented in our study group, and all reactions with the tested hen extracts are to be understood as the result of cross-reactivity. Moreover, hens have few pulviplumes and consequently produce only small quantities of antigen-containing material.

The immunologic cross-reactivity between individual bird species is of practical importance for sensitized patients with regard to preventive measures. In case of contact with cross-reacting bird species, the occurrence of allergic symptoms has to be considered.

It should be mentioned that structures and compositions of the plumage may be important for interpretation of the results (16, 18). For example, the plumage of pigeons consists of about 5800 feathers.

The feathers are coated with a very fine dust that resembles talcum powder (1, 3). Small amounts of this powder are shed by ordinary plumules and contour feathers. It is chiefly produced, however, by special feathers, the powder down (pulviplumes). The powder down is clustered in specific areas known as powder down patches; other powder down is interspersed among the contour feathers around the boundaries of the patches and, to a less degree, elsewhere on the body. The powder is derived from the middle of each of the many ridges of barb-forming tissue within a feather germ. The particles are about 1 µm in diameter and are granular, rod-shaped, or splinter-shaped. That is why they can be inhaled and deposited in the airways.

Pigeons (Columbiformes) and parrots (Psittaciformes) have the largest number of pulviplumes. Hens (Phasianiformes) and singing birds (Passariformes) have a smaller number of pulviplumes; ducks and geese, the smallest (11, 26). These differences in plumage among species may be of clinical significance and may explain why bird fancier's asthma and allergic alveolitis are predominantly reported from contact with pigeons or budgerigars.

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