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Antigens in Pigeon Breeders' Disease

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Summary. It has been shown that many pigeon materials contain one or more of four pigeon proteins—pigeon γ-globulin (PGG), pigeon serum albumin (PSA), pigeon β-globulin (PBG) and a protein cross-reacting immunologically with PGG termed XPGG. It is thought that the ability of seemingly unrelated sources of pigeon material, e.g. pigeon droppings, serum, egg yolk and white to react with sera from cases of pigeon breeders' disease is due to the presence of PGG, PSA, PBG or XPGG in these materials.

Only in pigeon droppings were important antigens other than PGG, PSA, PBG and XPGG found and it is possible that pigeon droppings are the only 'complete' source of antigens concerned with the disease unless specified conditions arise.

INTRODUCTION

Pigeon breeders' disease is one of a number of respiratory diseases associated with the inhalation of organic material to which the individual has become hypersensitive. The diseases have been variously termed as 'hypersensitivity pneumonitides' (Fink, Sosman, Barboriak, Schlueter and Holmes, 1968) or 'extrinsic allergic alveolitis' (Pepys, 1967) or 'organic dust pneumoconioses' (Frank, 1958), and in general precipitating antibodies to the inhaled material have been found in a high percentage of affected persons.

In pigeon breeders' disease, precipitating antibodies to a variety of pigeon materials have been found, e.g. droppings and serum (Fink, Barboriak and Sosman, 1967), egg white and feathers (Fink *et al.*, 1968) and egg yolk (Reed, Sosman and Barbee, 1965). In particular the pigeon serum proteins, γ-globulin (PGG), albumin (PSA) (Fink, Tebo and Barboriak, 1969), a β-globulin (PBG) (Reed *et al.*, 1965) and an antigen cross-reacting immunologically with PGG, termed XPGG (Edwards, Fink and Barboriak, 1969), are antigens associated with the disease.

While antibodies to pigeon droppings and pigeon feathers can be explained due to the inhalation of dropping and feather particles as part of the dust cloud in the pigeon loft, the presence of precipitins to the serum proteins PGG, PSA and PBG would presumably entail exposure to pigeon serum or other materials containing these (or immunologically related) proteins. Indeed recent work (Edwards *et al.*, 1969) has identified PGG and PSA in freshly voided pigeon droppings but extracts of pigeon droppings which had been left at room temperature for over 1 month did not contain detectable PGG and PSA due to their breakdown by gastro-intestinal tract enzyme activity persisting in the droppings after voiding.

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Since dehydration is necessary to reduce the semi-solid 'fresh' droppings to a dust, it is not known whether the trace amounts of PGG and PSA in 'fresh' droppings are reduced to negligible quantities by the time the droppings are capable of forming part of the pigeon loft dust cloud. There may, therefore, be other sources of antigens responsible for producing antibodies to PGG and PSA.

Also it may be that PGG, PSA, PBG and XPGG and possibly other antigens are common to several pigeon materials; thus exposure to one material may produce antibodies reacting with other materials; certainly antibodies to pigeon feathers have been absorbed out by pigeon-dropping extracts (Barboriak, Sosman and Reed, 1965), and the absorption of sera from patients with pigeon breeders' disease with pigeon serum reduced the number of precipitin lines to pigeon droppings (Fink *et al.*, 1967).

The present investigation was undertaken to determine:

1. The distribution of PGG, PSA, PBG, XPGG and other pigeon proteins reacting with pigeon breeders' disease sera in several pigeon materials.

2. The influence of XPGG on the production of antibodies to PGG and *vice versa.*

3. The relationship of antigens in the various pigeon materials to the production of precipitins in the sera of pigeon breeders' disease cases.

MATERIALS AND METHODS

Preparation of pigeon materials

1. Blood was obtained from pigeons by cardiac puncture and PGG and PSA isolated as previously described (Fink *et al.*, 1969). PBG was also isolated from the starch-block electrophoresis of pigeon serum; it produced only one immunodiffusion line at the dilution tested against rabbit anti-pigeon serum.

2. Intestinal contents and 'fresh' droppings extracts were prepared as previously described (Edwards *et al.*, 1969).

3. An 'old' droppings extract was prepared by saline extracting 2–3-month-old pigeon droppings, dialysing for 2 days against running tap water and centrifuging at 10,000 ***g*** for 10 minutes. After sterilization by passage through a Millipore filter (0·20μ) the solution was lyophilized and made up at 10 mg/ml for immunoelectrophoresis.

4. Pigeon 'urine' was the liquid voided from the cloaca by pigeons under ether anaesthesia. Often the liquid had intestinal material present but the separation of a clear solution could easily be obtained.

5. Pigeon egg white and yolk were obtained from freshly laid pigeons eggs. Pigeon egg white was first separated from the yolk by gentle suction. The yolk was then removed from the yolk sac with a syringe and wide bore needle.

6. Pigeon egg surface extract was prepared by washing intact freshly laid eggs with 5 ml distilled water, lyophilizing the aqueous solution and resuspending in 0·25 ml water.

7. Pigeon 'oviduct' extract was the saline (2 ml) washing from the interior of the isolated female pigeon reproductive tract and was used without concentration.

8. 'Gastric' juice was obtained from the gizzard of a pigeon at the time it was killed.

9. Pigeon 'saliva' was obtained by washing 0·25 ml saline around the buccal cavity of anaesthetized pigeons.

10. Pigeon 'crop' fluid was the liquid that occasionally copiously flows from the mouths of pigeons under ether anaesthesia.

11. Pigeon plume was scraped off clean galvanized sheeting hung vertically near the pigeon cages. An aqueous extract of this was lyophilized and resuspended in one-tenth its original volume of water.

12. Pigeon lachrymal fluid was obtained by mechanical irritation of the pigeon cornea. The fluid was collected by capillary tubing, evaporated to dryness in a dessicator and resuspended at 40 mg/ml in water.

When not in use all extracts were kept at $-20°$. Immunoelectrophoresis was carried out on all materials and developed with high titre sera from cases of pigeon breeders' disease and also rabbit anti-pigeon serum. Materials producing immunoelectrophoretic arcs that were tentatively determined to be PGG, PSA, PBG or XPGG were tested with isolated PGG, PSA or PBG by the method of Osserman (1960).

In order to differentiate between PGG and XPGG each material was also developed with a high titre pigeon breeders' disease serum known to contain specific anti-PGG and anti-XPGG precipitating antibodies. This serum was rendered specific for XPGG by absorption with PGG and a further aliquot of the serum rendered specific to PGG by absorption with lyophilized 'old' droppings. Basically, 'old' droppings contain XPGG in high titre but no demonstrable PGG, PSA or PBG.

The relationship of XPGG to PGG antibody production and *vice versa* was investigated by absorbing eight other pigeon breeders' disease sera with (a) PGG, or (b) 'old' droppings. PGG absorbed sera were used to develop an 'old' droppings extract on immunoelectrophoresis, and 'old' droppings absorbed sera were used to develop PGG on immunoelectrophoresis.

Absorption by PGG

A 1 ml aliquot of each serum was mixed with PGG (300 μg), incubated for $2\frac{1}{2}$ hours at 37°, centrifuged at 2000 ***g*** for 10 minutes then left overnight at 4° and again centrifuged at 5000 ***g*** for 10 minutes. The supernatant was tested for excess PGG by immunodiffusion. If necessary further 300 μg quantities of PGG were added until PGG was determined to be in excess by immunodiffusion. The serum was then concentrated to its original volume with Lyphogel (Gelman Instrument Company). Total amounts of PGG added varied from 300 to 1800 μg.

Absorption by 'old' droppings

5 mg lyophilized 'old' droppings were added to 1 ml of each serum and processed as per PGG. 5 mg was sufficient to absorb out XPGG from all nine sera.

RESULTS

DETECTION OF SPECIFIC PIGEON PROTEINS

The distribution of PGG, PSA, PBG and XPGG as determined by immunoelectrophoresis, Osserman and absorption techniques was widespread in the pigeon materials tested although only five of these materials contained all four antigens; the pigeon 'oviduct' extract in particular produced a good immunoelectrophoretic pattern of all four

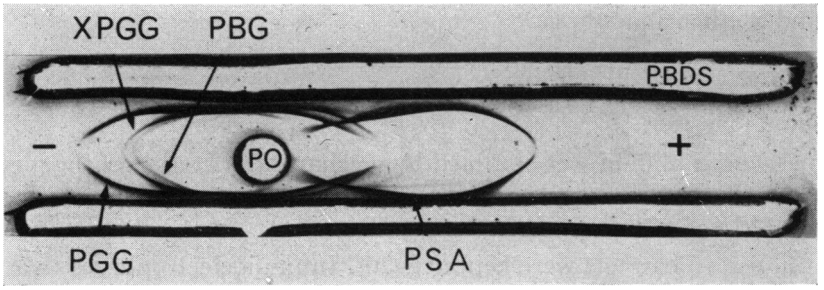

FIG. 1. Immunoelectrophoresis of pigeon 'oviduct' (PO) washings developed with serum from a case of pigeon breeders' disease (PBDS). Specific arcs identified are: PSA, pigeon serum albumin; PGG, pigeon γ-globulin; XPGG, a pigeon protein cross-reacting immunologically with PGG; PBG, pigeon β-globulin.

antigens (Fig. 1). Six pigeon materials contained a lesser number of the four antigens and in three pigeon materials the antigens were not detected (Table 1).

Other antigens were detected in the pigeon materials: pigeon 'gastric' juice, intestinal contents, 'saliva', 'crop' fluid and 'oviduct' extracts exhibited weakly reacting antigens whereas in both 'fresh' and 'old' droppings extracts strongly reacting antigens were found besides PGG, PSA, PBG and XPGG.

TABLE 1

PIGEON PROTEINS DETECTED IN VARIOUS PIGEON MATERIALS BY IMMUNOELECTROPHORESIS, OSSERMAN AND ABSORPTION TECHNIQUES

	Pigeon material			
	PSA	PGG	XPGG	PBG
Serum	+	+	−	+
Droppings 'Fresh'	(+)	(+)	+	(+)
Droppings 'Old'	−	−	+	−
Intestinal contents	(+)	(+)	+	(+)
'Urine'	−	−	−	−
Egg white	(+)	(+)	+	+
Egg yolk	+	+	−	+
Egg surface	(+)	(+)	(+)	−
'Oviduct' washings	+	+	+	+
'Gastric' juice	−	−	−	−
'Saliva'	(+)	(+)	(+)	−
'Crop'	(+)	(+)	(+)	(+)
Plume	−	−	−	−
Lachrymal fluid	+	+	−	−

(+) = Trace amounts.

PSA, pigeon serum albumin; PGG, pigeon γ-globulin; XPGG, a pigeon protein cross-reacting immunologically with PGG; PBG, pigeon β-globulin.

ABSORPTION WITH PGG AND XPGG

Four out of nine pigeon breeders' disease sera reacted to XPGG after absorption with PGG and seven out of nine reacted with PGG after absorption with XPGG, whereas all sera when previously tested reacted with PGG and XPGG. Only two out of nine did not react with either PGG or XPGG after absorption and four out of nine reacted with both PGG and XPGG after absorption.

DISCUSSION

The presence of PGG, PSA, PBG and XPGG in so many pigeon materials satisfactorily explains the precipitation reactions obtained with such varied pigeon materials as droppings, serum, egg white, egg yolk etc., in that exposure to any one of these materials could result in the production of precipitating antibodies capable of reacting with identical or related antigens in other materials. It is not known whether PGG, PSA and PBG in these materials are derived from pigeon serum or are secreted by specialized cells into fluids other than pigeon serum whereas XPGG seems to be the product of such specialized cells since it is not detected in pigeon serum but found in high concentration in certain pigeon secretions, e.g. pigeon droppings (Edwards *et al.*, 1969), and in particular in the pigeon 'oviduct' where the absence of enzyme activity, as compared with droppings (Edwards *et al.*, 1969) allows a clear immunoelectrophoretic distinction to be made between XPGG and PGG. Note is made of the similarity in distribution of XPGG in pigeon 'external' secretions, i.e. 'saliva', 'oviduct', 'crop' fluid, intestinal contents, droppings and that of raised IgA levels in human 'external' secretions (Tomasi, 1969).

The absorption of the nine pigeon breeders' disease sera with PGG and XPGG has shown antibodies to PGG and XPGG to fall into three groups.

1. Those specific for PGG.
2. Those specific for XPGG.
3. Those reacting with antigenic determinants common to PGG and XPGG, similar to the common antigenic determinants in light chains of human IgG and IgA (Martin, 1969).

It would, therefore, appear that patients with specific antibodies against both PGG and XPGG have been exposed to materials containing both proteins or alternatively they may have been exposed to a combination of materials such as egg yolk or serum and 'old' droppings; however, certain conditions must prevail before pigeon serum or pigeon egg yolk could become part of the loft environment, e.g. pigeon serum could be derived from pigeons killed by exsanguination and egg yolk and white may be released into the loft by breaking the pigeon egg shell. It has been suggested that ingestion of pigeon meat may be responsible for the development of antibodies to pigeon serum proteins (Barboriak, Fink and Scribner, 1968), but heat inactivation studies (Edwards, 1968) on pigeon serum show that neither PGG nor PSA are detectable by immunoelectrophoresis after heating pigeon serum for 15 minutes at 100°. It is less likely that antibodies to pigeon egg yolk and white are due to ingestion. Specialized conditions must also exist before 'saliva', 'crop' fluid, 'gastric' juice and lachrymal fluid are released into the pigeon loft.

In view of the presence of PGG, PSA, PBG and XPGG in so many pigeon materials and also due to the antigenic determinants shared by PGG and XPGG it is difficult to determine which material or materials are those responsible for the precipitating antibodies found in pigeon breeders' disease sera.

An indication of the most likely antigenic source might be found with those materials containing strongly reacting antigens other than PGG, PSA, PBG and XPGG; and of the materials tested only pigeon droppings contained such antigens reacting strongly with pigeon breeders' disease sera. It would therefore appear that pigeon droppings are the only pigeon material with a full 'complement' of major antigens concerned with pigeon breeders' disease and should be considered as the most important source of antigenic material in this hypersensitivity pneumonitide.

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