Excretion of Pigeon Serum Proteins in Pigeon Droppings[1] (34334)

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Precipitating antibodies to a variety of pigeon materials have been demonstrated in the sera of individuals with the hypersensitivity pneumonitis pigeon breeders' disease (1, 2). Several of these materials, in particular pigeon serum (PS) and pigeon droppings, contain identical or related antigens (3). Individuals with pigeon breeders' disease have a high titer of antibody against PS proteins (4), but do not usually come into contact with PS. This study was therefore designed to determine whether the serum proteins pigeon gamma globulin (PGG) and pigeon albumin (PSA) were present in the droppings. Further, the effects of intestinal and droppings enzyme activity on these proteins were assessed.

Materials and Methods. Droppings extracts from pigeons receiving labeled PS proteins. The PGG and PSA were prepared by starch block electrophoresis of pooled PS (4). The PSA was further purified by gel filtration on Sephadex G200 and fractions containing only PSA were pooled and concentrated with polyacrylamide gel.[2] One mg each of PSA and PGG were labeled with ^{131}I by the iodine monochloride method of McFarlane (5). After dialysis against saline for 2 days, the specific activity of PGG was 100 μCi/mg, and that of PSA was 600 μCi/mg; over 95% of the radioisotope was precipitated by 10% trichloroacetic acid (TCA).

Pigeons were injected intravenously with ^{131}I-PGG (500 μg) or ^{131}I-PSA (500 μg); and their droppings were collected daily. Extracts were prepared from droppings by emulsifying them with three times their dry weight in water, equilibrating for 10 min and centrifuging at 10,000*g* for 10 min; the supernatant constituted the extract.

Extracts were tested by immunoelectrophoresis (6) against a pigeon breeders' disease serum known to contain antibody against PGG and PSA, and the presence of radioactive material determined by radioimmunoelectrophoresis (7).

Droppings extracts from control pigeons. "Fresh" droppings obtained by overnight collection from 10 pigeons were pooled and immediately extracted, as described above. "Old" droppings were collected from the same 10 pigeons during normal cage cleaning, stored at room temperature for at least 1 month, and extracted as above. These "old" droppings were considered to be typical of pigeon droppings that might be used for making extracts used in serological studies of pigeon breeders' disease.

Both "fresh" and "old" droppings extracts were tested by immunoelectrophoresis against rabbit antipigeon serum (RAPS). In addition, the method of Osserman (8) was used to identify PGG or PSA in the droppings.

Digestion of PSA and PGG. The contents of isolated pigeon intestines were gently extruded and mixed with 4 ml of saline. After equilibration for 10 min the suspension was centrifuged at 10,000*g* for 10 min, and the supernatant was used as an "intestinal contents" extract. The activity of the "intestinal contents" extract in digesting PSA or PGG was compared with that of "fresh" and "old" droppings extracts by incubation at 40° with ^{131}I-PSA or ^{131}I-PGG. The rate of digestion was followed by sampling the mixtures at various time intervals and assessing the TCA

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[2] Lyphogel, Gelman Instrument Company, Ann Arbor, Michigan.

precipitability of radioactive material using 1:10 PS as carrier protein. The remaining or undigested PGG or PSA was expressed as:

$$\frac{\text{counts per min (cpm) precipitated with TCA}}{\text{cpm precipitated with TCA} + \text{cpm TCA soluble}} \times 100 \text{ in } \%.$$

Controls were: (i) "intestinal contents" extract heated at 100° for 5 min prior to incubation; (ii) "intestinal contents" extract to which the specific protease inhibitor Trasylol (9) had been added at 10,000 KIU/ml;[3] and (iii) saline in volumes equal to the "intestinal contents" extract. The controls were incubated with ^{131}I-PSA or ^{131}I-PGG at 40°, and the TCA precipitability was tested at the same time intervals as the other extracts.

Results. Pigeons injected with ^{131}I-PSA or ^{131}I-PGG. The excretion of ^{131}I in birds receiving ^{131}I-PSA or ^{131}I-PGG was maximal in the overnight period following injection. Thus, immunoelectrophoresis was carried out on the initial overnight droppings extract and precipitin arcs with the electrophoretic mobilities of PSA or PGG were observed. Radioimmunoelectrophoresis confirmed the presence

[3] KIU: kallikrein inhibitor unit.

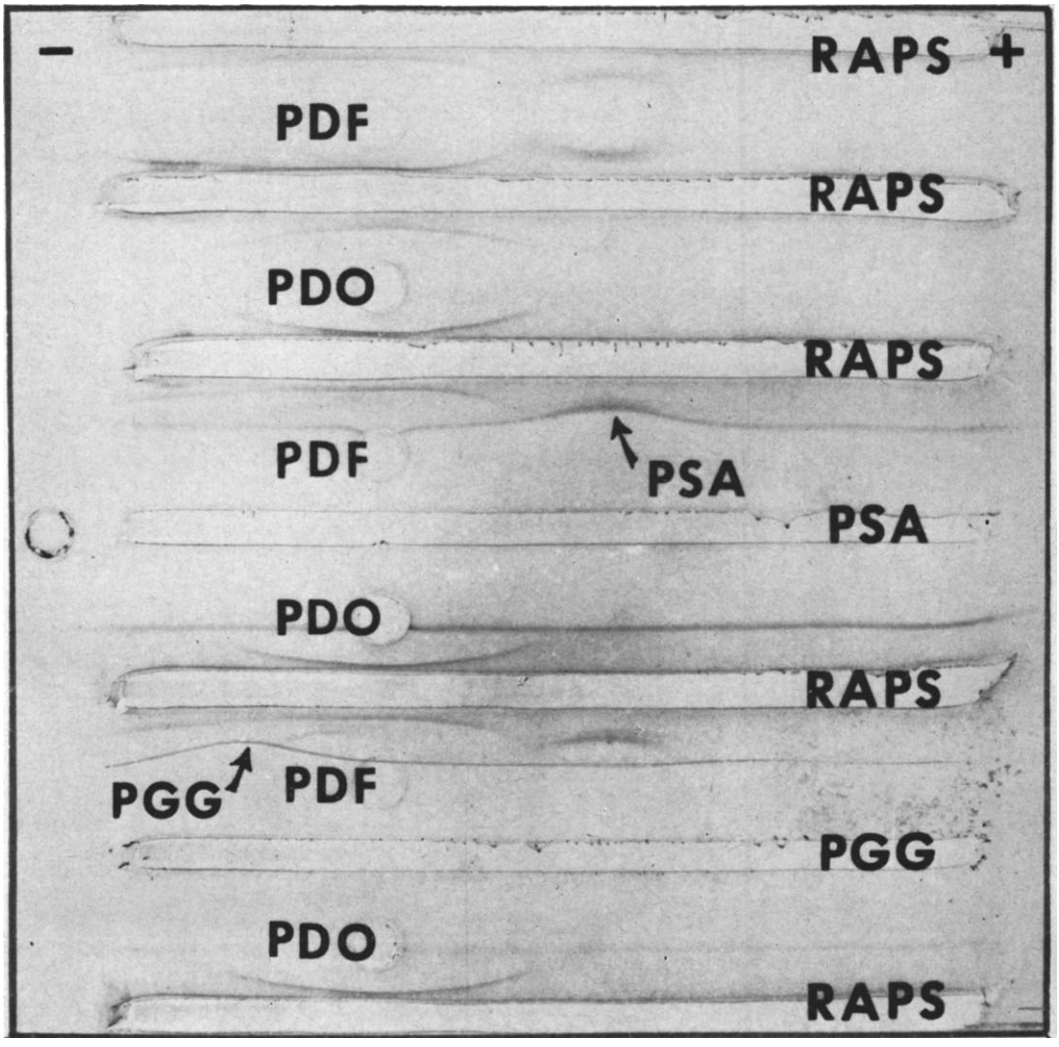

FIG. 1. Immunoelectrophoresis of "fresh" droppings extract (PDF) and "old" droppings extract (PDO) developed with RAPS and detection of PSA and PGG by the method of Osserman (8); [precipitin arc of PGG (arrows) emphasized for photographic purposes].

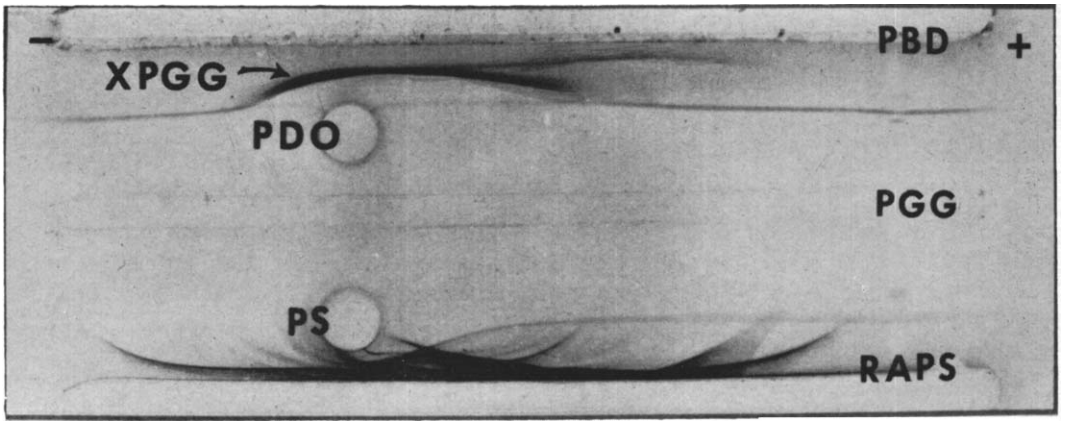

FIG. 2. Immunoelectrophoresis of "old" pigeon droppings extract developed with serum from a case of pigeon breeders' disease (PBD) and PS developed with RAPS; PGG in center trough forms a continuous precipitin line with PGG in PS, whereas, in "old" droppings the PGG precipitin line partially fuses with the XPGG precipitin arc indicating XPGG to be an antigen of partial immunological identity.

of PSA or PGG in the droppings. These proteins could also be demonstrated in pigeon "intestinal contents" extracts from the birds receiving ^{131}I-PSA or ^{131}I-PGG.

Control pigeons. Immunoelectrophoresis of "fresh" droppings tested against RAPS produced precipitin arcs in both PSA and PGG regions. These arcs were demonstrated to be PSA and PGG by the method of Osserman (Fig. 1). In contrast, PSA and PGG were not detected in the "old" droppings extracts (Fig. 1).

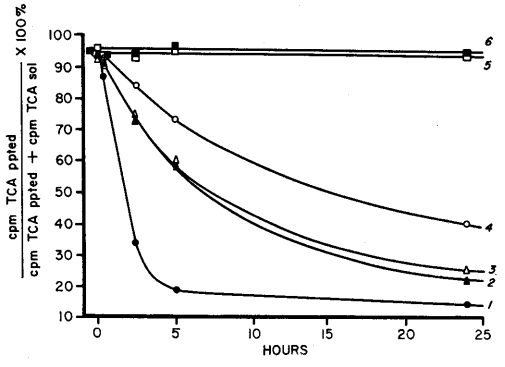

FIG. 3. The percentage TCA-precipitable counts of ^{131}I-PSA incubated at 40° with (1) pigeon "intestinal contents" extract, (2) "fresh" droppings extract, (3) "old" droppings extract, (4) pigeon "intestinal contents" plus Trasylol at 10,000 KIU/ml, (5) saline, and (6) pigeon "intestinal contents" heated at 100° for 5 min.

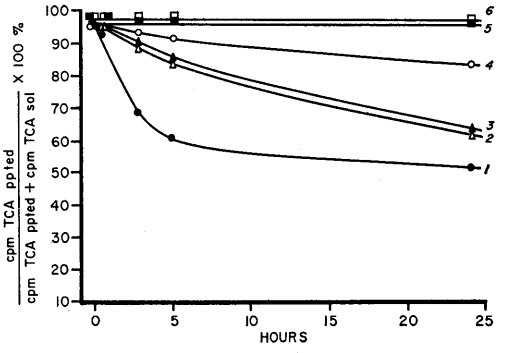

FIG. 4. The percentage TCA-precipitable counts of ^{131}I-PGG incubated at 40° with (1) pigeon "intestinal contents" extract, (2) "old" droppings extract, (3) "fresh" droppings extract, (4) pigeon "intestinal contents" plus Trasylol at 10,000/ml, (5) saline, and (6) pigeon "intestinal contents" heated at 100° for 5 min.

In both "fresh" and "old" droppings an antigen was observed that cross-reacted with PGG but had a different electrophoretic mobility (Fig. 2). The cross-reacting antigen was termed XPGG and appeared little affected by storage at room temperature for over 1 month; its only alteration was a slight increase in electrophoretic mobility.

Digestion of PSA and PGG. "Intestinal contents" were most active in digesting PSA and PGG (Figs. 3 and 4). Inhibition by Trasylol was observed while the heat inac-

tivated and saline controls had no activity. Considerable activity remained in extracts of both "fresh" and "old" droppings; the digestion curves for these extracts were virtually identical with both PSA and PGG. Comparatively, the rate of digestion of PSA was faster and more complete than that of PGG; 51% PGG remained TCA precipitable after incubation with "intestinal contents" extract for 24 hr, a finding similar to the effects of pepsin or papain on chicken gamma globulin (10).

Discussion. PSA and PGG are known to be antigens associated with pigeon breeders' disease (4, 11) and have been used in quantitative studies on the disease (4). However, until the present investigation it has been difficult to explain the finding of antibodies to the pigeon serum protein PSA and PGG in the sera from cases of pigeon breeders' disease, since these individuals are exposed by inhalation to droppings rather than serum. Work presented here suggests a possible solution in that PSA and PGG are present in small quantities in "fresh" droppings only, but are not detected in "old" droppings commonly used in preparing droppings extracts. Incubating PGG or PSA with "intestinal contents" and "fresh" or "old" droppings extracts show that digestion of these purified proteins (presumably enzymic) can continue, but at a lesser rate, after intestinal material is voided as droppings. Thus, the small quantities of PSA and PGG in "fresh" droppings might in time be reduced to undetectable levels. Also, certain methods of preparing pigeon droppings extracts, involving long periods of equilibration in aqueous solutions (2, 3), would presumably enhance enzyme activity and reduce further any remaining PSA and PGG. Presumably, PGG performs some function in the pigeon gastrointestinal tract although its role has not been established. Perhaps PGG forms part of the pigeon gastrointestinal tract defense mechanism similar to guinea pig copro-antibody in experimental cholera (12) or gastrointestinal γA antibodies in humans following oral polio vaccine (13), and is excreted selectively for this purpose. However, the additional presence of PSA and other proteins reacting with RAPS in "fresh" droppings suggests a simple transudation of serum proteins into the gastrointestinal tract. If proteins are secreted into the pigeon gastrointestinal tract for defensive purposes, it is more likely that the XPGG represents pigeon gastrointestinal tract immunoglobulin since it is present in relative abundance and apparently stable to gastrointestinal tract digestion. Immunoelectrophoresis indicates that XPGG is not present in serum to the degree it is in droppings so that transudation, followed by resorption (or digestion) of most of the other PS components, would have to occur to achieve a higher concentration of XPGG in pigeon droppings. Alternatively, there may be active transport of XPGG from PS or again XPGG may be the product of pigeon gastrointestinal tract secretory cells similar to γA antibodies that are predominant in gastrointestinal tract and other external secretions (14). Finally, XPGG may reflect a class of pigeon γA antibodies.

Summary. The pigeon serum proteins albumin (PSA) and gamma globulin (PGG) were present in extracts of pooled pigeon droppings collected within 16 hr of voiding, but were not detected in extracts of pooled pigeon droppings from the same birds that had remained at room temperature for over 1 month. The results of incubating ^{131}I-labeled PGG or PSA with a pigeon "intestinal contents" extract, an overnight droppings extract, or a month-old droppings extract suggest that continued enzyme activity could account for the absence of PSA and PGG in extracts of month-old droppings.

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