Immunoglobulins in Respiratory Secretions Obtained from the Canine Tracheal Pouch[1] (35127)

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The secretory immunoglobulins of several species have been characterized. In man, the primary secretory immunoglobulin is an IgA which appears to be derived from glandular plasma cells. It is both antigenically and immunochemically distinct from serum IgA (1–6). Studies of rabbit colostrum and intestinal secretions (7) and rat intestinal secretions (8) indicate that IgA is also the predominant secretory immunoglobulin in these species. The principal immunoglobulin in the colostrum and saliva of the cow and sheep differs from the other species reported because it is an IgG (9).

Studies of the secretory immune system of the respiratory tract are important in relation to protection against infection by local antibody production and to hypersensitivity phenomena. Characterization of the respiratory immunoglobulin system has been difficult because of the problems inherent in obtaining sterile, normal respiratory secretions. Recently a technique has been described which permits the repeated collection of canine tracheal secretions, free of demonstable microorganisms and containing only minimal numbers of cells, in amounts suitable for analysis (10). To prepare a canine tracheal pouch, a 5–6-cm segment is separated surgically from the cervical trachea and anastomosed to reestablish a patent airway. The bisected segment is fashioned *in situ* into a subcutaneously buried pouch located lateral and anterior to the anastomosed trachea. The pouch has been shown to remain histologically normal and physiologically functional. Its original innervation and blood supply remain intact, and its pharmacological responses are analogous to those of the untreated normal trachea. Milliliter samples of tracheal secretions may be withdrawn from the pouch at regular intervals throughout a prolonged period. These secretions have been used in the study of the immunologic characteristics of canine respiratory mucous by means of Ouchterlony double gel diffusion, immunoelectrophoresis, and gel filtration chromatography.

The present studies have been aided by the selection of potent antisera directed against the alpha and mμ chains of human IgA and IgM. These antisera cross-react with their canine analogues (11). Therefore, they may be used to identify the immunoglobulin classes in canine respiratory secretions in a manner similar to that used for identification of canine serum IgA and IgM (11).

Materials and Methods. Collection and preparation of tracheal secretions (ts). Normal dogs of mixed breed weighing between 10 and 15 kg were used. Tracheal pouches were constructed in the manner previously described (10). Tracheal secretions were aspirated weekly and examined for the presence of blood. Samples that were free of blood as demonstrated by the guaiac test (12) for hemoglobin were used for study.

To prepare extracts of tracheal pouch secretions for study, an equal volume of 0.15 *M* NaCl, pH 7.5 was added to the extracts, and the mixture was agitated vigorously on a vortex mixer for three min. The mixture of mucous and saline was incubated at 37° for 60 min and then centrifuged at 1500*g* for 30

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min. The supernatant was removed, and the sediment was extracted two additional times. The 3 vol of saline used for the extractions of respiratory mucous were then combined.

Preparation of rabbit anticanine tracheal secretions (ts). An emulsion of 1 ml of ts in 3 ml of complete Freund's adjuvant containing *Mycobacterium tuberculosis* was administered subcutaneously to a rabbit. This immunization was repeated 17 and 32 days later. The rabbit was bled 24 and 55 days after the initial injection, and the serum was tested by gel diffusion for precipitating antibody against ts. Thirty ml of pooled precipitating antisera were collected and stored at —20°.

Other antisera. Rabbit anticanine serum and rabbit anticanine IgG were prepared as previously described (13). Goat antihuman IgM Hyland and sheep antihuman IgA (Melpar) were used in Ouchterlony double gel diffusion studies.

Immunologic techniques. Ouchterlony double gel diffusion and immunolectrophoresis were done by standard techniques (14).

Gel filtration chromatography (15) was done with Sephadex G-200 (Pharmacia). Eight ml of ts extract containing 75 mg of protein/ml and dextran blue, to determine the void volume, were applied to a 2.5 × 100-cm Sephadex G-200 column which had been equilibrated with 1 *M* NaCl in 0.1 *M* Tris–HCl buffer, ph 8.0. The same buffer was used to elute the column. Five-ml samples were collected at 4°. The optical density of the samples was read at 280 mμ on a Beckman DU spectrophotometer. The collected fractions were then tested by the double gel diffusion technique, using antiserum specific for the alpha, mμ, and gamma chains.

Results. Detection and estimation of relative concentrations of IgA, IgM, and IgG in ts and canine serum (cs). Using the double gel system, peripheral wells were charged with serial 2-fold dilutions of ts or cs. The central well contained either anti-IgA, anti-IgM, or anti-IgG. The formation of precipitin bands was determined, and the highest dilution giving a positive precipitin band was read as the end point.

The results (Table I) indicated that IgA was present in greater concentration in ts than in cs. In contrast, IgG and IgM were present in greater concentration in serum than in ts.

Immunoelectrophoresis. Immunoelectrophoresis of the cs and ts developed with rabbit anti-cs (Fig. 1) shows the major immunoglobulin arc to be of gamma 1 mobility. A slower migrating immunoglobulin of cs is present and appears to be IgG. Arcs consistent with albumin are seen in both ts and cs. No proteins migrating as beta globulins are evident in ts.

Figure 2 shows the immunoelectrophoretic patterns of ts and cs after development with rabbit anti-ts. Several beta migrating proteins are evident in ts, whereas cs has little evidence of proteins of beta mobility that react with anti-ts. An arc adjacent to the ts well

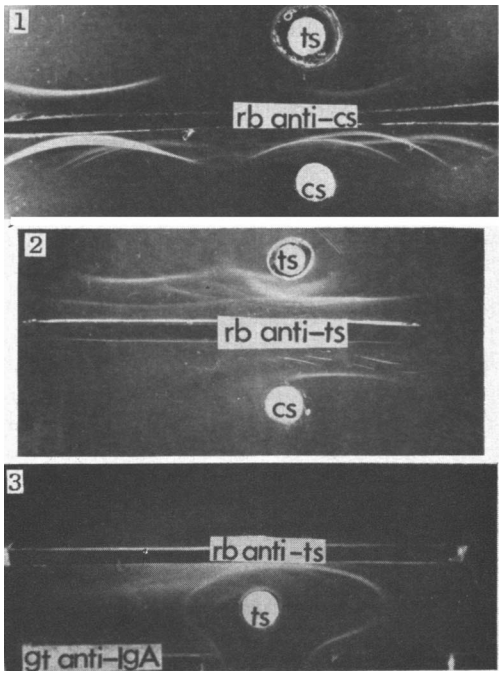

FIG. 1. Immunoelectrophoresis of canine tracheal secretions (ts) and canine serum (cs) developed with rabbit anticanine serum (rb anti-cs).

FIG. 2. Immunoelectrophoresis of canine tracheal secretions (ts) and canine serum (cs) developed with rabbit antitracheal secretions (rb anti-ts).

FIG. 3. Immunoelectrophoresis of canine tracheal secretions (ts) developed with rabbit antitracheal secretions (rb anti-ts) and goat antihuman IgA (gt anti-IgA). Although not labeled, both lower troughs contain goat antihuman IgA.

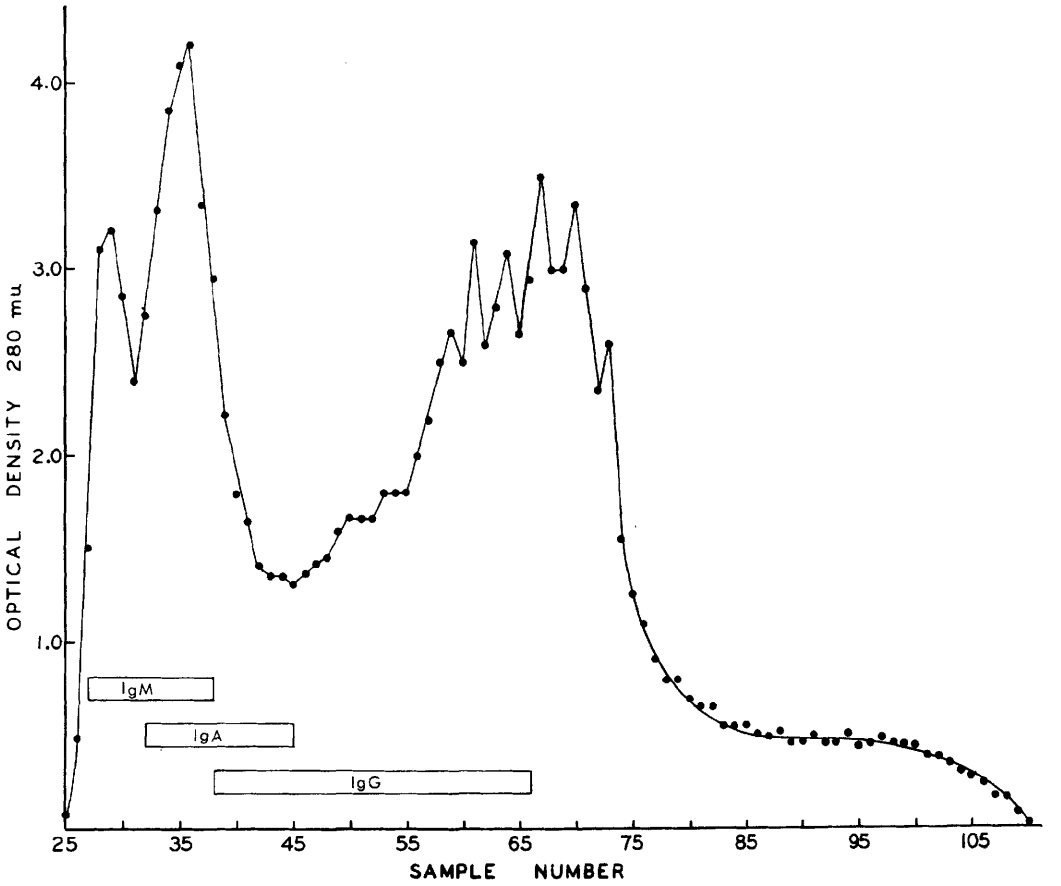

FIG. 4. Sephadex G-200 fractionation of canine tracheal secretions.

that may be IgM is visible. As in Fig. 1, there is one major immunoglobulin precipitin arc in ts. This major arc in both Figs. 1 and 2 is consistent with IgA. Proof of its identity as IgA is seen in Fig. 3 when immunoelectrophoresis of ts is developed with sheep anti-human IgA in one trough and rabbit anti-ts in a second trough.

TABLE I. Relative Concentrations of Immunoglobulins of ts and cs.

	Highest positive dilution[a]	
Antiserum	ts	cs
Anti-IgA	1:32	1:8
Anti-IgG	1:16	1:1024
Anti-IgM	Undiluted	1:8

[a] Recorded as the highest serial 2-fold dilution giving a positive precipitin reaction with each antiserum in the Ouchterlony double gel diffusion system.

Gel filtration chromatography. Three distinct peaks were obtained when the ts extract was fractionated on Sephadex G-200 (Fig. 4). Each fraction was tested for immunoglobulin class specificity by double gel diffusion. IgM was localized to peak 1 and peak 2. IgA was localized in peak 2, and IgG was found in peak 3 and the trailing edge of peak 2.

Discussion. The immunoglobulin pattern of canine respiratory secretions obtained from the tracheal pouch is consistent with that observed in certain other secretory fluids of the dog (16). IgA appears to be the primary immunoglobulin of canine tracheal secretions. The results of double gel diffusion tests indicated that there is approximately 4 times as much IgA in ts as in cs, and that IgG and IgM are found in relatively low concentra-

tions in ts. The technique used for these studies was a semiquantitative method.

The reasons for the high concentrations of IgA in ts have not been established. Local antibody production or selective concentration from the serum are two mechanisms which might account for this phenomenon. Most evidence indicates that local production followed by a unique method of secretion dependent upon the addition of a "secretory piece" accounts for the increased amounts of IgA present in human secretions (17). At the present time, no conclusive demonstration of a canine "secretory piece" has been reported. Evidence suggesting the existence of a canine "secretory piece" can be inferred from the fact that colostral secretory IgA has a higher molecular weight than serum IgA (18). This suggests polymerization, possibly accomplished by the addition of a coupling piece.

The immunoelectrophoretic patterns of ts also reveal that the major precipitin arc is IgA. IgG, IgM, and albumin are also apparently present. The numerous arcs that appear in the beta region between ts and anti-ts are not identifiable at this time. However, their absence between ts and anti-cs and between cs and anti-ts would indicate several antigenic components in ts that are either not present in cs or are present in a quantity insufficient to demonstrate. These components may be mucoproteins.

The absence of blood and the relatively small concentrations of IgG and IgM in ts suggests that the tracheal pouch remains intact, and that serum transudate does not provide a large volume of the secretions present in the pouch. In addition, the accessibility of the pouch as a source of secretions indicates that it should be of value in future immunologic studies of these secretions.

Summary. Canine tracheal secretions obtained from a new system for studying respiratory tract mucous were examined by Ouchterlony double gel diffusion, immunoelectrophoresis, and gel filtration chromatography. Immunoglobulins of three classes, IgA, IgM, and IgG, were identified. The main immunoglobulin of canine tracheal secretions appears to be IgA. The tracheal pouch provides a useful model in which to study the secretory immune system of the dog.

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