

PRESENCE OF REAGIN AND γ_{1A} -GLOBULIN IN SALIVA

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APPPLICATION of ragweed allergen to the mucous membranes, such as the conjunctiva and respiratory tracts, of ragweed sensitive individuals induces an allergic reaction. This fact suggested to us the possibility that reagin might be present on or in the mucous membrane and that secretions from these membranes or glands might contain reagin. The present investigation was undertaken to detect reagin in saliva from asthmatic patients. In view of recent evidence¹⁻⁴ that reagin is associated with the γ_{1A} class of immune globulins, some studies were carried out to identify serum proteins in saliva. The results indicate that saliva contained γ_{1A} -globulin, whereas neither γ_2 - nor 19S γ -globulin was detected.

MATERIALS AND METHODS

Saliva and Allergen.—In most cases, saliva was collected by expectoration from a ragweed sensitive patient and immediately centrifuged at $24,000 \times g$ for 30 minutes by Spinco preparative ultracentrifugation to remove insoluble precipitates. One specimen of saliva was collected from the parotid duct. All specimens were kept in a frozen state (-20°C.) until used. A commercial ragweed test allergen* was used to induce skin responses in humans.

Human Serum Proteins.—A γ_2 -globulin fraction was purified from human Cohn Fr. II† by starch block electrophoresis in barbital buffer of pH 8.6 and ionic strength of 0.1.⁵ Eluates from the slowly migrating γ -globulin region were pooled and concentrated by pervaporation in the cold. Gamma $_{1A}$ (β_{2A}) globulin was purified from fresh human serum by the method of Heremans and associates.¹ 19S γ -globulin was obtained through the courtesy of Dr. H. Fudenberg. Crystalline human serum albumin† was used in the experiments without further purification.

Double Diffusion in Agar and Immunoelectrophoresis.—Double diffusion on agar plates was carried out by the method of Ouchterlony.⁶ Immunoelectrophoresis was performed following the modification of Scheidegger⁷ on micro-

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scopic slides. The antisera used in these tests were obtained from Hyland Laboratories. They were goat antisera against whole human serum and those specific to 7S (γ_2) globulin (No. 173), β_{2A} (γ_{1A})-globulin (No. 206), and β_{2M} (19S γ)-globulin. Precipitin bands were stained with azocarmine.

Prausnitz-Küstner (P-K) Reactions.—Prausnitz-Küstner reactions were carried out on nonallergic individuals who had negative skin test reactions to ragweed allergen (500 N units per milliliter). One-twentieth to one-tenth milliliter of appropriate dilutions of allergic serum or of saliva was injected intracutaneously into the forearm. Twenty-four hours later, 0.02 ml. of ragweed allergen containing 2 N units was injected into the same sites. Erythema-wheal reactions appeared 15 to 20 minutes after the injection of allergen, and the reactions were traced on a transparent plate applied to the area.

EXPERIMENTAL RESULTS

Serial dilutions of reaginic sera from ragweed sensitive individuals were used for the sensitizing activity in P-K reactions. It was found that a 1:4000 dilution of serum from an allergic patient (A) and a 1:400 dilution of serum from another allergic patient (B) gave definite erythema-wheal reactions with allergen. The average diameter of these reactions was 35 to 40 mm. Saliva was collected from the 2 patients and from 2 normal individuals, diluted 1:5 with borate buffered saline, and centrifuged at $24,000 \times g$ for 30 minutes. Supernatant fluids were sterilized by filtration through a Seitz filter, and 0.1 ml. of each was injected intracutaneously into 3 normal individuals. Slight erythema appeared 3 to 5 hours after the injection and disappeared within 24 hours in most cases. Allergen was injected into all sites 24 hours after the injection of saliva. The sites receiving the patient's saliva gave definite erythema-

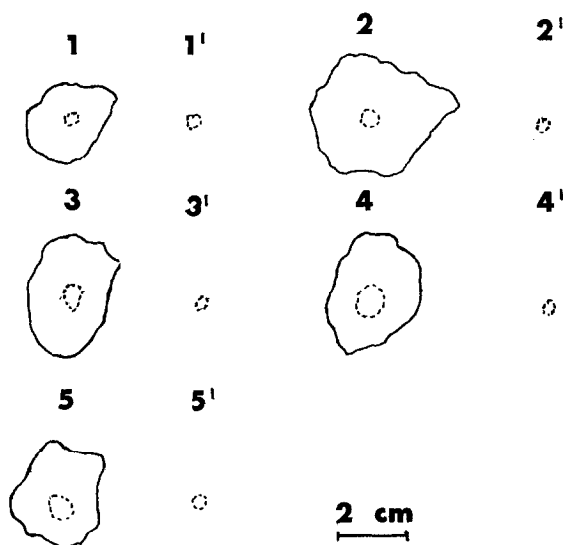


Fig. 1.—Direct tracings of some of the erythema-wheal reactions in normal human skin produced with saliva and ragweed allergen. 1 and 2: Saliva obtained by expectoration from patient A. 3: Saliva from patient B. 4: Saliva collected from parotid duct of patient A. 5: Protein fraction of saliva from patient A. Control sites 1' and 2' received saliva from a normal individual and allergen 24 hours later. Other control sites (3' to 5') received allergen alone.

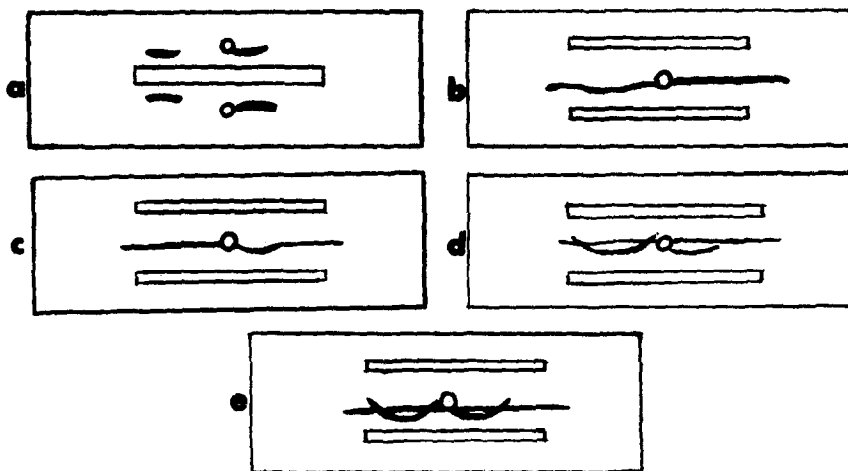


Fig. 2.—Diagrams of immunoelectrophoresis of saliva. *a*, Saliva from a normal (top) subject and patient A. The trough was filled with anti-human serum. *b* to *e*, Saliva from patient A. Upper trough in these slides were filled with albumin (*b*), γ_{1A} -globulin (*c*), 19S γ -globulin (*d*), and γ_2 -globulin (*e*). Lower troughs were filled with anti-human serum. All specimens were obtained by expectoration, except (*c*), which was collected from the parotid duct.

wheal reactions, whereas the control sites, into which normal saliva had been injected, did not. A similar experiment was carried out using twice-diluted saliva from patient B with comparable results (Fig. 1).

In order to eliminate nonspecific reactions by saliva, its protein fraction was utilized for passive sensitization. Three volumes of 4M ammonium sulfate at pH 7.0 to 1 volume of saliva from patient A was added and stirred for 30 minutes. This mixture was centrifuged for 30 minutes at $24,000 \times g$. The resulting precipitate was dissolved in saline equal to the original volume and dialyzed against saline for 24 hours. After sterilization, 0.1 ml. of the preparation was injected intracutaneously into normal individuals. A definite skin reaction was observed upon injection of allergen in the same site (Fig. 1). It is evident that the protein fraction of a patient's saliva can sensitize human skin for erythema-wheal reactions.

The question arose whether the presence of reagin in saliva might be the result of contamination with either nasal secretion or sputum. Thus, experiments were repeated with saliva collected by cannulation of the parotid duct of patient A. One-tenth milliliter of this saliva, twice diluted in saline, was used for passive sensitization. It was confirmed that the specimen could sensitize human skin (Fig. 1).

Serum proteins in saliva were studied by immunoelectrophoresis. Specimens used for the above experiments and those from 2 normal individuals were concentrated 3 to 4 times by pervaporation in the cold and dialyzed against borate-buffered saline (pH 8.0). They were electrophoresed and reacted with anti-human serum. The specimens obtained by expectoration gave 2 precipitin bands (Fig. 2, *a*). One of these bands, in the albumin region, was not present in the specimen obtained from the parotid duct of patient A (Fig. 2, *c*). Another precipitin band in the β -globulin region was observed in all cases. In order to

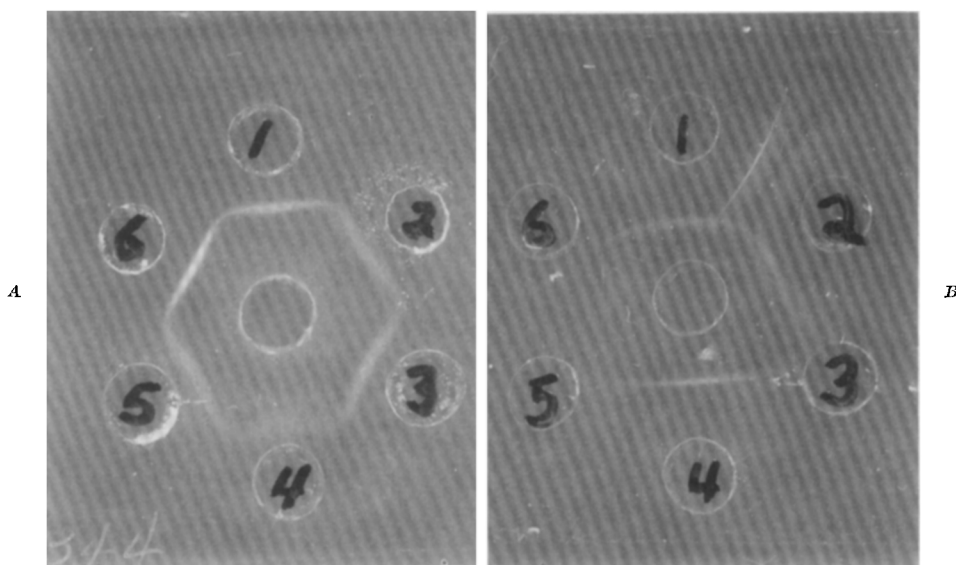


Fig. 3.—Ouchterlony plate analysis of saliva. *A*, Peripheral wells were filled with saliva from patient *A* (1) and normal individuals (2 to 6). Center well was filled with anti- γ_{1A} -globulin serum. *B*, Center well was filled with saliva (protein fraction) from patient *A*. Peripheral wells were filled with anti-human serum (1) and anti- γ_{1A} -globulin (2 and 4) and anti- γ_{2S} -globulin (6). Wells 3 and 5 were not used. Precipitin bands between the anti-human serum and other antisera are due to human proteins present in the specific antisera.

identify the proteins involved in the precipitin reactions, Osseman's technique was applied.⁸ After electrophoresis of the saliva, 1 trough was filled with anti-human serum and another with albumin, γ_{1A} -globulin, 19S γ -globulin, or γ_{2S} -globulin. The concentration of the proteins was 200 to 400 μ g N per milliliter. As shown in Fig. 2, *b* and *c*, the fast migrating protein had identical specificity to serum albumin and the slower migrating protein was γ_{1A} -globulin. In a specimen obtained from patient *A* by expectoration, a trace of γ_{2S} -globulin was detected by this technique. Other specimens did not contain γ_{2S} - or 19S γ -globulin (Fig. 2, *d* and *e*).

Presence of γ_{1A} -globulin in saliva from 6 normal and 2 allergic individuals (*A* and *B*) was confirmed by Ouchterlony plate analysis using a specific anti- γ_{1A} -globulin serum (Fig. 3, *A*). When the center well was filled with anti- γ_{2S} - or anti-19S γ -globulin serum, no precipitin band was observed with any of the specimens. The protein fraction of the saliva from patient *A*, which was used in the P-K reaction in the above experiments, was concentrated 3 times by pervaporation and tested for the presence of serum proteins. As shown in Fig. 3, *B*, the preparation gave a single precipitin band of identical specificity with anti-whole human serum and with the anti- γ_{1A} -serum. Again, no γ_{2S} -globulin was detected.

DISCUSSION

The data described in this paper clearly show that saliva from ragweed sensitive individuals contains reaginic antibody. It was also found that γ_{1A} -

globulin, but neither γ_2 - nor 19S γ -globulin, was present in the saliva of allergic and normal individuals. The specimens obtained by expectoration usually contained albumin, which was barely detected in the specimen from the parotid duct. Most albumin in the former specimens may be due to contamination with nasal secretion or sputum which contains serum albumin.^{10, 11}

While the present work was in progress, Fudenberg⁹ made a similar observation that γ_{1A} -globulin was the only serum protein present in saliva from normal individuals. When one of the authors was in his laboratory in July, 1963, his findings were confirmed using 10 times concentrated saliva collected from the parotid duct. Presence of a trace amount of γ_2 -globulin in one of our specimens obtained by expectoration from patient A is probably the result of contamination of nasal secretion and/or sputum. It has been reported that these secretions from either asthmatic or infected individuals contain γ_2 -globulin.^{10, 11, 12}

Association of antibody activity with γ_{1A} -globulin was suggested by Schultze.¹³ Recently, Heremans and co-workers¹⁴ found diphtheria antitoxin and antibody to *Brucella abortus* in pure γ_{1A} -globulin of human serum, and Kunkel and Rockey¹⁵ detected γ_{1A} -globulin in purified isohemagglutinin preparations. With respect to reaginic activity in allergic serum, Heremans and Vaerman¹ detected reagin in γ_{1A} -globulin preparations containing only a small amount of γ_2 -globulin, and Fireman and associates² found that removal of γ_{1A} -globulin from the sera of ragweed sensitive individuals by precipitation with a specific antiserum was accompanied by loss of all detectable skin sensitizing activity. It was also found in our previous studies³ that normal γ_{1A} -globulin, but neither γ_2 - or 19S γ -globulin, blocked passive sensitization of human skin with reagin. In view of much evidence that blocking of passive cutaneous sensitization in guinea pigs is obtained by proteins having common physiochemical properties to sensitizing antibody,^{16, 17} the results support the idea that reagin is associated with γ_{1A} -globulin. The present findings provide another set of correlated observations between reagin and γ_{1A} -globulin through the demonstration that γ_{1A} -globulin is the only serum protein detectable in saliva possessing reaginic activity.

Presence of reaginic activity and γ_{1A} -globulin, but not γ_2 -globulin, in saliva may be of clinical importance. In our more recent studies on sputum, it was found that over 80 per cent of the mucus specimens from asthmatic patients contained γ_{1A} -globulin, whereas about 50 per cent of these specimens contained γ_2 -globulin. Even in these cases, the ratio of the concentrations between these proteins (γ_2/γ_{1A}) in sputum was lower than that in serum. It has been reported by Perlmutter and co-workers¹⁸ that blocking antibody is associated with γ_2 -globulin. The possibility is therefore considered that reagin is normally secreted through the mucous membrane, whereas blocking antibody is not.

SUMMARY

Reaginic activity was detected in saliva from ragweed sensitive individuals. These specimens, as well as saliva from normal individuals, contained γ_{1A} -

globulin and possibly a trace of albumin, but no other serum protein was detected.

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