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Research Article

Serum Proteins and Antibody Activity in Human Nasal Secretions *

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The presence of serum proteins and antibody activity against a variety of bacteria and viruses has been described in certain human secretions and excretions: vaginal (2, 3), salivary (4, 5), intestinal (6), prostatic (7), urine (8, 9), and feces (10, 11). The capacity of human nasal secretions to inactivate viruses was first described by Amoss and Taylor in 1917 (12) and confirmed by Howitt in 1937 (13). In 1939 Burnet, Lush, and Jackson (14) published the results of their studies with a number of viruses including influenza and poliomyelitis. These authors were doubtful whether the protocols of Amoss and Taylor and of Howitt were sufficient to justify the conclusion that any specific viricidal agent was concerned and concluded from their own data that the inactivating agent is most likely an enzyme. In 1941 Francis and Brightman (15) reported the development or increase in the viral inactivating capacity of nasal secretions of individuals in the acute and convalescent stages of influenza and later, the enhanced capacity of nasal secretions to inactivate the virus when vaccination with influenza virus induces a significant increase in titer of neutralizing antibodies in the blood (16). In 1948 Bell (17), working with polio virus, published additional evidence that the neutralizing substance is specific antibody, although in two instances in which the nasal secretions neutralized the virus,

the corresponding serum did not. The over-all results of the studies cited above suggest that neutralization of virus by nasal secretions is due to antibody and that such antibody may be of importance in host resistance to those agents.

If viral neutralizing antibody is present on the surface of the nasal mucosa, other antibodies may be present as well. The present studies, undertaken to determine the nature of the proteins found in nasal secretions, have revealed γ-globulins in the nasal secretions of all normal and allergic individuals examined. Reagenic antibody activity was found in the nasal secretions of allergic individuals and antibacterial antibodies in the nasal secretions of normal volunteers.

Methods

Human volunteers, from whom nasal secretions were obtained, were divided into two categories: normal and allergic (defined by a history of nasal allergy and skin testing). The allergic persons did not have signs or symptoms of clinical allergy at the time the secretions were collected. The technique employed for collection of the secretions was similar to that used by Anderson, Riff, and Jackson (18), who allowed 5 ml of distilled water to run into each nostril while the subject held his head in hyperextension with the tongue elevated and the glottis closed. Washings were collected in a sterile Petri dish held under the nose as the head was tipped forward. However, in the present study 0.15 M NaCl was employed rather than distilled water because the latter was found to be irritating. Results of immunodiffusion studies with nasal secretions collected using 0.15 M NaCl were the same as those with secretions collected using distilled water. The washings were dialyzed in Visking tubing ($\frac{1}{4}$ inch) for 24 hours against distilled water and lyophilized, and sufficient 0.15 M NaCl was added to bring the powder into solution. This was centrifuged at 8,000 rpm for 30 minutes and the supernatant fluid stored at $-20°$ C. Before obtaining nasal secretions, a smear was gently taken from the nasal mucosa with a
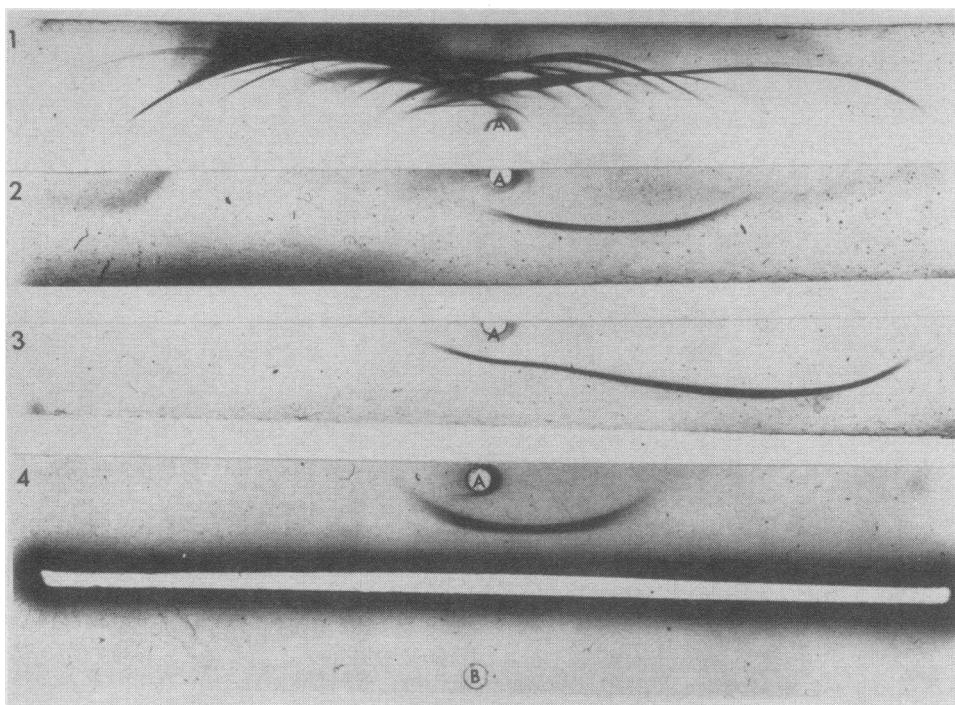
FIG. 1. IMMUNOELECTROPHORETIC CHARACTERIZATION OF THREE ANTIBODIES USED IN THE PRESENT STUDIES. Antiserum troughs are not shown except in part 4. A = normal human serum; B = 5% serum 7 S γ_2-globulin; 1 = horse antihuman serum; 2 and 4 = goat antihuman γ_{1A}-globulin used for immunoplates; 3 = antihuman 7 S γ_2-globulin.

cotton swab and the material applied to microscope slides. The preparations were stained with Wright's stain. No red blood cells were observed in the mucosal smears of any of the volunteers. The nasal secretions of three subjects contained large numbers of polymorphonuclear leukocytes and were not used.

Immunoelectrophoresis. The micromethod of Scheidegger (19) was employed with 1% agar in barbital buffer pH 8.2 (μ 0.05). After the precipitin lines appeared to be at optimal intensity (24 to 72 hours), the agar was washed in 0.15 M NaCl, and the precipitin lines were stained with Ponceau S.

Double diffusion in agar gel. The technique of Ouchterlony was used (20). In some instances, a micromethod was employed as previously described (9).

Antisera.[1] Goat antihuman 7 S γ-globulin, no. G10-63A and no. 6173-9P71562; horse antihuman β_2-macroglobulin, no. H137 2–63; goat antihuman β_2-macroglobulin, no. G37-98/3-3163; horse antihuman serum, no. H76138 53062; horse anti-F II no. H205PABS; and goat antihuman β_{2A} (γ_{1A})-globulin, no. G206-7ABS-3163 were used. Before use, the last antiserum was adsorbed with normal human cord serum, as well as with a serum that lacked γ_{1A}-globulin obtained from a patient with dysgammaglobulinemia. A single precipitin line resulted

from the reaction of the adsorbed anti-β_{2A} antiserum with normal human serum [Figure 1 (4) and Figure 2 (3)].

Analytical ultracentrifugation. A Spinco model E ultracentrifuge equipped with Schlieren and Rayleigh optics was used. Sedimentation velocity experiments were performed at 52,640 rpm in a double-sector 12-mm cell with rotor temperature maintained at 20° C. Measurements of the photographic plates were made in a Nikon microcomparator.

Electrophoresis. Cellulose acetate strips were used with a barbital buffer at pH 8.6 (μ 0.075). After drying, the strips were stained with Ponceau S. Starch-block electrophoresis was performed in borate buffer at pH 8.6 (μ 0.064). The separated proteins from serum or nasal secretions were eluted from 1-cm segments of the blocks. Nasal secretion protein fractions with electrophoretic mobilities corresponding to those of individual serum proteins were pooled and dialyzed against distilled water. The fractions were lyophilized, resuspended in 0.15 M NaCl, and centrifuged at 8,000 rpm for 30 minutes to remove insoluble starch granules. In some experiments, a polyvinyl chloride[2] preparation was used instead of starch.

Quantitative estimation of proteins. Immunodiffusion: Initially, the technique described by Feinberg was used

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[1] Purchased from Hyland Laboratories, Los Angeles, Calif.

[2] Pevikon, C-870, Stockholms Superfosfat Fabriks Aktiebolag, Stockholm, Sweden.
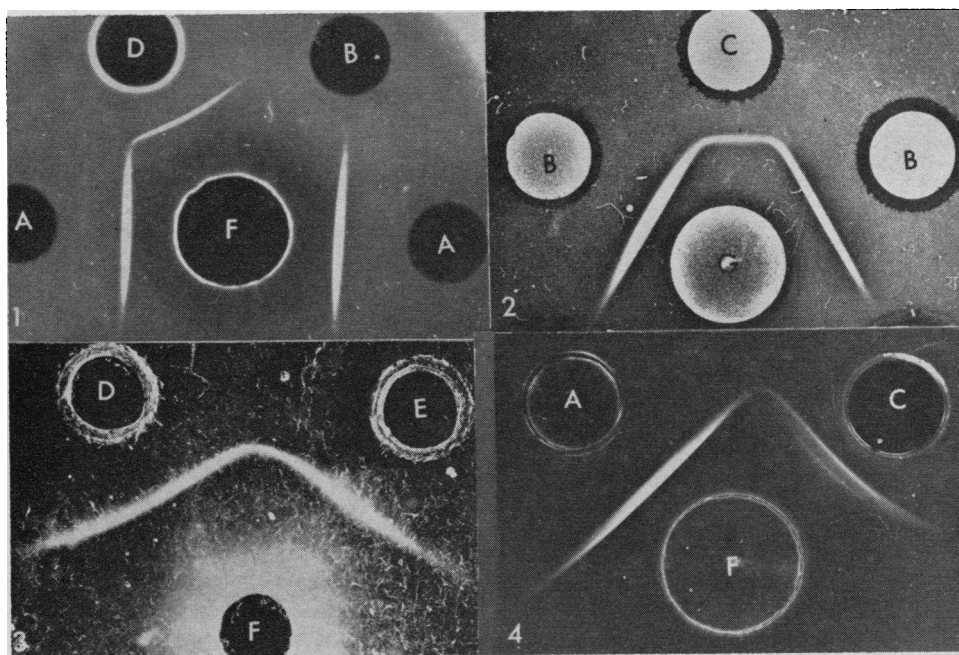
FIG. 2. DOUBLE DIFFUSION IN AGAR GEL OF PURIFIED IMMUNE GLOBULINS AND PROTEINS OF NASAL SECRETIONS. A = purified γ_{1A}-globulins from serum; B = purified 7 S γ_2-globulins from serum; C = Fraction I from starch block (see Figure 3); D = normal nasal secretions; E = normal human serum; F = goat anti-γ_{1A}-globulins; G = goat antihuman 7 S γ_2-globulin [same as used in Figure 5 (1)].

(21). The values obtained with the Hyland immuno-plates [1] for β_{2A}- and 7 S γ-globulins agreed very well with those obtained with the Feinberg method and thus were substituted in later studies. The results of the reaction between the antisera used in the immunoplates and normal human serum are shown in Figure 1 (2, 3). A single precipitin line was noted in each instance. Serial 2-fold dilutions were made of sera from 1:2 through 1:16, and a sample of the undiluted serum and each dilution was placed in a well of the immunoplates. The corresponding nasal secretions were placed undiluted in alternate wells on the same plates. After incubation for 4 to 6 hours at 37° C in the case of the antihuman γ-globulin plate, and for 16 hours at room temperature for the antihuman γ_{1A} plate, the diameter of the precipitin rings was measured for each of the serum dilutions. The results were plotted on semilogarithmic paper, the log scale representing the percentage of the total serum protein in the sample (e.g., the undiluted serum represented 100%, 1:2 dilution, 50%, etc.) and the linear scale, the diameter of the zones. In this manner a straight line was obtained, and the zone diameters about the well of nasal secretion were applied to the standard curve to obtain the relative concentration of specific protein in the sample. By using the values for the total protein in the serum (average, 8.1%; range, 7.9% to 8.9%) and samples of nasal secretions (average, 0.31%; range, 0.1% to 0.5%), the ratio N/S of the total γ_2-globulins and γ_{1A}-globulins in the nasal secretions to

their concentrations in the corresponding serum was calculated from the formula:

$$\frac{N}{S} = \frac{C_{nig}}{C_{tnp}} \times \frac{C_{tsp}}{C_{sig}},$$

where C_{nig} = nasal secretion immune globulin concentration (immunoplate), C_{sig} = serum immune globulin concentration (immunoplate), C_{tnp} = total nasal secretion protein concentration (refractometry), and C_{tsp} = total serum protein concentration (refractometry).

Refractometry. Protein concentrations were determined on isotonic solutions by using a commercial refractometer designed for serum protein determinations.[3] This method was used because it is nondestructive to the material measured and does not depend on the individual nature of the proteins measured (22, 23). The results obtained using refractometry agreed satisfactorily with those obtained in separate experiments using the micro-Kjeldahl technique.

Passive transfer. Reaginic activity in serum and nasal secretions was determined by the Prausnitz-Kustner test (24). 0.25 ml of the serum or concentrated nasal secretion solution was injected intradermally through a Swinny filter adapter containing a 0.47-μ Millipore filter. Twenty-four to 48 hours later, the site was challenged with 0.05 ml of a concentrated and purified orchard grass

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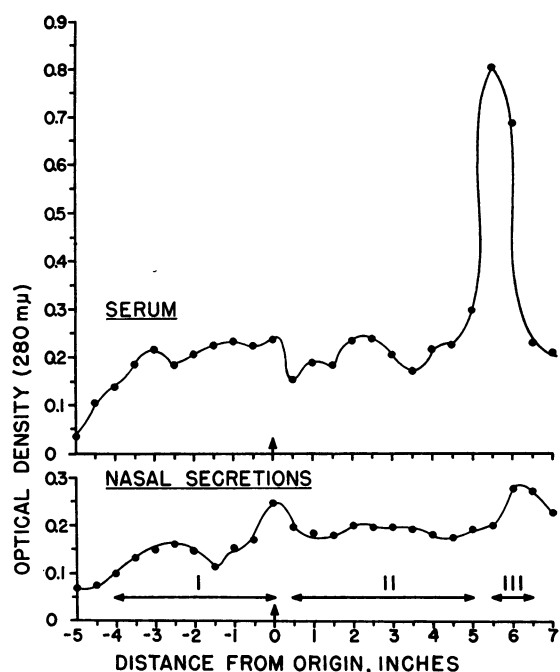[3] Atago protometer, National Instrument Co., Baltimore, Md.

allergen dissolved in 0.15 M NaCl. [The antigen was
a 16 mg per ml isotonic solution of an orchard grass
pollen preparation purified by ammonium sulphate pre-
cipitation to remove hemagglutinating antigen followed
by ethanol precipitation of inert ingredients (25)]. The
reactions were graded from negative (control) to 4 +
(1 +, 5-mm wheal with erythema; 2 +, 10-mm wheal
with erythema; 3+, 15-mm wheal with pseudopods and
erythema, and 4 +, 20-mm wheal with pseudopods and
erythema).

Immunization. Volunteers were immunized with either
0.5 ml of tetanus toxoid [4] or diphtheria toxoid.[5] Blood
and nasal secretions were obtained from each person
just before and 14 days after immunization. In cases
of primary immunization, blood and nasal secretions
were collected again at 30 days. Sera were obtained also
from individuals with high antistreptococcal antibody
titers due to natural infection.

Hemagglutination. Human, group O, Rh-negative,
red blood cells were used. The hemagglutination tests
were performed as described for type specific strepto-
coccal antibody by Vosti and Rantz (26) and for tetanus
and diphtheria antibodies as described by Levine, Wy-

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[4] Cutter lot G 4822, Cutter Laboratories, Berkeley,
Calif.

[5] Purified diphtheria toxoid lot no. KP 59 A, Massa-
chusetts Department of Public Health, Division of Bio-
logic Laboratories, Boston, Mass.

man, Broderick, and Ipsen (27). Four volumes of a
1/320 dilution of the stock semipurified M protein or of
37.5 Lf (the amount of toxin that produces optimal floc-
culation with 1 U of antitoxin) of tetanus or diphtheria
toxoid was incubated for 15 minutes at room temperature
with 1 vol of tanned cells. Lyophilized nasal secretions
used for determination of hemagglutinins were reconsti-
tuted in a volume of 0.5 ml of 1/100 normal rabbit serum,
and each specimen was adsorbed with packed human type
O red blood cells (ratio, 1 : 2) to remove nonspecific he-
magglutinins. Twofold dilutions of the serum or nasal
secretions to be tested were made in 1% rabbit serum that
had been adsorbed with human type O red blood cells.
The sensitized cells were added, and the test was read by
the pattern technique after incubation at room temperature
for 2 hours (28). The agglutination patterns obtained
with nasal secretions appeared the same as those with se-
rum and ranged from folded edges and matting (4 +)
through a granular carpet (3 +) and varying ring sizes
(2 + to ±). The titer was read as the reciprocal of the
last dilution giving a 3 + or greater reaction.

Hemagglutination inhibition was performed using a
1 : 10 dilution of the stock tetanus antigen (1,250 Lf) or
of the undiluted streptococcal antigens. Before the ad-
dition of sensitized cells, 0.05 ml of the diluted antigen
was added to each tube of the diluted specimen of serum
and nasal secretion, and the mixtures were incubated at
room temperature for 30 minutes.

Preparation of protein fractions. γ_{1A}-Globulins were
separated as follows. To 1 vol of serum was added 1¼
vol of saturated ammonium sulfate. The precipitate was
removed by centrifugation, dissolved, and dialyzed against
distilled water. After removal of the euglobulin precipi-
tate by centrifugation, the supernatant fluid was further
processed by a modification of the zinc precipitation
technique of Heremans (29). An equal volume of 0.1 M
$ZnSO_4$ was added to the supernatant fluid and the pH
immediately adjusted to 6.95 with aqueous sodium carbon-
ate (saturated). After standing at room temperature
for 1 hour, the precipitate was removed by centrifuga-
tion. The supernatant fluid was concentrated by the ad-
dition of 1¼ vol of ammonium sulfate and the light pre-
cipitate removed by centrifugation and dissolved in 0.15
M NaCl. This preparation was placed on a column of
Sephadex G-100 (4 × 120 cm) and eluted with 0.15 M
NaCl. The optical density of the eluates at 254 mμ was
recorded directly. Contents of tubes comprising portions
of the elution patterns within each peak were com-
bined, dialyzed against distilled water, and lyophilized.
The resulting powders were resuspended in small vol-
umes of 0.15 M NaCl. Each fraction was studied for
antigenic purity by immunoelectrophoresis and double dif-
fusion in agar. The material from the first peak off the
G-100 column contained the γ_{1A}-globulins.

[7] S γ-globulins were prepared by chromatography of
Cutter poliomyelitis immune globulin lot no. E 3217 on
DEAE cellulose in phosphate buffer, 0.0175 M, pH 6.3.
Urinary γ-globulins and albumin were prepared as
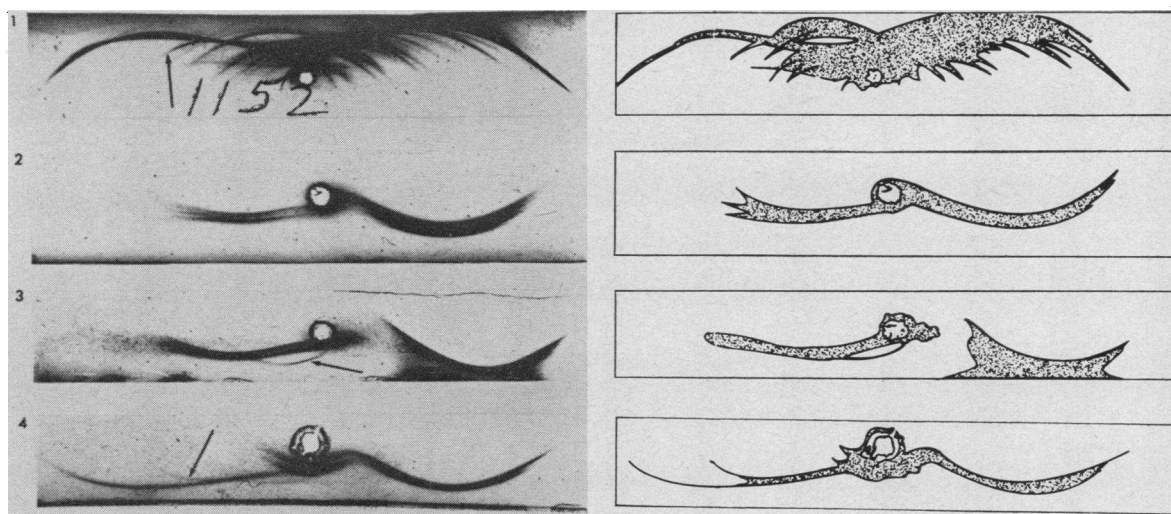previously described (30).

FIG. 4. IMMUNOELECTROPHORETIC PATTERNS OF NORMAL NASAL SECRETIONS REACTED WITH HORSE ANTIHUMAN SE-
RUM. Right = diagrammatic drawing of patterns shown on left. 1 = normal human serum (arrow points to γ_{1A}-
globulin line) ; 2, 3, 4 = nasal secretions (see text). Arrow in 1 and 4 points to γ_{1A}-globulin arc; arrow in 3 points to
an unidentified β-globulin arc.

Chromatography. Fractionation of nasal secretions
was performed on columns of DEAE cellulose [6] at 15° C.
Concentrated pools of nasal secretions were dialyzed
against 0.0175 M phosphate buffer at pH 6.3. The se-
cretions were then placed on a column previously equili-
brated with starting buffer until the effluent had a pH of
6.3. Columns were connected to a continuous flow ul-
traviolet absorption meter, and the optical density of the
eluates at 254 mμ was recorded directly. Elution with
0.0175 M phosphate buffer was continued until there was
no further ultraviolet absorption. Contents of tubes com-
prising the portion of the elution pattern within the peak
were combined, dialyzed against distilled water, and ly-
ophilized. The resulting powders were brought into sol-
ution with 0.15 M NaCl.

Results

Electrophoresis. A representative pattern of
the separation of the proteins of nasal secretions
by starch block electrophoresis is shown in Figure
3. When the pool of concentrated eluates from
the area corresponding to Fraction I of the starch
block was analyzed by immunoelectrophoresis with
an antiserum prepared against whole human se-
rum, a single precipitin line appeared that corre-
sponded to the area of electrophoretic mobility of
the γ_{1A}-globulins of normal serum run in parallel
on the same slide. No precipitin line was present
in the γ_2-globulin area of the Fraction I subjected
to electrophoresis. However, analysis of the frac-

tion in an Ouchterlony plate with an antiserum
prepared against 7 S γ_2-globulins resulted in a pre-
cipitin line that gave a reaction of complete identity
with serum 7 S γ_2-globulins [Figure 2 (2)]. The
anti-7 S γ_2-globulin antiserum used in these stud-
ies did not cross-react with γ_{1A}-globulins. A simi-
lar setup to that depicted in Figure 2 (2), but
with an unadsorbed anti-γ_{1A} antiserum, resulted in
a spur formation of the precipitin line formed by
C over that formed by B. When analyzed by im-
munoelectrophoresis with an antiserum to whole
human serum, Fractions II and III each gave a
single precipitin line corresponding in electro-
phoretic mobility to normal serum albumin.

Electrophoresis of the concentrated, pooled na-
sal secretions on cellulose acetate resulted in
bands corresponding in mobilities to the albumin
and β-globulins of normal human serum. In ad-
dition, three bands were present in the patterns of
the nasal secretions that did not appear in those
of serum. One occurred intermediate to the mo-
bilities of the albumin and α_1-globulins of serum;
one between those of β- and γ-globulins; and the
third cathodic to the serum γ-globulins. A precipi-
tin line did not result when this last slow-moving
component was reacted in agar with an antiserum
prepared against 7 S γ_2-globulins.

Immunoelectrophoresis. Nasal secretions were
obtained from 67 normal individuals. The mu-

[6] Selectacel 70 standard grade, Brown Co., Berlin, N. H.

FIG. 5. IMMUNOELECTROPHORESIS OF NORMAL NASAL SECRETIONS. A = normal human serum;
C = normal nasal secretions; F = goat anti-γ_{1A}-globulins; G = goat antihuman 7 S γ_2-globulins.

cosal smears from each of them showed a few squamous cells and a few polymorphonuclear leukocytes; no red cells were noted. Each sample was analyzed with antisera prepared against normal human serum, 7 S γ_2-globulins, and γ_{1A}-globulins. A typical pattern obtained when a sample of nasal secretions was reacted with an antihuman serum is shown in Figure 4 (2). The patterns of all 67 samples of nasal secretions showed precipitin lines that corresponded to the electrophoretic mobility of serum albumin and γ_{1A}-globulins. In most cases, these two precipitin lines were the only ones visible. In 7 of the 67 patterns a precipitin line appeared also in the β area, [Figure 4 (3)], and in 4 of the 67 a clear precipitin line appeared in the area corresponding to the 7 S γ_2-globulins of serum [Figure 4 (4)]. The majority of specimens obtained from females gave very faint lines on immunoelectrophoresis when compared with the patterns of specimens obtained from males. Figure 5 depicts the results of the reaction between samples of nasal secretions, antihuman 7 S γ_2-globulins, and antihuman γ_{1A}-globu-

lins. Those nasal secretions, which reacted with the anti-7 S γ_2-globulin antiserum to give a precipitin line corresponding in electrophoretic mobility to the γ_2-globulins of serum, also contained γ_{1A}-globulins [Figure 5 (1)]. Usually no precipitin arc was noted in the γ_2 region [Figure 5 (2)]. Figure 5 (3) shows the electrophoretic mobility of the γ_{1A}-globulins of serum and nasal secretions to be similar.

No precipitin arc resulted from the reaction of any sample of nasal secretions with an antiserum to β_{2M}-globulins.

Nasal secretions were collected from 20 persons with histories of nasal allergy and documented evidence of immediate skin reactivity to allergens. The immunoelectrophoretic patterns obtained with the nasal secretions of these individuals did not appear to differ from those of normal persons. In three instances, a precipitin line was present that appeared to correspond to the line in the β-globulin area of the normal nasal secretions [Figure 4 (3)]; in no instance was a precipitin line noted in the γ_2-globulin area. Even though

there appeared to be little difference in the immunoelectrophoretic patterns of the nasal secretions of allergic and normal individuals, there was a marked difference in their nasal smears. Mucosal cells, polymorphonuclear leukocytes, and eosinophiles appeared more frequently in the smears of the allergic patients.

Double diffusion in agar. Although the majority of samples of nasal secretions failed to reveal 7 S γ_2-globulins when analyzed by immunoelectrophoresis, all of them formed a precipitin line that gave a reaction of complete identity with serum 7 S γ_2-globulins when analyzed by the Ouchterlony method. Figure 2 (1) shows the results of the reaction between normal nasal secretions and purified γ_{1A}-globulins with an antiserum prepared against γ_{1A}-globulins. All samples gave a reaction of complete identity. In several patterns
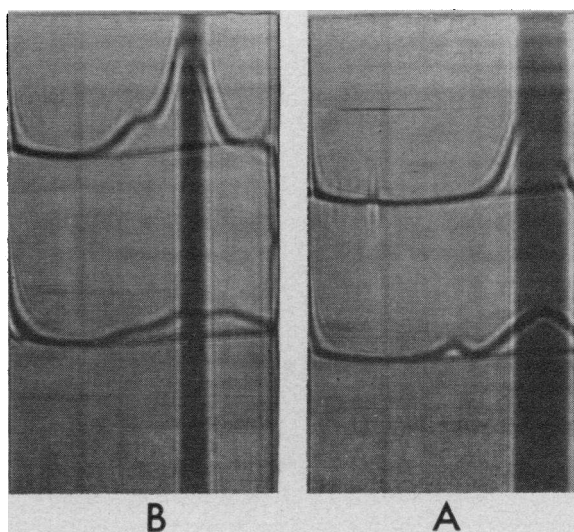

FIG. 6. ULTRACENTRIFUGE PATTERNS OF NORMAL HUMAN SERUM (above) AND POOLED NORMAL NASAL SECRETIONS (below). Sedimentation from right to left. Pictures were taken at 48 minutes (A) and 96 minutes (B).

TABLE I

Relative immune globulin quotients: nasal secretions to serum

Sample no.	γ_{1A}(N/S*)	Total γ_2(N/S)
1	9.8	0.4
2	6.5	1.2
3	2.0	0.5
4	4.0	0.5
5	5.2	0.4
6	6.1	0.9

* N = fraction of total nasal proteins; S = fraction of total serum proteins.

of the nasal secretions, a second precipitin line was noted between the major precipitin line and the antibody well. This second precipitin line was noted also with a γ-globulin preparation obtained by starch block electrophoresis of one of the pools of nasal secretions [Figure 2 (4)].

A reaction of complete identity occurred between the precipitin line formed by the γ-globulins of nasal secretions and the precipitin line formed nearest the well (7 S γ-globulin line) containing urinary γ-globulins, when these antigens were reacted against an antiserum to 7 S γ_2-globulins. Reactions of complete identity were also noted when an antiserum to human serum albumin was reacted in agar against serum, nasal secretions, and urinary albumin.

Ultracentrifugation. Ultracentrifugal analyses were performed on two pools of concentrated

nasal secretions. One pool represented five normal individuals and the other eighteen. Figure 6 shows representative Schlieren diagrams obtained with the pool from the eighteen persons. Patterns obtained with both pools showed four peaks with $S_{20,w}$ values of 12.3, 6.3, 4.3, and 2.4. Patterns obtained with the γ-globulins of nasal secretions that were separated by chromatography on DEAE cellulose resin revealed a major peak with an $S_{20,w}$ of 1.1 and a minor peak with an $S_{20,w}$ of 6.1. A similar Schlieren pattern was obtained with Fraction I from the starch block.

Quantitation of immune globulins. The concentrations of both 7 S γ_2-globulins and γ_{1A}-globulins in the proteins of nasal secretions from six normal individuals were compared with the total concentrations of these globulins in the proteins of corresponding sera obtained at the same time as the nasal secretions. The results are shown in Table I. Fractional γ_{1A}-globulin concentrations in nasal secretions ranged from 2 to 10 times their fractional concentration in the corresponding serum. In nasal secretions, γ_2-globulins were usually present in lower fractional concentrations than in the corresponding serum.

Antibody studies. 1) Tetanus. Nasal secretions collected from 13 persons who had received booster doses of tetanus toxoid were analyzed for

TABLE II

Tetanus hemagglutinins in sera and nasal secretions of three volunteers

Specific inhibitor	Serum no.			Nasal secretion no.			
	1	4	5	1	4	5	Pool*
Absent	655,360†	2,621,440	1,310,720	160	640	80	2,560
Present	20	N.D.‡	N.D.	10	40	10	<10

* Concentrate of 15 samples.
† Reciprocal hemagglutinin titer.
‡ Not done.

the presence of serum proteins by immunoelectrophoresis. The patterns did not differ from those of the normal volunteers. Seven samples of nasal secretions were pooled and reacted against tetanus toxoid in an Ouchterlony plate. The resulting single precipitin line gave a reaction of complete identity with that resulting from the reaction of the corresponding pool of serum with tetanus toxoid. The precipitating activity of another similar pool, separated by starch block electrophoresis, was found only in the γ-globulin fraction. The results of hemagglutination tests with the nasal secretions and the corresponding serum of three individuals are shown in Table II. Significant inhibition of hemagglutination resulted if serum or nasal secretions were incubated with antigen before addition of sensitized cells. Each of the remaining three specimens of nasal secretions and the corresponding sera were all found to have positive titers by hemagglutination.

2) Streptococcus. A comparison of type specific streptococcal hemagglutinating antibody in the serum and nasal secretions of a volunteer is shown in Table III. Specificity of the reaction was shown by the ability of the antigen to inhibit the reaction in both serum and nasal secretion. Antibodies were found in nasal secretions only when they were present in the corresponding serum.

TABLE III

Type specific streptococcal hemagglutinins in serum and nasal secretions of a volunteer

Test antigen	Inhibitor	Titer	
		Serum	Nasal secretion
Type 17	None	5,120*	2,560
Type 17	Type 17	<10	10
Type 12	None	<10	<10

* Reciprocal hemagglutinin titer.

3) Diphtheria. Control serum and nasal secretions were obtained from five adults. Two of these patients, who were shown to have levels of serum hemagglutinins to diphtheria toxoid in titers of > 1:100,000, had antibody in their nasal secretions. Of the remaining three subjects, two had serum titers of < 1:64, and one had no measurable titer at 1:4. All three had no antibody in their nasal secretions. After immunization, these three patients showed greatly increased titers of antibody in their serum, and antibody appeared in their nasal secretions. The antibody activity was found in the γ-globulin fraction obtained by starch block electrophoresis. Again, as with the other antigens, inhibition of hemagglutination could be accomplished by incubation of the serum or nasal secretions with the antigen before the addition of the sensitized red blood cells.

Passive transfer studies. Serum and nasal secretions were obtained from six individuals with known allergies and immediate skin hypersensitivity to grass pollen allergens. The nasal secretions were analyzed by immunoelectrophoresis, and the patterns of each showed precipitin lines only in the albumin and γ_{1A} areas. Similar specimens were collected from four persons without known allergy who did not react to an intradermal inoculation of grass pollen allergens. Recipients of passive transfer experiments met the same criteria as the latter group. Only serum and nasal secretions from allergic individuals caused a positive Prausnitz-Kustner reaction. However, one sample of nasal secretions from an allergic person failed to give a Prausnitz-Kustner reaction despite a 3 + reaction elicited with corresponding serum. Except for the degree of intensity (3 + to 4 + for serum and 1 + to 3 + for nasal secretions) reactions at the sites of the injected nasal secretions did not differ from sites prepared with serum. Heating serum or nasal secretions at 56° C for 2 hours destroyed their capacity to react in the Prausnitz-Kustner test.

Discussion

This study describes certain immunochemical and physicochemical properties of the γ-globulins present in normal human nasal secretions. These γ-globulins possess hemagglutinating, precipitating, and reagenic antibody activities.
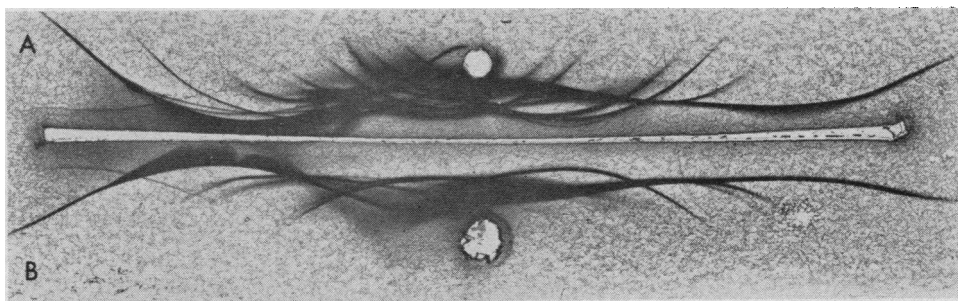
FIG. 7. IMMUNOELECTROPHORESIS OF NASAL SECRETIONS OBTAINED WITH COTTON SOAKED IN 10% NACL. A = normal human serum; B= nasal secretions; antiserum = horse antihuman serum.

To obtain nasal secretions as they occur normally, special precautions were taken in the collection of the samples to prevent damage to the mucous membrane that might cause a leak of serum proteins. Collections of nasal secretions with cotton or gauze, either dry (14, 17, 31) or soaked with 10% saline (32), were not used because of the possibility of local irritation. Preliminary results in our laboratory with immunoelectrophoretic analyses of nasal secretions collected with cotton soaked in 10% NaCl gave completely different results from those obtained from the same individuals using a 0.15 M NaCl wash as described in the present studies. The former method caused an outpouring of serum proteins (Figure 7) probably representing a response to local irritation and injury due to the foreign body or hypertonic solution, or both. Nasal secretions collected using such methods cannot be considered physiological, and the finding of antibodies in the secretions would not be surprising (32).

Analysis of normal nasal secretions by immunoelectrophoresis revealed γ_{1A}-globulins and albumin to be the two serum proteins most commonly present. In some instances, γ_2-globulins and an unidentified β-globulin were also present. With the Ouchterlony method, 7 S γ_2-globulins were shown to be present in all samples of nasal secretions despite the fact that the corresponding precipitin line was relatively uncommon on the immunoelectrophoretic patterns. The finding of a protein in nasal secretions more electropositive than serum γ_2-globulins on the cellulose acetate electrophoresis patterns was of interest in view of the previous observation of a protein of similar mobility in normal human urine (30). This may be lysozyme, which is known to migrate cathodic to serum

γ-globulin (33). Immunodiffusion patterns obtained with nasal secretions of allergic persons did not appear to differ significantly from those of normal individuals despite the fact that the former often contained many eosinophils on cytological examination.

The results of the quantitative analyses indicate that the γ-globulins in nasal secretions are primarily composed of the γ_{1A} component. Thus, a selective mechanism is probably involved in the delivery of this serum protein into the nasal secretions. Tomasi and Zigelbaum (34) recently reported finding relatively high concentrations of γ_{1A}-globulins in other body fluids when compared to the corresponding sera and have suggested that factors in addition to simple transudation from serum are in operation. The lack of other α- and β-globulins in nasal secretions, in amounts which can be discerned by immunoelectrophoresis, is difficult to explain. This absence cannot be explained simply by differences in molecular weight of the serum proteins, since a portion of the purified γ-globulins obtained from nasal secretions have an $S_{20,w}$ of at least 6.1. Anderson, Riff, and Jackson (18), in studies of nasal secretions collected during a common cold, noted that 10 to 20% of individuals tested before challenge with the virus had γ-globulins in addition to albumin in their nasal secretions. The authors stated that these specimens either contained hemoglobin or were taken from subjects who demonstrated polymorphonuclear leukocytes on smears taken at the time nasal washings were done. They concluded that γ-globulins are absent in normal nasal secretions. The protein concentration in the samples they analyzed was constant at 3 g per 100 ml, a value far in excess of the concentration of most of

the samples analyzed in the present study. The reason for the differences in results between the present study and those of Anderson and associates is not known.

Studies using double diffusion in agar gel failed to reveal a pattern suggestive of low molecular weight γ_2-globulins in nasal secretions. However, analytical ultracentrifugal studies of the purified γ-globulins of normal nasal secretions revealed a slow sedimenting component with an $S_{20,w}$ of 1.1 in addition to the 6.1 S component. These sedimentation coefficients did not vary significantly in samples prepared from starch block electrophoresis or chromatography on DEAE cellulose. Although the low molecular weight component represents the major fraction of the purified γ-globulins, it may be in too low concentration in the preparations to give a visible precipitin arc. In the case of urinary γ-globulins, the 7 S fraction, which constituted 10% or less of the total preparations, formed a much clearer precipitin line with the antibody than did the highly concentrated low molecular weight fraction (30). In some instances, the reaction of γ-globulins of total nasal secretions with an antiserum prepared against γ_{1A}-globulins resulted in 2 precipitin lines. Whether each precipitin line represents a species of molecule of different molecular weight or simply antigenic heterogeneity, or both, is not known.

The presence of antibacterial antibodies in normal nasal secretions suggests that the secretions may play an important role in host defense against bacteria. The occurrence of carriers of diphtheria bacilli and of streptococci may be influenced by the presence or absence of antibodies in secretions from the nasal and nasopharyngeal mucosae. The studies by MacLeod, Hodges, Heidelberger, and Bernhard (35) indirectly suggest such an active role of nasopharyngeal secretions as a protective mechanism against the carrier state of pneumococci. Immunization of humans with a variety of capsular polysaccharides of pneumococcus significantly lowered the carrier rate for those pneumococcal types as compared with controls.

The precipitating and hemagglutinating antibody activity was noted in the samples of whole nasal secretions as well as in the preparations of total γ-globulins. The small amounts of γ_2-globulins present in the secretions could be responsible for the

antibody activities. Whether or not the γ_{1A}-globulin fraction of the total γ-globulins contained antibody was not determined in this study. Of interest is the recent report of Heremans, Vaerman, and Vaerman (36) that described antibody activity to diphtheria in the γ_{1A}-globulin fraction of the serum of a subject vaccinated with diphtheria toxoid.

Reagenic antibodies have been shown recently to reside, at least in part, in the γ_{1A}-globulin fraction of the serum immune-globulins (37). Because of the limited amounts of material, the protein fraction of the nasal secretions that contain reagenic activity was not identified. The presence of local reagenic antibodies in the nasal secretions may be one of the host's first lines of defense against inhaled allergens. Clinical symptoms that follow such inhalation may be due to the release of histamine as a result of the reaction between allergen and reagin. That reagin-bearing serum is capable of sensitizing nasal mucous membranes was shown experimentally by Sherman, Kaplan, and Walzer (38), who elicited reactions at the sensitized sites (inferior turbinate and septum) by topical application of the allergen. The liberation of histamine into the blood plasma has been shown after incubation of whole blood of a clinically sensitive individual with ragweed antigen (39, 40). An analogous situation may take place in the nose. Mast cells in addition to goblet cells and eosinophiles are seen in significant numbers on cytological examination of nasal secretions obtained from allergic persons (41), whereas mast cells are infrequently seen in most nasal smears from persons with colds or chronic infections. When the increase in number of mast cells is marked, the allergic reaction is usually severe. The prominent role of the mast cell in the allergic nasal response may be to release histamine (42). Thus, the finding of relatively high concentrations of γ_{1A}-globulins in association with reagin activity in the nasal secretions may be more than of theoretical interest and may lead to a better understanding of the mechanisms of nasal allergy.

Summary

Serum proteins and antibody activities were found in nasal secretions of normal and allergic individuals. The serum proteins in highest con-

centrations were γ_{1A}-globulins and albumin. γ_2-Globulins were present in all specimens but in lower concentrations. Antibody activity to tetanus and diphtheria toxoids and streptococcal M protein was found in nasal secretions of normal volunteers whose sera showed high titers to the same antigens. Reagenic antibody activity was demonstrated in the nasal secretions of allergic individuals but not in those of nonallergic persons. The possible role of nasal secretions in host immunity and nasal allergy is discussed.

Acknowledgments

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