polio antibody could be detected in the IgM fraction in sera of the 17 adults studied by us. This is not surprising since none of these individuals had been recently immunized and poliovirus infections are at present rare in the New York metropolitan area, where all of our subjects live. The 7S antibodies were readily determined to be of both the IgG and IgA globulin classes.

The sensitivity of the method described here was equal to the one-hour neutralization test. A serum with a neutralization titer of only 1:3 was able to bind poliovirus to give a positive radioautograph. The only serum in the series which did not have neutralizing antibody did not show any binding on the radioautograph. A serum with a neutralizing titer of 1:12 gave by radioimmunodiffusion, on 2 separate trials, titers of 1:8 and 1:16.

Summary. (1) By techniques of radioimmunoelectrophoresis and radioimmunodiffusion, using radioactive poliovirus, it was shown that the quantity of poliovirus antibodies in a serum and the immunoglobulin class of these antibodies can be determined. (2) By these techniques it was shown that adult human serum contains A Immunoglobulin (IgA) poliovirus antibodies as well as G Immunoglobulin (IgG) polio antibodies. (3) Some sera contain a higher concentration of A Immunoglobulin (IgA) polio antibodies than G Immunoglobulin (IgG) polio antibodies.

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Protein Composition of Nasal Secretion During Respiratory Virus Infection. (30406)

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Study of nasal secretions during virus infection of the respiratory tract offers a unique opportunity to assess the local effects of virus replication and to observe the interaction of local and sytemic factors in the host response to infection. Previous investigation of the composition of nasal secretion has shown that it normally contains albumin, γ A globulin and smaller amounts of γ G globulin(1), and that during coryzal illness it may assume some of the protein characteristics of an inflammatory exudate(2). The present study was undertaken to extend the observations on the protein composition of nasal secretion by investigating the changes occurring in the nasal secretions of volunteers experimentally infected with the respiratory virus, Coxsackie A-21.

Materials and methods. Volunteers. Subjects were 18 adult males from federal correctional institutions. Details of the clinical and virological studies on these volunteers have been reported elsewhere(3) and are summarized below.

Eleven volunteers, free of detectable serum neutralizing antibody to Coxsackie A-21, and one volunteer with a low titer of serum antibody were infected either by aerosol in doses of 50-676 $TCID_{50}$* (10 volunteers), or by intranasal instillation of 32 $TCID_{50}$ in a 0.5 ml inoculum (2 volunteers). Virus shedding began 1-3 days after inoculation and continued throughout the period of observation; only 4 of 8 volunteers tested developed a 4-fold or greater rise in serum neutralizing antibody titer during this period (16-17 days), but all 12 volunteers had done so by 4 weeks. Eleven of the 12 infected volunteers developed a respiratory illness, in each case with rhinitis as a component. These illnesses began 3-4 days after inoculation and lasted 1-7 days.

Six volunteers were not infected. One of these was inoculated with an aerosol containing a low dose of Coxsackie A-21 (50 $TCID_{50}$). Three received an aerosol containing Coxsackie A-21 inactivated by means of specific hyperimmune guinea pig antiserum. The remaining 2 were inoculated orally with a rhinovirus contained in enteric-coated capsules which proved toxic for the virus. Absence of infection was demonstrated in all 6 by the failure to culture virus from multiple nasal, rectal, pharyngeal and/or nasopharyngeal wash specimens, and the failure to show a rise in serum antibody against the agent administered. One of the volunteers given antiserum-inactivated virus and one man given rhinovirus by capsule had an unexplained mild coryzal illness.

Collection of nasal secretions. The volunteer sat with his neck hyperextended while 5 cc sterile lactated Ringer's solution† was pipetted into each nostril. He then tilted his head forward and blew through his nose, catching the fluid and secretions in a sterile beaker. This material was homogenized in clean glass tissue grinders at 4°C and then centrifuged at 1400 *g* at 4°C for 2 hours. The clear supernatant and the sediment were stored separately at −60°C.

Test for occult blood. A portion of the centrifuged sediment was tested by the guaiac method.

Quantitation of total protein. Total protein concentration was measured by the Biuret method.

Immunoelectrophoreses (IE). IE was carried out on microslides using the LKB Co. model #6800A.‡ All runs were done at 5 V/cm for 2 hours at room temperature using 2% Agar Noble in pH 8.6 barbital buffer, ionic strength 0.09. The IE patterns were developed with anti-whole human serum (Goat IEP lot #3802 UI).§

Agar gel diffusion. This was performed according to the technique of Ouchterlony(4). The following mono-specific antisera were used, all produced by Hyland Labs., Inc.: goat anti-β_2M (γ-M) globulin, Lot #G37-98 P3-3163; goat anti-alpha-2 macroglobulin, Lot #G70Abs P7-1961; goat anti-transferrin (siderophilin), Lot #s G197 P4-1363 & G197 P12-63; goat anti-orosomucoid, Lot #G88, goat anti-β lipoprotein, Lot #G7-363; sheep anti-β_1 A/C globulin, Lot #Sp 6-63; goat anti-β_1 A/C globulin, Lot #G97-63; goat anti-ceruloplasmin, Lot #G154 P11-961 & Lot #GP 1263; goat anti-haptoglobin, Lot #G219 P-1963; goat anti-7S γ (γ G) globulin, Lot #GP 11-63 & Lot #GP 4-63; goat anti-β_2A (γ A) globulin Lots #G206 7Abs 8962, G-206 7Abs 3163, G-206 7P1063; goat anti-albumin, Lot #G155 P10-961. All preparations of anti-β_2A (γ A) antiserum gave a single precipitin line in IE against whole human serum and had no reactivity against purified γ G globulin in agar gel diffusion.

Identification of specific serum protein components. Eleven components could be independently confirmed both by IE and the monospecific antisera. One additional component was recognized frequently by IE alone. This nasal secretion component formed a continuous α-1 precipitin arc in IE with a protein present in the perchloric acid filtrate of normal human plasma, and failed to be absorbed from serum or nasal secretion with an excess of anti-orosomucoid antiserum. Thus, it was tentatively identified as an α-1 glycoprotein(5).

* 50% tissue culture infectious dose.

† Baxter Laboratories, Inc., Morton Grove, Ill.

‡ LKB Produkter A. B., Stockholm, Sweden.

§ Hyland Laboratories, Inc. Los Angeles, California.

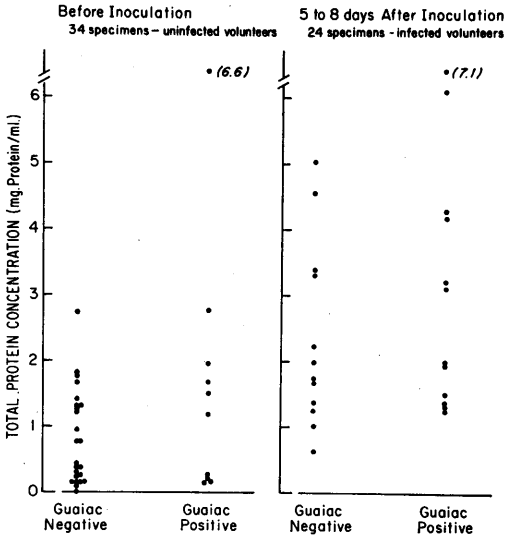

FIG. 1 (Left). Protein concentrations of "normal" nasal secretions from 18 volunteers free of upper respiratory infection. (Right). Protein concentrations of nasal secretions following infection during the period when maximum concentrations were observed (12 infected volunteers).

Quantitative determination of γ A globulin and γ G globulin. The single diffusion precipitin method in agar, described recently by Tomasi(6), was adapted to our requirements. Lot #s G206 7Abs 8962, GP 7964F goat anti-β_2A (γ A) antiserum or Lot #GP 11-63 goat anti-7S γ (γ G) antiserum‖ were used. Nasal secretions were concentrated up to 10-fold for these determinations by lyophilization. Serial dilutions of a normal serum with a known γ A and γ G content¶ were used as reference standards on each agar plate. The minimum detectable concentration of γ G globulin was 0.10 mg/ml (± 0.04 mg/ml = 1 SD), and γ A globulin was 0.08 mg/ml (± 0.05 mg/ml = 1 SD). All determinations were done in duplicate or triplicate.

Results. Preinoculation nasal secretions. Total protein concentration. Though none of the 34 preinoculation specimens contained grossly visible red coloration, sediments from 10 (29%) were guaiac positive. Protein concentrations in the guaiac negative group (Fig. 1) ranged from 0 to 2.7 mg/ml (mean = 0.82 ± 0.72 SD) and in the guaiac positive group from 0.2 to 6.6 mg/ml (mean 1.67 ± 1.86 SD). The difference in the mean protein concentrations of the 2 groups was not statistically significant (p>.10).**

Protein constituents. As shown in Fig 2, albumin and γ A globulin were identified in practically all of the 34 supernates from preinoculation specimens. Only 2 specimens had no detectable γ A globulin. One of these was the single specimen which lacked albumin and gave no Biuret reaction. The other possessed a faint trace of albumin but had a total protein of less than 0.1 mg/ml. Gamma G globulin was found in 11 of 24 specimens with guaiac negative sediments and 6 of 10 with guaiac positive sediments. The remaining protein components listed in Fig. 2 were found with decreasing frequency.

Four protein components, β_1 A/C globulin, α_2 macroglobulin, α_1 glycoprotein and slow (β) lipoprotein were identified significantly more frequently in supernates with guaiac positive sediments (in each case, p≤ .01).†† Since all 4 were found together in the 3 specimens with the highest total protein concentrations of the guaiac positive group, the detection of these 4 components was probably interdependent.

Changes in nasal secretion with nasal virus infection. Virus shedding, illness, total protein and occult blood. Fig. 3 relates the amount of virus in the nose (top panel), and the number of men ill (bottom panel), to the

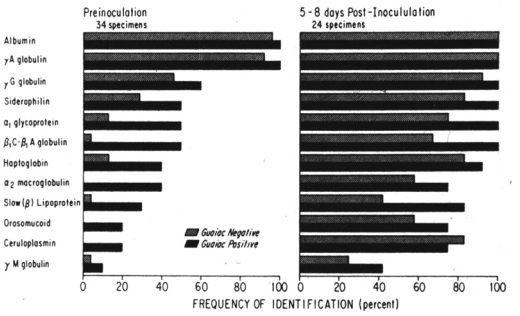

FIG. 2 (Left). Frequency with which 12 serum proteins were identified in nasal secretion by I.E. before upper respiratory infection or illness (18 volunteers). (Right). Maximum change in protein composition of nasal secretion by I.E. during viral rhinitis (12 infected volunteers).

‖ Hyland Laboratories, Inc.

¶ Kindly determined by Dr. Eugene McKelvey, Laboratory of Immunology, N.C.I., N.I.H.

** T test(7).

†† Fisher exact test(8).

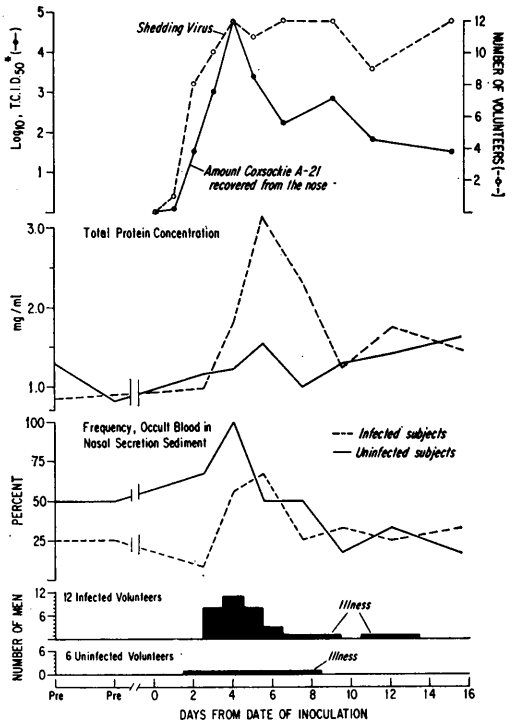

FIG. 3 (Top). Quantitative nasal virus shedding for 12 infected volunteers. *Titers expressed are mean titers per 2 ml nasal swab specimens, 8 men, or per 1 ml nasopharyngeal wash specimens, 4 men(3). (Middle). Mean protein concentration and frequency with which guaiac positive reactions were observed in nasal secretion from infected and uninfected subjects. (Bottom). Shaded blocks reflect number and duration of upper respiratory illnesses in both volunteer groups. Two of the 6 uninfected volunteers had common colds. Their illnesses occurred sequentially.

protein concentration and to the presence of detectable hemoglobin in the nasal secretion (middle panels). The mean virus concentration in the noses of infected volunteers reached its maximum on day 4 at the time when the greatest number of infected volunteers were ill. The greatest elevation in nasal secretion mean protein concentration was observed on the following day. This elevation in protein concentration persisted until the 8th day and was statistically significant when compared either to pre-inoculation values for the same men ($p<.01$),‡‡ or to concurrent determinations from uninfected volunteers ($p<.05$).§§ The period 5-8 days post-inoculation when protein concentrations were significantly elevated coincided temporally with the decline in virus titers and a decreasing frequency of illness. Relatively small amounts of virus were shed for the remainder of the study, and the protein concentrations of the nasal secretion were indistinguishable from control values after the 8th day.

There was no significant increase in the frequency of positive guaiac tests from the sediments of the nasal secretion in the infected volunteers ($p>.12$),‖‖ and the increase in positive guaiac tests during the first week after inoculation in both volunteer groups was not significant at the 5% level ($p = .07$).‖‖

Fig. 1 presents the total protein concentrations of all nasal secretion supernates collected from infected volunteers on days 5 through 8. There was no correlation ($p>.24$)¶¶ between the presence of occult blood in the sediment and the total protein concentration.

Identification of protein components—IE patterns. Photographs of the IE patterns of representative nasal secretions from 2 infected volunteers before inoculation and 5-8 days after inoculation are shown in Fig. 4. All 4 specimens were concentrated 10-fold before electrophoresis for good photographic reproduction. Volunteer No. 3 experienced a 7-day febrile illness, while Volunteer No. 10 had no discernible illness. However, the qualitative change in the IE patterns from these volunteers was characteristically similar.

Fig. 2 summarizes the protein composition of nasal secretion in 24 specimens collected 2 from each of the 12 infected volunteers 5 to 8 days after inoculation. These specimens were equally divided in terms of the guaiac reaction of their sediments, and there was no significant association between the presence of occult blood and the frequency with which any of the 12 protein components was identified ($p>.10$).††

Seven protein components were detected with significantly greater frequency after inoculation ($p\leq.02$).‖‖ These were α-1 glyco-

‡‡ Signed rank test(7).
§§ Rank sum T′ test(7).
‖‖ Sign test(7).
¶¶ Rank sum T′ test(7).

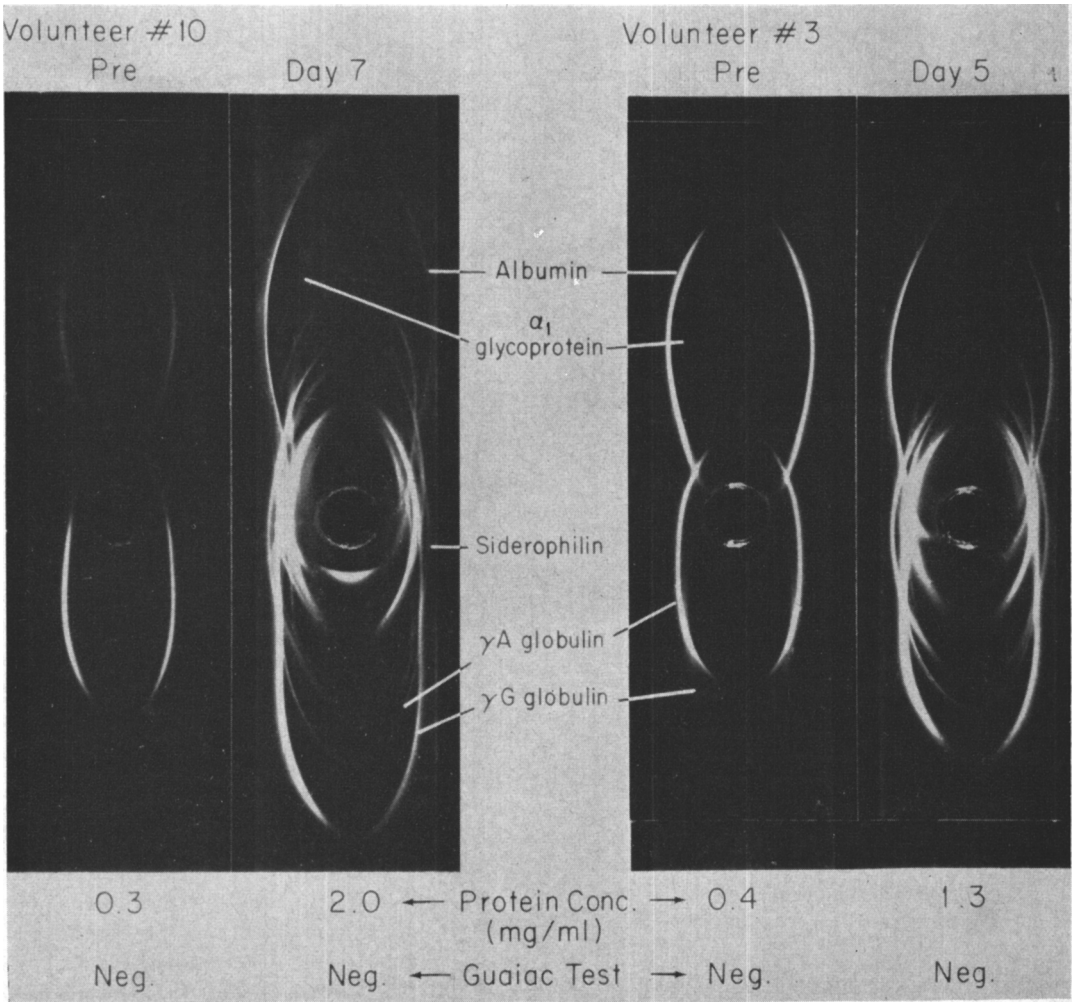

FIG. 4. Immunoelectrophoresis of 10-fold concentrated nasal secretion from 2 infected volunteers before, and 7 and 5 days, respectively, after virus inoculation. These patterns were developed with a 1:2 dilution of anti-whole human serum in the left trough and a 1:3 dilution of the same antiserum in the right trough.

protein, B_1A/C globulin, haptoglobin, α_2 macroglobulin, slow (β) lipoprotein, orosomucoid, and ceruloplasmin. Gamma M globulin was found relatively infrequently at all times, while albumin, γ A globulin, γ G globulin, and siderophilin were found with such high frequency before inoculation that a significant increase subsequently was not detected. There was no significant change in the frequency with which any protein component was identified in specimens from uninfected volunteers ($p > .22$) throughout the study.

Quantitation, γ A and γ G globulin. Table I compares the nasal secretion concentrations of γ A and γ G globulin with the total protein concentration before inoculation and at intervals in the post-inoculation period. The values for γ A and γ G globulin in Table I have been corrected for the concentration to which specimens were subjected prior to determination of γ A and γ G content. Nasal secretion concentrations of γ A globulin were almost uniformly higher than γ G globulin.

In addition, comparison of paired pre- and post-inoculation specimens from the same volunteers suggested that the changes in γ A and γ G globulin concentration closely paral-

TABLE I. γA and γG Globulin Concentration in Nasal Secretion.

Vol No.*	Before inoculation				4-8 Days after inoculation				13-17 Days after inoculation			
	Total protein, mg/ml	Conc γA, mg/ml	% γA in total protein	% γG in total protein	Total protein, mg/ml	Conc γA, mg/ml	% γA in total protein	Conc γG, mg/ml	% γG in total protein	Total protein, mg/ml	Conc γA, mg/ml	% γA in total protein	Conc γG, mg/ml	% γG in total protein
1	1.76	.24	14	2	1.95	.08	4	.05	3	.24	Tr	<1	0	0
2	1.97	.16	8	5	1.36	.15	11	.08	6	2.34	.24	10	.13	6
3	2.77	.37	13	6	3.16	.20	6	.15	5	.86	.05	6	Tr	<1
4	.16	Tr†	<1	0	3.13	.13	4	.09	3	1.79	.10	6	.05	3
5	.20	.05	25	0	7.06	.18	3	.17	2	4.44	.20	5	.07	2
6	1.33	.23	17	<1	1.98	.33	17	.05	3	.73	.06	8	0	0
7	1.84	.22	12	3	2.22	.24	11	.07	3	1.88	.19	10	.05	3
8	1.35	.01	<1	<1	1.51	.12	8	.07	5	.67	.06	9	.04	6
9	.31	.01	3	<1	1.25	.07	6	.06	5	.94	.05	5	.04	4
10	.25	.01	4	4	5.03	.25	5	.18	4	2.06	.17	8	.13	6
11	.39	.01	3	<1	4.19	.19	5	.36	9	1.49	.09	6	.03	2
12	.24	.01	4	<1	1.99	.14	7	.11	6	2.81	.22	8	.26	9

* All 12 subjects infected with Coxsackie A-21. † Concentration too low to quantitate. ‡ None detectable.

leled one another ($p<.02$),*** and followed the changes in total protein concentration ($p\leqq.05$).*** Thus, although there was a sizable non-specific increase in the number and concentration of serum protein components in nasal secretion following infection, the proportion of γ A to γ G globulin and the proportion of both to the total protein concentration did not significantly change.†††

Discussion. The demonstration that γ A globulin remains the predominant immunoglobulin in nasal secretion during nasal virus infection suggests that the mechanisms responsible for the presence of plasma proteins in these secretions may be more complex than has previously been appreciated. In a previous study of nasal secretion during upper respiratory infection, Anderson *et al*(2) suggested that normal nasal secretion contains a transudate of plasma and that during infection an inflammatory response produces a progressive break in capillary permeability resulting in exudation of the larger plasma proteins. The observation by Remington *et al*(1) that nasal secretion contains a higher concentration of γ A globulin than plasma in proportion to γ G globulin or total protein content provided a means of testing this hypothesis. In the current study, Table I shows that a high concentration of γ A globulin was maintained in nasal secretions despite an average 3-fold increase in total protein during infection. These data suggest that the mechanisms responsible for the selective accumulation of γ A globulin in nasal secretions showed a continuous and possibly accelerated activity during virus infection. A large, unselective outpouring of plasma protein into the nasal secretion could not alone account for these findings since it would have produced concentration ratios of γ A to γ G globulin more similar to those in plasma.

*** Rank correlation test(7).

††† Subsequent work has shown that lyophilization causes a systematic reduction in the concentration of γA and γG globulin detectable by immunodiffusion assay. Thus, the actual values for the data shown in Table I may be substantially greater than reported, but the proportional relationship between γA and γG globulin concentrations remains as demonstrated.

Because of the association of antibody activity with γ A globulin both in serum(9,10, 11) and in nasal secretions(12), secretions collected serially for 16-17 days following inoculation from 8 of the volunteers were assayed for neutralizing activity against Coxsackie A-21. Antibody titers in nasal secretion as in the serum of these volunteers (see *Materials and methods*) were negligible during the period of observation. The absence of specific neutralizing activity in almost all these secretions for over 2 weeks following infection suggests that local antibody synthesis was not responsible for the increase in γ A globulin content of nasal secretions seen during the immediate post-inoculation period. If the γ A globulin appearing in nasal secretion came from the plasma pool, the preferential excretion of this protein may be a factor in prevention or attenuation of infection in individuals who, unlike the present volunteers, already possess sufficient concentrations of circulating antibody at the time of challenge.

Tomasi *et al*(13) have accounted for the high concentration of γ A globulin in parotid secretions by demonstrating that this gland is involved either in *de novo* synthesis or modification of the plasma protein for transport into the parotid secretion. Possibly, glandular elements in the nasal membranes have a similar capacity. Whether the other proteins identified in the nasal secretion(14) are carried along by the same mechanisms which effect the preferential excretion of γ A globulin is not presently evident. However, the infrequent identification of plasma proteins other than albumin and γ A globulin suggests that a more random process, possibly passive transudation, may be responsible for the presence of the less commonly identified proteins in "normal" nasal secretions.

During infection, the increased frequency with which a number of plasma proteins were identified and the simultaneous rise in the total protein concentration suggests that there was an increase in plasma protein content of the nasal secretion at a time when virus titers and the indices of illness were declining. The data do not indicate whether altered vascular permeability or, possibly, secretory activity on the part of the nasal membranes was principally responsible for these findings. However, the changes in protein composition were probably not secondary to overt capillary rupture since they occurred independent of the presence of guaiac positive material in the nasal wash sediments and were characterized by persistently high concentrations of γ A globulin. For the same reasons, inflammatory exudation was probably not exclusively responsible for these changes, even though the IE patterns of the nasal secretion during infection resembled those observed with inflammatory exudates. The mechanism by which immunoglobulins gain access to nasal secretion and the contribution of their activity and the activity of other humoral elements in nasal secretion to the host response to respiratory illness are being investigated.

Summary. During the latter portion of upper respiratory illness induced with Coxsackie A-21 virus, there was a rise in total protein concentration and an increase in the frequency with which several plasma proteins were identified in nasal secretions. These changes in protein were independent of the presence of guaiac positive material in the nasal secretion specimens. Concentrations of γ A globulin before and during infection were higher in nasal secretion, in relation to the total protein, than are found in plasma, but antibody titers against Coxsackie A-21 in these secretions were negligible for up to 17 days following inoculation. The enhanced excretion of γ A globulin in nasal secretion during infection, therefore, probably did not contribute significantly to the clinical recovery of these initially antibody-free volunteers.

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Inhibition of Arboviruses by a Constituent of a Staphylococcus.* (30407)

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In the course of studies on the phagocytosis of inactivated staphylococci by primary monolayer cultures of human amnion cells (1) it was found that cells exposed to a suspension of these bacteria were resistant to Sindbis virus. Investigation of this phenomenon revealed that a constituent of the bacterium conferred protection by inhibiting viral multiplication. Experiments reported here attempted to explore the mode of action of this inhibitor and to define some of its physical and chemical properties.

Methods and materials. At the outset it was found that a constituent of several different strains of staphylococci inhibited multiplication of Sindbis virus. The bacterium employed in the experiments outlined below was recovered by Mrs. Dixie Kaslick (Dept. of Clinical Laboratories, Children's Hospital Medical Center) as a rare organism from the urine of a patient with cystic fibrosis. The organism was classified as a staphylococcus according to the criteria of Wilson and Miles (2). The various fermentation reactions and its growth anaerobically were compatible with this classification. It possessed characteristics of both a *Staphylococcus albus* and a *Staphylococcus aureus*. It was coagulase negative; it did not liquify gelatin; and it fermented mannitol only after 5 days. On blood agar plates or agar slants it produced a pale golden yellow pigment.

Preparation of the inhibitory factor. Bacteria were propagated in 2 liters of Difco beef heart infusion broth in a gyrotory shaker water bath at 37°C for 60-72 hours. At this time there were approximately 5×10^8 viable organisms per ml. Although slight viral inhibitory activity was demonstrated in the supernatant after centrifugation (3000 rpm for 30 minutes) of the culture fluid, only the inhibitory factor found to be associated with the bacteria was systematically investigated.‡ Accordingly, the bacterial sediment was suspended in phosphate buffer and centrifuged at 3000 rpm for 15 minutes. One-half ml of concentrated bacteria was resuspended in 4.5 ml of distilled water; 5 ml of #12 Ballotini glass beads were added, and the bacteria were disrupted at 4°C in a Mickle disintegrator.

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† Career Development Award, Nat. Inst. of Allergy & Infect. Dis., NIH, USPHS, Bethesda, Md.

‡ The total amount of inhibitor present in the 2 liters of supernatant exceeded that present in the 2.5 ml of sedimented bacteria. It was more practical, however, to obtain inhibitor of high titer from extraction of the smaller volume of bacteria than from concentration of the larger volume of supernatant.