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## SEROLOGIC ACTIVITY OF ANTIGEN ISOLATED FROM THE BODY FLUID OF *ASCARIS SUUM*<sup>1</sup>

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An antigen prepared from the body fluid of *Ascaris suum* by fractionation on Sephadex G-200 and purification by cellulose acetate electrophoresis was evaluated for the diagnosis of visceral larva migrans (VLM) in adults and children. The antigen was highly reactive with sera (95%) from children with VLM. Among the sera of adults from an area endemic for ascariasis, 65% were positive. It is suggested that positive reactions in these cases are due to an antigen present in migratory phases, which is also present in the body fluid of *Ascaris*. The high incidence of reactions of the antigen with *Ascaris* antibody may serve as a useful serologic adjunct in the diagnosis of visceral larva migrans in suspected cases among children.

Improvement of the serologic diagnosis of infection with *Ascaris lumbricoides* and in the specific diagnosis of visceral larva migrans (VLM) due to infection with *Toxocara canis* is at present dependent on the finding and evaluation of new antigens. Most of the antigens used today are complex substances of low specificity. For the diagnosis of VLM, acid-soluble protein fractions prepared by the method of Melcher (1) from *T. canis*, *A. lumbricoides* and *Ascaris suum* are employed, as well as saline extracts of *Ascaris* whole worms or tissues. Hemagglutination, bentonite flocculation, fluorescent antibody, and *in vitro* precipitate formation by living larvae have been the main techniques employed in evaluating these antigens (2-5). The incrimination of various larval species of other parasites as causative agents for the VLM syndrome has made the problem of serologic diagnosis more complicated.

The purpose of this study was to evaluate the serologic activity of a substance isolated from the body fluid of *Ascaris suum*. If such an antigen were selectively more reactive in an individual with migrating *Ascaris* larvae or with larvae encysted in his tissues, the antigen might be useful in confirming a diagnosis of systemic

VLM infection due to larvae of *T. canis* and/or the larvae of other parasitic helminths. Toward this end, sera from children with the VLM syndrome, sera from adults and children infected with *A. lumbricoides*, sera from individuals infected with other parasitic diseases and sera from non-infected controls were evaluated with this antigen.

### MATERIALS AND METHODS

The body fluid was collected from fresh *Ascaris suum* either with a 1-ml syringe and 27-gauge needle, or by severing the posterior tip of the worm. The fluid was kept at 6°C until used. Fractionation of the fluid through columns of Sephadex<sup>2</sup> G-200 was accomplished as previously described (6). Cellulose acetate electrophoresis of the first peak filtrate revealed two substances soluble in saline which when used together are designated as the unpurified fraction. Figure 1 shows the two substances X and Y as compared with fractions observed in the whole fluid and with fractions from normal human serum. Dialysis or dilution in demineralized water, pH 5.5, effectively precipitated one of the fractions (X) which is the one migrating in the region of  $\gamma$

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<sup>2</sup> Use of trade names is for identification only, and does not constitute endorsement by the Public Health Service, or the United States Department of Health, Education and Welfare.

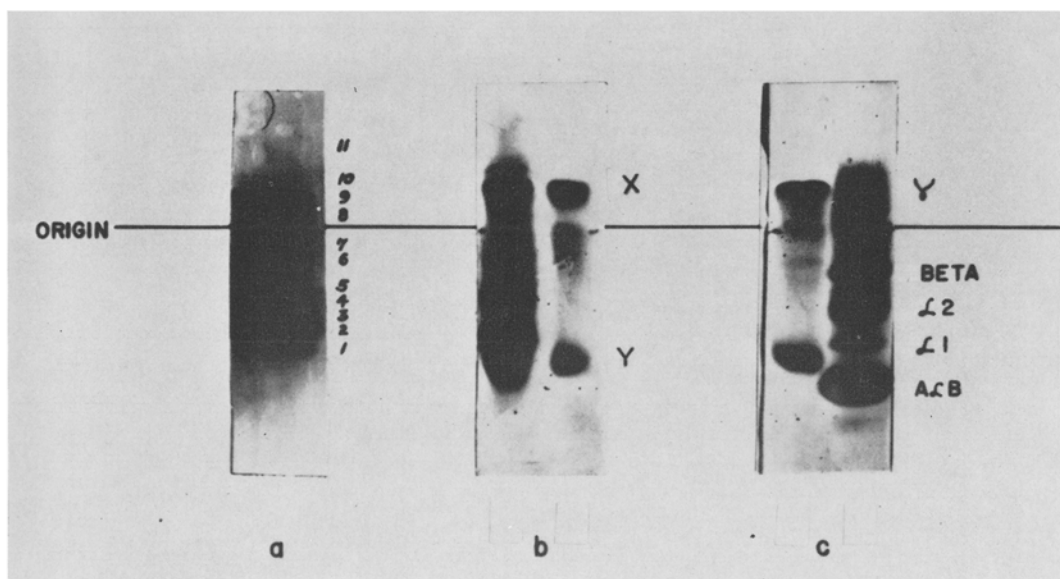


Figure 1. Cellulose acetate electrophoresis of unpurified fraction (b) showing substances X and Y corresponding to 9 and 10 of the whole body fluid (a) and with mobilities similar to  $\gamma$  globulin and albumin of normal human serum (c).

globulin. The precipitate was washed once with acidified water and dissolved in 0.85% saline. Further purification of this fraction (the antigen employed in this study) was accomplished by elution following cellulose acetate electrophoresis for 2 hr at a current of 1.5 ma/strip in Veronal (Winthrop, New York, N. Y.) buffer, pH 8.6, and ionic strength 0.75. A guide strip was stained for protein using Ponceau S and the areas in unstained strips corresponding to the protein staining areas in the guide strip were cut out and eluted in saline. The eluate was dialyzed for 48 hr against demineralized water and the entire dialysate was distributed in 1-ml vials and was lyophilized. Figure 2 shows the purified fraction after disc electrophoresis.

Sera were evaluated by the indirect hemagglutination technique of Boyden (7). Human erythrocytes of group O-RH+ were washed three times in 0.85 saline, treated with 1:20,000 tannic acid and then exposed to dilutions of purified fraction antigen. Titration of antigen was performed daily using sera prepared in rabbits against the unpurified fraction. Two rabbits were injected during a 3-week period; each animal received a total of 100 mg of material and was bled on the 10th to the 15th day after the last injection. Sera were titrated in twofold dilutions

beginning at a dilution of 1:10, and the tests were read after 2 hr at room temperature and at 4°C overnight. The highest dilution of antigen used was that which gave marked positive reactions against positive control rabbit and human sera. Positive control sera yielded clear end points, and no reactions with negative rabbit and human sera were observed. The agar gel double diffusion technique was also used to test the rabbit sera for precipitins.

Sera from the following groups of individuals all natives of Puerto Rico, were evaluated:

1. Twenty-one children from 3 to 5 years of age with VLM syndrome, diagnosed on the basis of history, clinical signs and symptoms, and eosinophilic counts above 25%.<sup>3</sup> These children came from areas where *Ascaris* infections are endemic. At the time when blood was taken, only 13 children had stools positive for *Ascaris* eggs. The stools from another five became positive from 2 to 4 weeks later. Two saline smears were examined from each stool sample, using about 2 mg of material/smear.

2. Thirty-five healthy children from 2.5 to 5

<sup>3</sup> The authors are grateful to Dr. Margarita Cáceres de Costa, Department of Pediatrics, School of Medicine, for supplying the sera.

years of age, who were enrolled in a Head Start Program in San Juan. One stool specimen was examined from each of these children; only one was positive for *Trichuris* eggs and the remainder were negative for all parasites.

3. Twenty-three adults infected with *Schistosoma mansoni*. Three of these individuals had stools positive for eggs of *Trichocephalus trichiurus*. The remainder of the stool specimens were negative for all parasites except for eggs of *S. mansoni*.

4. Adults with diseases such as tuberculosis, leprosy, lupus erythematosus, rheumatoid arthritis and various types of cancer. In these individuals there was no way of determining the presence or absence of eggs in stools.

5. One hundred thirty-three hospitalized adult males and females coming from an area where *Ascaris*, hookworm and *Trichuris* are highly endemic. In another study, the infection rate of

508 individuals from the area was 69%, 44% and 95% for each parasite, respectively. Upon hospitalization for parasitic therapy, the 133 individuals in this study did not complain about symptoms specifically related to any disease condition, and none gave a history consistent with visceral larva migrans. Eosinophilia ranged from 4% to 15%.

## RESULTS

The antigen isolated from the body fluid of *Ascaris suum* was very soluble in saline, had an electrophoretic mobility equivalent to that of serum  $\gamma$  globulin, was stained with Ponceau S for protein and was PAS-positive. Protein content, determined by the biuret method, varied from 1.75 to 19.25  $\mu\text{g}/\text{ml}$  of solution. The antigen dilutions used to coat the human and sheep erythrocytes contained 10 to 12  $\mu\text{g}/\text{ml}$ .

*Hemagglutinin titers.* Titers of sera from rabbits artificially immunized with the unpurified fraction, as tested against human group O erythrocytes coated with the antigen, were high, varying from 1:81,920 to 1:327,680. Titers against human sera varied from 1:10 to 1:10,240.

The sera from 20 of the 21 (95%) Puerto Rican children with VLM syndrome gave positive hemagglutination reactions. Titers varied from 1:160 to 1:5,120 in children with and without *Ascaris* eggs in their stools (Tables I and II). The tests with this group of sera were repeated five times on different occasions, and the results were essentially the same.

In the group of 133 heavily parasitized adults undergoing parasite chemotherapy, 65% were serologically reactive for hemagglutinating antibody. Within this group, 100 had ascariasis eggs in their stools, and 70 of these gave hemagglutination titers ranging from 1:320 to 10:240. The remaining 30 were negative reactors. The sera from 17 of the remaining 33 individuals with no eggs in the stools gave hemagglutinin tests with titers ranging from 1:320 to 1:5120.

The serum reactivity observed in the sera from 35 healthy Puerto Rican school children was negative except in one case with a titer of 1:40; for 23 adults infected with *Schistosoma mansoni* three were reactive with titers ranging from 1:20 to 1:160; and from 115 individuals with other diseases, five were reactive with titers ranging from 1:10 to 1:80 (Table II).

Only one band of precipitate was observed when

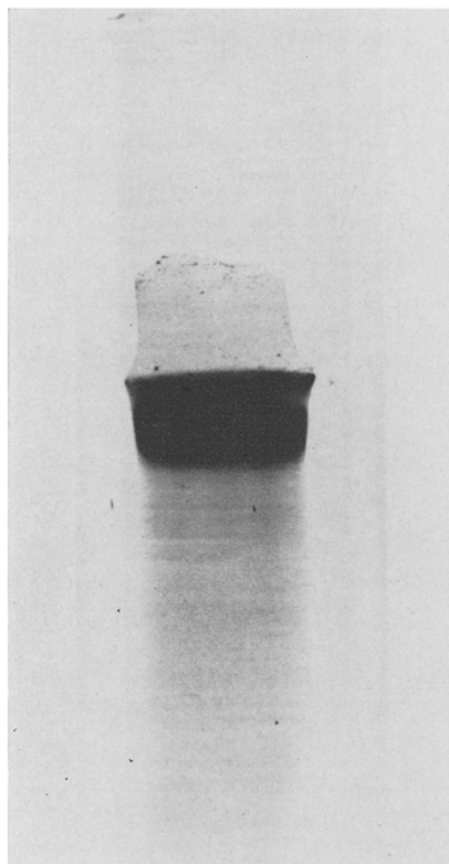


Figure 2. Purified fraction used as antigen after disc electrophoresis.

TABLE I

*Hemagglutination reactions of sera from adults and children with positive or negative stools for Ascaris lumbricoides, against antigen from body fluid of Ascaris suum*

| Group Description                | Hemagglutination of Stools Positive |             |              | Hemagglutination of Stools Negative |             |              |
|----------------------------------|-------------------------------------|-------------|--------------|-------------------------------------|-------------|--------------|
|                                  | Positive (%)                        | Titer range | Negative (%) | Positive (%)                        | Titer range | Negative (%) |
| Adults from Ascaris endemic area | 70 (70.0)                           | 320         | 30 (30.0)    | 17 (51.5)                           | 320         | 16 (48.5)    |
|                                  |                                     | 10,240      |              |                                     | 5,120       |              |
|                                  |                                     | 160         |              |                                     | 320         |              |
| Children with VLM                | 13 (100)                            | 5,120       | 0 (0)        | 7 (87.5)                            | 5,120       | 1 (12.5)     |
|                                  |                                     |             |              |                                     |             |              |

TABLE II

*Hemagglutination reactions of sera from children and adults with various disease conditions against antigen from body fluid of Ascaris suum*

| Disease Condition      | No. Of Cases |           | No. Positive (%) | Titer Range |
|------------------------|--------------|-----------|------------------|-------------|
|                        | Children     | Adults    |                  |             |
| Visceral larva migrans | 21           | 20 (95.2) |                  | 160         |
|                        |              |           |                  | 5120        |
| None                   | 35           | 1 (2.8)   |                  | 40          |
|                        |              |           |                  | 20          |
| Schistosomiasis        | 23           | 3 (13.1)  |                  | 160         |
|                        |              |           |                  | 0           |
| Tuberculosis           | 14           | 0         |                  | 10          |
|                        |              |           |                  | 80          |
| Leprosy                | 9            | 1 (11.1)  |                  | 0           |
|                        |              |           |                  | 20          |
| Lupus erythematosus    | 15           | 0         |                  | 40          |
|                        |              |           |                  | 20          |
| Cancer                 | 48           | 2 (4.1)   |                  | 80          |
|                        |              |           |                  |             |
| Rheumatoid arthritis   | 29           | 2 (6.9)   |                  |             |
|                        |              |           |                  |             |

the antigen was tested against the sera from rabbits artificially immunized with the unpurified fraction.

DISCUSSION

The antigen from the body fluid of *Ascaris suum* used to coat human group O erythrocytes is very reactive against sera from children with clinical VLM. The battery of VLM sera tested was selected from cases that were carefully studied and the serologic reactivity in this group was 95%.

The antigen was also reactive against sera from adults and children coming from areas highly endemic for *Ascaris*. Thus 87 of 133 adults (65%) reacted. If presence or absence of *Ascaris* eggs in

stools is to be considered, 70 sera of 100 individuals with positive stools gave serologic reactions, and 17 of 33 individuals with negative stools were reactive.

Serum reactivity observed in adults and children with and without *Ascaris* eggs in the stools suggests that the antigen involved is not related to the presence of adult worms in intestine, but probably to antigens in migrating larvae. Since the area where these individuals reside is highly endemic for *Ascaris*, one can speculate that in a high percentage of individuals larvae may be migrating through the tissues, either as part of their normal cycle or as aberrant forms. This also postulates that in individuals with long standing adult *Ascaris* in the intestine, antibodies related to migrating stages diminish in titer and are not detectable. This problem is being elucidated in experimental infections of hogs and guinea pigs, in the first of which the larvae mature to adults, whereas in the other the larvae do not develop completely.

Larva migrans in children is due to aberrant migration of larval stages, chiefly of *T. canis*, but the larvae of *Ascaris lumbricoides* and other parasites have also been incriminated. Substances similar to the antigen from *Ascaris* body fluid have been obtained in the senior author's laboratory from larvae of *Toxocara canis* isolated from mouse lung, from adults and cercariae of *Schistosoma mansoni*, from larvae of *Trichinella spiralis*, from 3rd and 4th stage larvae of *Ascaris suum* isolated from guinea pig liver and from adult *Dirofilaria immitis* (Conde del Pino, E. and Oliver-Gonzalez, J., unpublished). This finding, combined with the results presented above, suggests that antigens which may be responsible for antibodies present in the sera from cases of

visceral larva migrans are common to human, pig and dog *Ascaris* larvae, and possibly to larvae from other helminthic species. This in accord with Jung and Pacheco (4), who suggested that the host reaction in VLM is against an acute larval substance and is not specific for the etiologic agent.

The antigen from the body fluid exhibited a high degree of specificity for the serologic diagnosis of visceral larva migrans. The majority of sera from noninfected children and from adults with other disease conditions yielded negative results. In those in which a positive reaction was observed, the titer was below 1:160. In these individuals, however, it is possible that tissue migrating forms could have been involved. Investigations should be continued, using different protein concentrations of antigen to rule out low titered reactions, and, whenever possible, fecal examinations should be done to determine whether positive reactions actually represent parasitic infections.

Another question to be considered is whether

the antigen isolated from the body fluid reacts only with migrating stages of *Ascaris lumbricoides*, *Toxocara canis*, and other helminthic larvae, and that upon maturation of infection, these antibodies become undetectable. If such were the case, this antigen would be of great value for the diagnosis of early larval infections. This is in addition to the fact that it is a fractionated antigen of high purity and can be readily prepared.

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## ANTIBODY IN NASAL SECRETIONS AFTER INTRAMUSCULAR INJECTION OF INFLUENZA VIRUS VACCINE IN PERSONS WITH CHRONIC BRONCHOPULMONARY DISEASE<sup>1</sup>

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Intramuscular injection of influenza virus vaccine was an inefficient means of inducing nasal antibody even when the resulting serum titers were quite high. Persons with chronic bronchopulmonary disease and individuals of comparable age with normal bronchopulmonary status reacted similarly to the vaccine in terms of serum and nasal antibody production. An inverse relationship was observed between prevaccination serum antibody titers and the rise in antibody titer after vaccination. Nasal antibody titers were statistically correlated with nasal IgA levels and not with serum antibody titers. These results have been discussed in relation to the site of synthesis of antibody in the respiratory secretions. It was concluded that the combination of preexisting serum antibody with injected antigen could result in ineffectual antigenic stimulation of secretory antibody-producing cells in the respiratory tract.

Specific influenza virus neutralizing antibody in nasal secretions was demonstrated initially in 1940 (1). Interest has been renewed in such antibody in secretions of the respiratory tract because of the demonstration that it is principally immunoglobulin A (IgA) (2, 3) and has a protective effect against infection with influenza virus (4).

The danger of influenza to persons with chronic bronchopulmonary diseases has led to routine parenteral administration of influenza virus vaccine to such individuals. However, probably because secretory antibody is formed locally (5, 6), parenteral administration of the vaccine produces less antibody in nasal secretions than does vaccine introduced by the respiratory route (7, 8). Since influenza virus vaccine is of particular importance in persons with chronic bronchopulmonary disease, a study was conducted to determine the effects of parenteral immunization with the vaccine on the titers of antibody in nasal secretions in this clinical situation.

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<sup>2</sup> Veterans Administration Clinical Investigator.

### METHODS

Eleven hospitalized male patients (mean age 51) with emphysema, chronic bronchitis or bronchiectasis were investigated; 10 chronically ill hospitalized patients with no bronchopulmonary disease or apparent immunologic defect served as controls. A single 1.0-ml intramuscular dose of commercially prepared vaccine containing the A2/Japan/172/62, A2/Taiwan/1/64, and B/Massachusetts/3/66 strains of virus<sup>3</sup> was injected. The vaccine had been inactivated by irradiation with ultraviolet light and exposure to formalin. Serum antibody was evaluated by the hemadsorption-inhibition method (9) in rhesus monkey kidney tissue culture tubes<sup>4</sup> using 32 to 320 tissue culture infectious doses (TCID<sub>50</sub>) of A2/Taiwan/1/64 influenza virus.<sup>5</sup> Nasal washes were obtained by irrigating the nasal passages with 10 ml of buffered saline, and immunoglobulin determinations were performed with this material using a radial diffusion precipitin method as previously described (10). Both nasal wash and serum neutralizing antibody titers against

<sup>3</sup> Influenza virus vaccine, bivalent, lot 976671A, Parke, Davis & Co., Detroit, Michigan.

<sup>4</sup> Flow Laboratories, Rockville, Maryland.

<sup>5</sup> Obtained from Dr. Walter R. Dowdle, Chief, Respiratory Virus Infection Unit, National Communicable Disease Center, Atlanta, Georgia.

the influenza virus strains were determined by the same methods. Endpoints in both instances were determined by complete viral inhibition.

# RESULTS

The results of testing serum and nasal washes for neutralizing antibody and the levels of immunoglobulin in nasal washes are recorded in Table I. All except two subjects responded with fourfold or greater increases in serum antibody and a majority showed titers of 1:512 or greater after 2 weeks. The geometric mean increase in antibody titer among the patients with chronic respiratory disease was 11.1 and in the control

patients was 32.0. This difference occurred because subjects with the lowest initial serum antibody titers in both groups tended to respond with the greatest increase in titer, as shown in Figure 1, and patients with chronic bronchopulmonary disease had higher initial mean titers, probably as a result of frequent antecedent administration of vaccine.

Detectable antibody was demonstrated in the nasal washes of nine of 21 subjects in both groups with maximum titers of 1:8. There was no difference in response between the respiratory disease and control groups.

In several instances, high serum antibody

TABLE I  
*Antibody resulting from intramuscular injection of influenza virus vaccine*

| Subject              | Age             | Titer <sup>a</sup> |       |            |      |      | Nasal Wash Immuno-<br>globulins <sup>b</sup> |                 |
|----------------------|-----------------|--------------------|-------|------------|------|------|----------------------------------------------|-----------------|
|                      |                 | Serum              |       | Nasal wash |      |      | IgA                                          | IgG             |
|                      |                 | 0                  | 2 wk  | 0          | 1 wk | 2 wk |                                              |                 |
| <i>mg/100 ml</i>     |                 |                    |       |            |      |      |                                              |                 |
| Pulmonary disease    |                 |                    |       |            |      |      |                                              |                 |
| F. S.                | 30              | 512                | 512   | 2          | 4    | 4    | 128                                          | 188             |
| B. H.                | 50              | 32                 | ≥8192 | c          |      |      | 76                                           | 14              |
| G. B.                | 56              | 16                 | 128   |            |      |      | 84                                           | 114             |
| J. R. J.             | 66              | 8                  | 4096  |            |      | 8    | 147                                          | 27              |
| A. W.                | 57              | 512                | 2048  |            |      |      | 45                                           | 10              |
| J. M.                | 75              | 1024               | 4096  |            | 2    |      | 48                                           | 25              |
| M. L.                | 49              | 64                 | 256   |            |      |      | 62                                           | 20              |
| P. G.                | 42              | 256                | 1024  |            |      |      | 55                                           | 13              |
| J. R.                | 41              | 64                 | 512   |            |      |      | 52                                           | 16              |
| J. W.                | 49              | 64                 | 128   |            |      | 4    | 119                                          | 26              |
| H. S.                | 50              | 16                 | 512   |            |      |      | 35                                           | 20              |
| Mean                 | 51 <sup>d</sup> | 97 <sup>e</sup>    | 1045  |            |      |      | 77 <sup>d</sup>                              | 43 <sup>d</sup> |
| No pulmonary disease |                 |                    |       |            |      |      |                                              |                 |
| H. D.                | 43              | <4                 | 256   |            |      | 2    | 102                                          | 47              |
| J. K.                | 46              | 16                 | 512   |            | 8    | 8    | 232                                          | 46              |
| L. N.                | 72              | 8                  | 256   |            |      |      | 50                                           | 10              |
| C. R.                | 75              | 32                 | 512   |            |      | 2    | 60                                           | 48              |
| J. J.                | 42              | 8                  | ≥8192 |            |      | 2    | 53                                           | 19              |
| F. W.                | 46              | 64                 | 4096  |            |      |      | 48                                           | 16              |
| R. W.                | 51              | 8                  | 4096  |            |      |      | 37                                           | 15              |
| L. C.                | 57              | 32                 | 512   |            |      | 4    | 59                                           | 10              |
| B. H.                | 44              | 64                 | 256   |            |      |      | 34                                           | 10              |
| F. M.                | 64              | 32                 | 64    |            |      |      | 76                                           | 36              |
| Mean                 | 54              | 18.6               | 826   |            |      |      | 75                                           | 26              |

<sup>a</sup> Reciprocal of dilution completely neutralizing virus.

<sup>b</sup> Each value is the mean of 0, 1-week and 2-week specimens; specimens without detectable antibody were arbitrarily assigned value one-half that of sensitivity of test for calculations: IgA = 15; IgG = 10.

<sup>c</sup> Blank indicates lack of detectable antibody at 1:2 dilution.

<sup>d</sup> Arithmetic mean.

<sup>e</sup> Geometric mean: value of <4 considered = 2 and ≥8192 = 8192 for calculations.

titers were associated with absence of detectable nasal antibody. Indeed, no correlation between the maximal serum titers and the occurrence of nasal antibody (Spearman rank correlation coefficient,  $r_s = 0.07$ ) (11) could be demonstrated (Fig. 2). Nasal antibody was demonstrable in all subjects with nasal IgA levels of 100 mg/100 ml or greater. A positive correlation between nasal antibody and IgA levels was noted ( $r_s = 0.628$ ,  $p < 0.01$ ) (Fig. 3) (11). Influenza antibody

in the nasal washes of subject F. S., who had extensive bronchiectasis, may have resulted from the considerable amount of immunoglobulin G (IgG) in his nasal secretions.

# DISCUSSION

Hypodermic injection of influenza virus vaccine is not an efficient means of producing antibody in nasal secretions according to the data resulting from this and other studies (8) since serum titers as high as  $1 \geq 8192$  as noted here

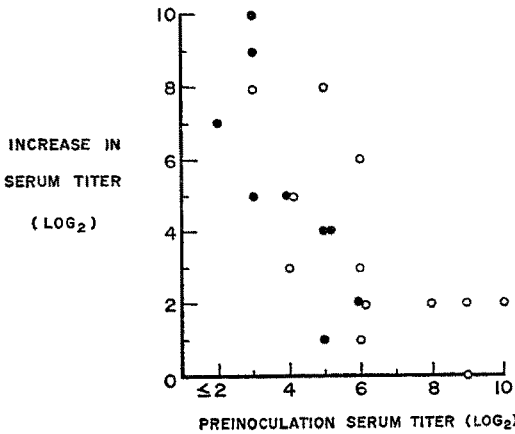


Figure 1. Serum influenza virus neutralizing antibody increase 2 weeks following parenteral administration of vaccine. ○, values from subjects with respiratory disease; ●, without respiratory disease.

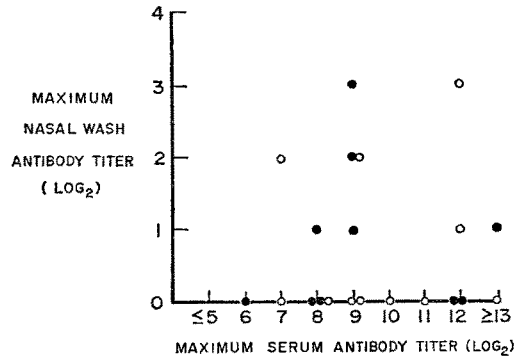


Figure 2. Serum and nasal influenza virus neutralizing antibody following parenteral influenza virus vaccination. Serum titers 2 weeks after vaccine are indicated and maximum nasal wash values 1 or 2 weeks after vaccine. ○, subjects with respiratory disease; ●, without respiratory disease.

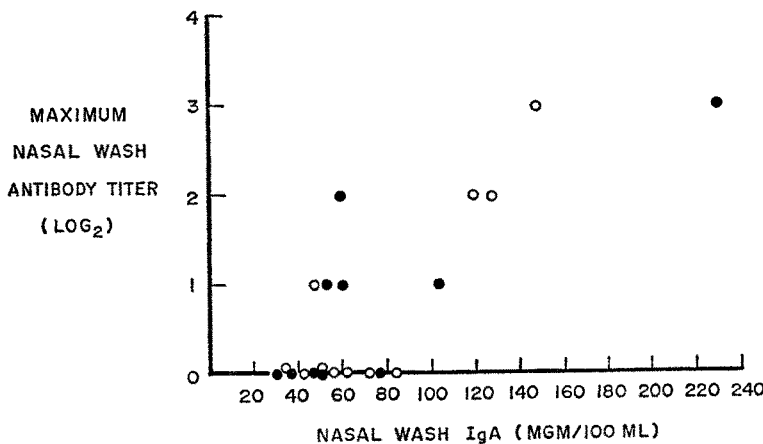


Figure 3. Nasal wash influenza virus neutralizing antibody and IgA concentration following parenteral administration of vaccine. Maximum antibody titers 1 or 2 weeks after vaccine. IgA values represent mean of values preinoculation, 1 week, and 2 weeks after vaccine. ○, subjects with respiratory disease; ●, without respiratory disease.

were not necessarily associated with detectable nasal antibody.

Lack of correlation between serum and nasal antibody titers in the present study and in another study of subcutaneous influenza virus vaccination (8) contrasts with the relationship which exists in well subjects with naturally acquired influenza antibody (2, 12), in individuals receiving formalin-inactivated influenza virus vaccine by the respiratory route (8), and in individuals with illness experimentally caused by inoculation of influenza virus into the respiratory tract (7, 12, 13). It seems reasonable that in these three circumstances the presence of antigen in the respiratory tract stimulates both local subepithelial IgA-producing cells and nearby IgG-producing cells. With hypodermic injection, in contrast, the site where antigen is introduced is remote from respiratory tract secretory IgA-producing cells, so that local synthesis of specific IgA and its subsequent occurrence in respiratory secretions would be expected to be less.

An inverse relationship between preinoculation serum antibody levels and increases in titer following parenteral inoculation of inactivated influenza virus was described almost 30 years ago (14). Since passively administered antibody can inhibit active antibody synthesis (15) by as yet incompletely understood mechanisms, it is reasonable to assume that circulating, specific influenza virus antibody also may limit the effectiveness of injected antigen. Much of the antigen probably combines with systemic antibody, thus preventing its stimulation of both systemic antibody-producing cells and cells that primarily produce secretory antibody. Parenteral immunization with killed vaccine in individuals with appreciable pre-existing titers of serum antibody may thereby result in very limited production of specific antibody in secretions of the respiratory tract. Recent studies using killed poliovirus (16) and parainfluenza virus (17) vaccines by the parenteral route have indicated similar results.

Before inoculation, detectable levels of nasal influenza virus antibody were absent in all except one subject; this contrasts with the results in a group of normal volunteers who in seven of 15 instances had nasal titers of 1:4 or greater (12). This could be due to the older age of the subjects in the current study since lower concentrations of nasal IgA can occur with advancing age (10).

A correlation between nasal IgA levels and specific influenza virus neutralizing antibody was demonstrated. This may only apply at low levels of detectable neutralizing antibody since high levels of nasal antibody were not produced in the present study. However, this relationship is not surprising since influenza virus antibody in the nasal secretions is predominantly secretory IgA (13). Variations among individuals in nasal IgA and specific influenza virus antibody may be due to the unique anatomy of each person's nasal passages, which results in consistently higher proportions of nasal secretions in the nasal washes of certain subjects. However, reproducible differences among persons with respect to the proteins found in their nasal washes (18) suggests individual variation in the synthesis or release of these proteins. The observed nasal wash values for IgA and influenza virus antibody in the present study could likewise result from intrinsic individual variation in the ability to synthesize and release antibody proteins into nasal secretions. Variation of this kind may alter susceptibility to infection by influenza virus in certain persons. However, patients with chronic bronchopulmonary disease as a group are not characterized by diminished nasal wash IgA concentration (10), and, although a small number of subjects was included in the present study, the data indicate that persons with chronic respiratory disease also exhibit levels of antibody to influenza virus in serum and nasal secretions that compare with those of other individuals of similar age.

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