Radioautographic Studies of Poliovirus Binding by Human Immunoglobulins.* (30405)

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A previous report from this laboratory demonstrated that radioactive poliovirus could diffuse through agar to combine with electrophoretically separated colostrum and serum antibodies(1). The technique as used was relatively insensitive, requiring concentrated, purified immunoglobulins and large volumes of poliovirus. A more sensitive technique, combining immunoelectrophoresis and radioautography with radioactive antigen as one of the reactants, has been described for demonstrating antibody activity. Thus antibodies which bind insulin, thyroglobulins and ragweed have been detected in the IgG,† IgA and IgM classes of immunoglobulins by this method(2-6). The adaptation and use of this radioimmunoelectrophoresis technique to the study of antibody directed against poliovirus is described in this paper.

Material and methods. Antigen. P^{32} labelled type I poliovirus was prepared by growing virus in HeLa cell cultures with citrate-Eagle's medium, 5% dialysed calf serum and sodium phosphate P^{32} (0.066 mc/ml). The labelled virus was purified by ultracentrifugation and DEAE column chromatography (7). The preparation used for diffusion contained approximately $10^{7.0}$ TID_{50} and 100,000 cpm per 0.1 ml.

Serum. Serum specimens were obtained from adult human males and females who had not been immunized within the past 2 years. Serum polio neutralizing antibodies were demonstrated by a standard one hour tube-neutralization method, using 100 TID_{50} of poliovirus, in HeLa cell cultures(8). The sera were fractionated by a combination of DEAE and Sephadex G-200 column chromatography(9). The type of immunoglobulin in the eluates was determined by radial immunodiffusion(10). The neutralization titer of each fraction was determined and correlated with the type of immunoglobulin present in the fraction.

Radioimmunoelectrophoresis was performed as described by Yagi(2) and Morse(3) using agar consisting of 1% Difco Special Agar Noble in a 25% solution of 0.1 M Veronal buffer (pH 8.6) on microscope slides. After electrophoresis of the serum, rabbit antiserum directed against human serum was added to the antiserum troughs. As soon as the antiserum had diffused into the agar, the radioactive poliovirus preparation was added to the trough. The plates were incubated 24 hours at room temperature and then washed for 48 hours with a 1% solution of NaCl. The slides were dried and then placed in contact with Kodak Royal Pan film for one week. Exposure was carried out in total darkness. After removal from contact with the film, the slides were stained with amido black. In some experiments the antiserum was allowed to react with the electrophoretically separated serum for 24 hours. These plates were washed for 48 hours and the radioactive virus was then added to the troughs. After 24 hours the slides were again washed, dried and placed in contact with the film in total darkness.

Radioimmunodiffusion was performed in the same agar system, using dilutions of the patient's serum. The slides were washed and printed as described above.

Results. The rabbit antiserum reacts with human serum components to form precipitation arcs. When the radioactive virus is added, it diffuses through the agar and is bound to these precipitates by the polioantibodies present in human serum. On the other hand,

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† The World Health Organization (Bull. 447, World Health Organ., v30, 1964) has recommended the following nomenclature for the classes of immunoglobulins: G Immunoglobulin (IgG) for 7S (γ_2) -globulin; A Immunoglobulin (IgA) for γ_{1a}-globulin and M Immunoglobulin (IgM) for γ_{1m}-globulin.

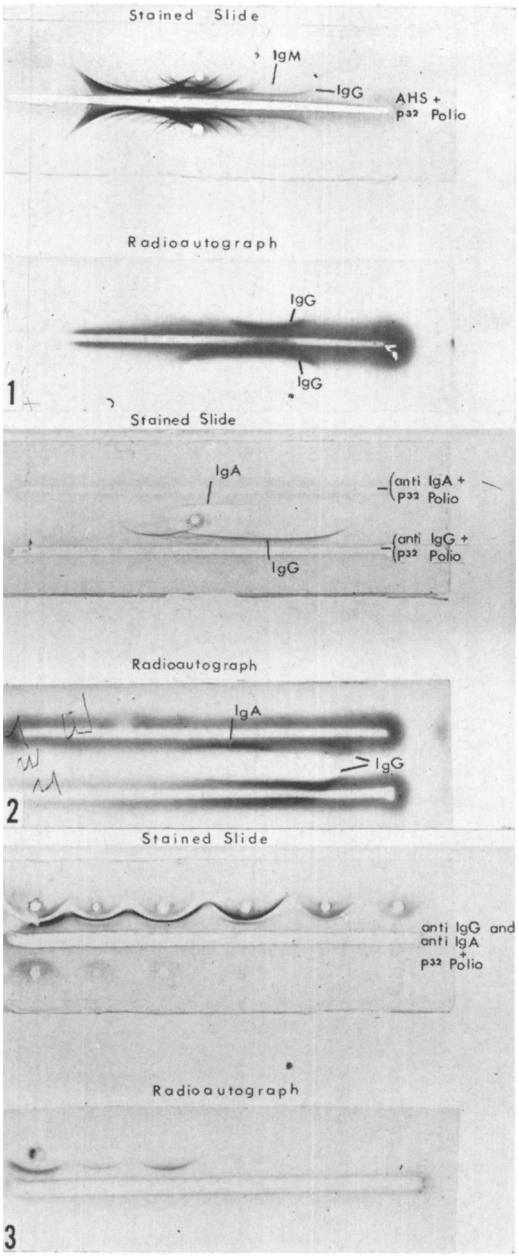

FIG. 1. Radioimmunoelectrophoresis of 2 human sera using rabbit anti-human serum (AHS) and P^{32} polio to develop stained precipitates and radioautograph.

FIG. 2. Radioimmunoelectrophoresis of human serum using rabbit anti-IgA serum (top trough), rabbit anti-IgG (bottom trough) and P^{32} polio to develop stained precipitates and radioautograph.

FIG. 3. Radioimmunodiffusion of human serum using rabbit anti-IgG, anti-IgA and P^{32} polio to develop stained precipitates and radioautograph. Serial 2-fold dilutions of the human serum were placed in each well. Radioautograph shows poliovirus binding in the first 4 concentrations (1:8).

virus not bound by the arcs diffuses through and is precipitated directly by antibodies in the human serum to form arcs visible only on the radioautographs (direct precipitation). Since these direct precipitates are difficult to identify they can be eliminated by using more potent rabbit antiserum or by washing the slide free of unprecipitated protein before adding radioactive virus.

Using rabbit anti-human sera of different specificities, sera of 17 individuals with various titers of neutralizing antibody were studied. After the radioautographs were developed, the radioactive arcs were compared with the stained slides and the component of the human serum binding the radioactive poliovirus identified.

When the rabbit antiserum was directed against whole human serum (AHS) precipitate arcs of many serum components were developed. The radioautograph, however (Fig. 1), showed that the radioactive poliovirus was bound only by immunoglobulins. In this example, both serum specimens show binding along the entire long arc of IgG. Although IgM as well as other protein arcs are visualized there is binding only with IgG. The difference in intensity of the 2 radioactive arcs can be explained by the difference in the neutralizing antibody titers of the 2 sera. The top serum had a neutralization titer of 1:14, the bottom serum had a neutralization titer of 1:100.

Since this antiserum did not develop the IgA arc and was unable to prevent doubling of the IgG arc by direct precipitation, we resorted to the use of other rabbit antisera. In Fig. 2 an example is given of the results obtained using 2 other rabbit antisera. In the top trough antiserum directed against colostrum IgA was used. As seen from the stained slide, this antiserum developed principally the arc of IgA. In the lower trough antiserum directed against serum IgG was used. This antiserum principally gives the arc of IgG and 3 other unidentified serum components. Neither of these sera was adsorbed to increase specificity, since this did not seem necessary. The radioautograph shows binding of radioactive proteins in both the IgG and IgA arcs of the human serum. High neutrali-

TABLE I. Poliovirus Antibodies in Human Serum Immunoglobulins.

Serum specimen	Column chromatography (neutralizing antibody)*			Radioautography (binding antibody)†		
	IgG	IgA	IgM	IgG	IgA	IgM
BE	++	±	—	++	+	—
LE	++++	—	—	++++	+	—
CA	+	++++	±	+	++++	—
BU	++	—	—	++	+	—
BR	++	—	—	++	+	—
DE	+	—	—	+	+	—
AU	+++	±	—	+++	++	—
AI	++	—	—	+++	+	—
NE	+++	+	—	+++	+	—
RE	++	+	—	+++	++	—
GR	++	—	—	++	+	—
JA	++++	+++	—	+++	++++	—
ZO	+++	+++	—	++++	++	—
HE	—	++	—	+	+++	—
ZE	—	—	—	++	+++	—
DA	—	—	—	—	—	—
BK	+++	—	—	+++	+	—

* The neutralization titers were graded + to ++++. ± indicates indecision whether the result was due to contaminating immunoglobulin.

† The binding of poliovirus was graded + to ++++ by judging the intensity of the arc formed and by quantitation using radioimmunodiffusion. + indicates a weak but definitely positive reaction.

zation titers were found in both the IgG and IgA globulins of this serum when it was fractionated by column chromatography.

Titrations of binding antibody were done by radioimmunodiffusion. Two-fold dilutions of the test sera were reacted with radioactive virus and an antiserum precipitating all the immunoglobulins (total binding titer) or with an antiserum made specific for one immunoglobulin by adsorption (fractional titer). Fig. 3 is an example of such a titration. The rabbit antiserum precipitated IgG and IgA. Radioactive binding is seen in the first 4 concentrations of the human serum (1:8).

The results obtained using radioautography and column chromatography are shown in Table I. In interpreting the results obtained from column chromatography, it was difficult to exclude the possibility that a low neutralization titer in the fractions rich in IgA globulins was due to small amounts of contaminating IgG antibody. The radioactive method solved this problem. By this method the polio antibodies were shown to be IgA globulins as well as IgG globulins.

Discussion. The methods of radioimmunoelectrophoresis and radioimmunodiffusion offer a rather easy and sensitive technique for determining the total polio antibody content of a serum as well as the class of the antibody. From this preliminary study it is evident that many individuals who have not been recently immunized have poliovirus antibodies not only in the IgG class of immunoglobulins but in the IgA globulins also. Several individuals, in fact, had a predominance of antibody activity in the IgA fraction. The small number of sera tested obviously precludes making any statement about the factors involved in determining whether the antipolio activity in an individual is predominantly in the IgA or IgG immunoglobulins. However, it is of interest to note that all serum specimens which showed strong IgA polio binding were obtained from females. None of the 5 males in this series showed strong IgA binding. A larger series is now being studied to determine whether males and females have different polio antibody patterns.

Svehag and Mandel(11) using zonal ultracentrifugation fractionation of human sera have shown that poliovirus antibody may be found in the 7S and 19S immunoglobulins. The 19S activity was transitory and disappeared several weeks after immunization. Their method did not distinguish between the two classes of 7S immunoglobulins. No

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.