COMPARATIVE STUDIES ON THE MODE OF NEUTRALIZATION REACTION OF POLIOVIRUS TYPE 2 WITH SERUM IgG AND SECRETORY IgA FROM MOTHER'S MILK AND FECAL EXTRACT

YORIYUKI AKAO, AKIRA SASAGAWA, SADASHI SHIGA AND REISAKU KONO

Central Virus Diagnostic Laboratory, National Institute of Health, Nakato, Musashimurayama, Tokyo 190-12, Japan

(Received: March 23, 1971)

SUMMARY: Serum and fecal specimens were collected from vaccinees receiving Sabin vaccine. Whey was separated from mother's milk collected from those who received Salk vaccine at their late pregnancy. Each specimen was tested for antibody to poliovirus type 2 (MEF 1) and the antibody-positive specimens were pooled separately with respect to serum, whey and stool extract. From each of three pools, the globulin fraction was precipitated at half saturation of (NH₄)₂SO₄. IgG/IgA ratios of globulin fractions from serum, stool and whey were 12.53, 4.06, and 0.76, respectively, but they showed no distinction in kinetics and in avidity of the neutralizing antibody for the poliovirus. The neutralization by each of the globulin fractions followed the first order reaction. The salting-out globulin fractions were subjected to Geon 72 S block electrophoresis and to step-wise elution with phosphate buffers of pH 7.5, 6.4, and 4.7 through a DEAE-cellulose column. The elutes were pooled into Fr. I, Fr. II, and Fr. III at three peaks in optical density at 280 m. Whey Fr. II showed two peaks, Fr. II-1 and Fr. II-2, and the latter was found by ultracentrifugal analysis to contain 11 S secretory IgA exclusively. Stool Fr. II was proved by immunophoretic and thin-layer chromatographic analyses to contain IgA. The modes of kinetic neutralization of poliovirus type 2 with serum IgG (Fr. I), secretory IgA from whey (Fr. II-2) and stool extract (Fr. II) were very much similar. However, stool Fr. I showed pronounced deviation from linearity. It was confirmed by serological analysis that this fraction was composed of degraded products of the IgA molecule, Fab or the like. In conclusion, the mode of neutralization of poliovirus type 2 was not different between serum IgG and secretory IgA derived from whey and stool extract.

INTRODUCTION

There has been increasing evidence indicating an important role of secretory IgA in the defence mechanism of the mucosal surface in viral and bacterial infections (Bellanti, 1968; Keller and Dwyer, 1968; Tourville et al., 1969; South et al., 1967; Hashimoto et al., 1970). We have been interested in the local immunity of the intestinal tract against enterotropic viruses. We found that coproantibody is synthesized in poliovirus infection (Kawakami et al., 1966; Kono et al., 1966) and that it was governed by a bursa- or gut-dependent lymphoid system by a model experiment with chickens and enterotropic Newcastle disease virus (Kono et al., 1969). The imp...
munoclonal nature of coproantibody was studied and, it was shown that the main component of coproantibody was secretory IgA (Keller and Dwyer, 1968).

Consequently, we became interested in the mode of action of the secretory IgA antibody in virus neutralization reactions. This paper presents comparative studies on the mode of neutralization of poliovirus type 2 with secretory IgA derived from mother's milk and fecal extracts and serum IgG.

MATERIALS AND METHODS

Stool extract: Stool samples were collected from infants and children, 3 months to 11 years of age, who had been orally immunized with two doses of trivalent Sabin vaccine. Samples were taken one month after the last vaccine administration. A 33% stool emulsion was made with Earle's balanced salt solution (BSS) for each specimen, then the emulsion was centrifuged at 3,000 rpm for 15 min. The supernatant was centrifuged again at 10,000 rpm for 30 min at 4°C. The final supernatants of 86 specimens diluted 1:3 were screened for the neutralizing antibody against 100 TCD₅₀ of poliovirus type 2. The screening was carried out by the method described before (Kawakami et al., 1966). Twenty-five antibody-positive supernatant were pooled and employed as the coproantibody-positive stool extract.

Mother's milk: Mother's milk was collected from 15 women who had received 1 ml of Salk vaccine twice during their late pregnancy. Collection was done 4 weeks after delivery. Whey was separated from fat and casein by centrifugation at 20,000 rpm for 30 min. Fourteen specimens of whey neutralizing in a dilution of 1:4 100 TCD₅₀ of poliovirus type 2 were pooled which served for further studies.

Human serum: Thirty serum specimens were collected from the vaccinees, from whom the stool specimens were collected. All these sera were preserved in a deep freezer at −20°C until processing. Thirty serum specimens neutralizing in a dilution of 1:10 100 TCD₅₀ of poliovirus type 2 were pooled. The pool was employed as the starting material for globulin fractionation.

Reagents for immunologic tests: Anti-human serum (rabbit) for immunoelectrophoresis was from the Research Institute for Microbial Diseases, Osaka University. Anti-human IgG, IgA and IgM sera (goat) were purchased from Hyland Lab. Inc., Los Angels, California. Anti-human Cohn Fraction II serum and fragments of human immunoglobulin (Fab, Fc, κ and λ chains) were obtained by the courtesy of Prof. T. Matuhasi of the Institute for Medical Science, University of Tokyo.

Separation of immunoglobulins: The procedure is shown in Fig. 1. Globulin was precipitated by making the sample half saturation with (NH₄)₂SO₄. The precipitate was dissolved in phosphate buffered saline (PBS) and made up to the original volume to 0.1 volume and to 0.01 volume with respect to serum, milk and stool, respectively. Each fraction was subjected to Geon 72S (polyvinyl chloridepolyvinyl acetate copolymer, Nihon Geon Co. Ltd., Tokyo) block electrophoresis after dialysis against 0.05 M veronal buffer, pH 8.6.

The Geon Block was cut into 1-cm sections. Each section was immersed in 20 ml of 0.1 M phosphate buffer. Each extract was lyophilized and then dissolved in 1 ml of distilled water. Each concentrated fraction was tested for the presence of IgA and other immunoglobulins by immuno-diffusion. The IgA-containing fractions from each of whey and stool specimens were pooled and concentrated by lyophilization to 1/4 and
NEUTRALIZATION WITH SECRETORY IgA

**Fig 1. Flow diagram of the procedure used for preparation of IgA from milk, stool and IgG from serum**

1/16 the volumes of whey and stool globulin fractions applied on the blocks, respectively. The IgG-containing fraction from serum was processed in the same way without concentration. Then, DEAE-cellulose chromatography was performed with each of the above materials by stepwise elution with phosphate buffers (0.01 M, pH 7.5; 0.10 M, pH 6.4; and 0.13 M, pH 4.7) according to the method of Tomasi et al. (1965) with a slight modification. The column was 1.5 × 60 cm and the flow rate was 4.5 ml/hr. Fractions of 5 ml were collected. The fractions showing the peak in optical density at 280 mλ were pooled and further concentrated by lyophilization to the volume of the sample added onto the DEAE-cellulose column.

Since the elute of mother’s milk (whey) at pH 6.4 showed two peaks in optical density, two pools were made of each peak. The elute of stool at pH 6.4 was pooled in one fraction.

**Virus and tissue culture:** MEF 1 strain of poliovirus type 2 was propagated by monkey kidney (MK) cell culture. Lactalbumin Earle’s (LE) medium was used for the maintenance and that enriched by addition of calf serum at 2% for the growth medium. The infectivity of virus was titrated by the plaque method.

**Measurement of neutralizing activity of globulin fractions:** The salting-out globulin fractions or DEAE-eluted fractions of stool, whey and serum were diluted appropriately in LE medium. Equal volumes of a virus suspension containing approximately 10³ PFU/ml and each diluted globulin fraction were mixed. The mixtures were incubated for one hour at 37 C and then overnight at 4 C. Two-tenth-ml portions of each mixture were inoculated into two bottles of MK monolayer. After one hour of absorption at room temperature, the monolayer cultures were overlaid with
melted nutrient agar and incubated for 5 days at 37°C, when plaque counting was performed. The dilution reducing plaque counts by 90% was taken as one neutralizing out.

**Kinetic neutralization**: Equal volumes of a prewarmed poliovirus type 2 suspension containing about 10^3 or 10^6 PFU and the globulin fraction so diluted as to contain 16 or 32 neutralizing units were mixed. The mixtures were incubated in a water bath at 37°C for various lengths of time (0, 2.5, 5, 15, 30, 60 and 120 min.). After the incubation, a 0.1-ml aliquot of each antibody-virus mixture was diluted with 9.9 ml of chilled PBS (1:100), from which 1:1,000 dilution was made. The surviving virus was assayed by the plaque method. The neutralization constant, K, was calculated by the equation (1),

\[ K = \frac{D}{t} \cdot \frac{2.3 \log \frac{V_o}{V_t}}{0.05} \]  

where Vo: initial quantity of active virus, Vt: the amount surviving at time, t: in min. D: neutralization unit of the immunoglobulin fraction instead of the serum dilution in ordinary use, and K: the neutralization constant.

**Multiplicity neutralization**: Equal volumes of prewarmed 10^6 PFU of poliovirus type 2 and each globulin fraction serially diluted were mixed and the mixtures incubated in a water bath at 37°C for 30 min. Thereafter, each mixture was chilled in an ice bath and the surviving virus was titrated.

**Quantitative measurement of the immunoglobulin**: The concentration of IgA and IgG of stool, whey and serum samples were measured by the radial immunodiffusion technique using immunoplates (Hyland Labl, Inc., Los Angeles, California).

**Ultracentrifugation**: Analytical ultracentrifugation was carried out in a Beckman analytical ultracentrifuge at 20 ± 0.2°C at 59,780 rpm.

**Immunodiffusion and immunoelectrophoresis**: Double diffusion in agar gel was performed by a microscopic slide modification of the Ouchterlony method. Immuno-electrophoresis was carried out on microscopic slides by the method of Scheidegger using 1.5% agar in 0.05 M barbital buffer, pH 8.6. The slides were kept at room temperature for 48 hr, washed and stained with amido-black 10B (Scheidegger, 1955).

**Thin layer gel chromatography**: A thin layer of Sephadex G-200, super fine (Pharmacia Fine Chemicals, Uppsala, Sweden) was made on a glass plate (200×60 mm) of 1 mm in thickness, at 20° of inclination. A M/12 phosphate buffer, pH 7.2, with an ionic strength of 0.10 was employed. After development, the gel layer was adsorbed by cellulose acetate membrane, which was stained with amido-black 10B.

**RESULTS**

**Characterization of Immunoglobulin Classes in Salting-out Fractions of Pooled Serum, Mother's Milk (Whey), and Stool Extract by the Double Diffusion Test and the Radial Immunodiffusion Method**

A double diffusion test in agar gel was performed with salting-out fractions of pooled serum, whey and stool extract and anti-human IgG, IgA and IgM goat sera. Figure 2 depicts the precipitation reaction with the whey fractions. Anti-IgG, IgA or IgM sera were placed in the central well; globulin fractions of serum, and milk or a mixture of both were in the circumferential wells. The bands formed with whey
Fig. 2. Double diffusion test in agar gel of the fractions precipitated by half saturation with (NH$_4$)$_2$SO$_4$ from pooled mother's milk (Whey) and serum.

Serum

Whey + Serum

Anti- Ig G

Whey + Serum

Whey

Serum

Whey + Serum

Anti- Ig A

Whey + Serum

Whey

Serum

Whey + Serum

Anti- Ig M

Whey + Serum

Fig. 2. Double diffusion test in agar gel of the fractions precipitated by half saturation with (NH$_4$)$_2$SO$_4$ from pooled mother’s milk (Whey) and serum.
and those of serum fused partially, and an additional spur was noticed when anti-IgG as well as anti-IgA sera were used. However, the bands completely crossed with anti-IgM serum.

When IgG and IgA contents of each globulin fraction were measured by the radial immunodiffusion method, as shown by Table 1, the IgG/IgA ratio of serum, milk and fecal extract globulin fractions were 12.53, 0.76 and 4.06, respectively. These figures are comparable with those reported by Chodirker and Tomasi (1963) who measured the ratio with serum IgA as a standard.

Table 1. Comparison in IgA and IgG concentrations and in neutralization quotients of globulin fractions precipitated at half saturation of \((\text{NH}_4)_2\text{SO}_4\) from pooled serum, mother’s milk (whey) and vaccinee’s stool extract

<table>
<thead>
<tr>
<th>Salting-out Globulin fraction from</th>
<th>Concentration of IgG (mg/dl)</th>
<th>Concentration of IgA (mg/dl)</th>
<th>IgG/IgA ratio</th>
<th>NT titer: IgG + IgA mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum*</td>
<td>940</td>
<td>75</td>
<td>12.53</td>
<td>1.86 × 10^3</td>
</tr>
<tr>
<td>Whey**</td>
<td>32</td>
<td>42</td>
<td>0.76</td>
<td>1.73 × 10^3</td>
</tr>
<tr>
<td>Stool***</td>
<td>134</td>
<td>33</td>
<td>4.06</td>
<td>1.67 × 10^3</td>
</tr>
</tbody>
</table>

* Restored to the original volume
** 10-fold concentrated in PBS
*** 100-fold concentrated in PBS

Neutralization Reaction of Salting-out Globulin Fractions of Pooled Serum, Whey and Stool Extract

Further investigation by the plaque reduction, kinetic neutralization and multiplicity neutralization tests was made so as to find difference in the mode of neutralization reaction to poliovirus type 2 among the three globulin fractions of different origins with different IgG/IgA ratios.

A hundred and twenty-three PFU of poliovirus type 2 was added to the globulin fraction of the serum, milk or fecal sample. After incubation of the mixture for 1 hr at 37°C and then for 20 hr at 4°C, the virus survival was titrated.

Figure 3 shows plaque reduction curves. Each plaque reduction curve was a straight line with the identical inclination from 10 to 90% reduction. Taking the end point at 90% plaque reduction, the titers of fecal, whey and serum globulin samples were 1:13, 1:128 and 1:2,048, respectively.

As shown in Table 1, the 90% plaque reduction titers were divided by the amount of IgA and IgG in each fraction; this is designated as the neutralization quotient. There is no significant difference between neutralization quotients of serum globulin fraction, which was mainly composed of IgG, and of the whey and stool globulin fractions, IgG/IgA ratio of which was smaller than that of the serum globulin fraction. Accordingly, it was considered that there was no marked difference in the avidity among serum, milk and coproantibodies for neutralization of poliovirus type 2.

As shown in Figure 4, the kinetics of neutralization with the whey globulin fraction against \(1.1 \times 10^6\) PFU...
Fig. 3. Plaque reduction tests with salting-out globulin fractions from pooled serum, mother's milk (whey) and stool extract. 32 neutralizing units each of salting-out globulin fractions and $1.1 \times 10^6$ PFU of the MEF1 virus were employed.

Fig. 4. Kinetic neutralizing curves of poliovirus (MEF1 strain) with salting-out globulin fractions from pooled serum and mother's milk (whey).
of MEF1 virus was similar to that of serum globulin. As shown in Fig. 5, kinetics of neutralization reaction with the fecal globulin fraction against $1.2 \times 10^3$ PFU of poliovirus type 2 was also similar to that of serum. It is very likely from these results that the neutralization reaction of the fecal and whey globulin fractions follows the first order reaction. The calculated neutralization constant, $K$, is shown in Table 2. There was no significant difference in $K$ value among the serum, whey and fecal globulin fractions.

The results of the multiplicity neutralization test with serum, whey and fecal samples are shown in Figs. 6 and 7. The log survival of virus was on the ordinate and log neutralizing units on the abscissa. In inverse proportion to the increase in units of the serum, whey and fecal globulin fractions, the virus survival decreases.

The above results show that the avidities of the neutralizing antibody to poliovirus type 2 are not different among the salting-out globulin fractions of serum, whey and stool, although they are different in the IgG/IgA ratio.
Table 2. Values of the neutralization constant, $K$, of various fractions from serum whey and stool against poliovirus type 2 calculated on the basis of viral survival after 2.5, 5 and 10 min of neutralization

<table>
<thead>
<tr>
<th>Neutralization time in min</th>
<th>Salting-out globulin fractions from</th>
<th>Final immunoglobulin fractions of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Whey</td>
</tr>
<tr>
<td>2.5</td>
<td>23.9</td>
<td>24.2</td>
</tr>
<tr>
<td>5.0</td>
<td>18.1</td>
<td>18.4</td>
</tr>
<tr>
<td>10.0</td>
<td>7.1</td>
<td>9.0</td>
</tr>
</tbody>
</table>

Neutralization units

<table>
<thead>
<tr>
<th>Virus dose (PFU)</th>
<th>Serum IgG</th>
<th>Whey IgA</th>
<th>Serum IgG</th>
<th>Stool IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.1 \times 10^6$</td>
<td>$1.8 \times 10^6$</td>
<td>$1.2 \times 10^6$</td>
<td>$1.3 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>$1.1 \times 10^6$</td>
<td>$1.8 \times 10^6$</td>
<td>$1.2 \times 10^6$</td>
<td>$1.3 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>$1.2 \times 10^3$</td>
<td>$1.0 \times 10^3$</td>
<td>$1.0 \times 10^3$</td>
<td>$1.0 \times 10^3$</td>
<td></td>
</tr>
</tbody>
</table>

N. T.: Not tested

The survival of $1.5 \times 10^6$ PFU of the MEF1 virus was titrated after mixing with various neutralizing units of the globulin fractions and incubation at 37°C for 30 min.

Fig. 6. Multiplicity neutralization curve of poliovirus type 2 (MEF1 strain) with salting-out globulin fractions of pooled serum and of mother’s milk (whey).
The survival of $1.5 \times 10^6$ PFU of the MEF1 virus was titrated after mixing with various neutralizing units of the globulin fractions and incubation at 37°C for 30 min.

Fig. 7. Multiplicity neutralization curve of poliovirus type 2 (MEF1 strain) with salting-out globulin fractions of pooled serum and of stool extract.

Neutralization Reaction of Poliovirus Type 2 with Purified Serum IgG and Secretory IgA Antibodies in Feces and Mother’s Milk (Whey)

To compare the mechanism of neutralization reaction of poliovirus type 2 with serum IgG to that of secretory IgA in feces and milk, the IgG and IgA fractions were separated by DEAE-cellulose column chromatography. Their neutralizing activities were tested by the same procedure as that used in the study of the globulin fractions.

The eluate of the serum specimen with 0.01 M PB, pH 7.5, designated as serum Fr. 1, was considered to be pure IgG.

As mentioned before, the optical extinction curve of the whey globulin fraction eluted with 0.10 M PB, pH 6.4, was bimodal; they were named whey Fr. II-1 and Fr. II-2. Other fractions eluted with 0.01 M PB, pH 7.5 and with 0.13 M PB, pH 4.7, showing peaks in optical density were whey Fr. I and Fr. III, respectively. It was demonstrated that Fr. II-2 was composed mostly of IgA, since no IgG or IgM precipitation ring developed in the immunoplate assay.

Three similar globulin fractions were obtained from fecal samples; they were also called as stool Fr. I, Fr. II and F. III. Fr. II was obtained with 0.10 M PB, pH 6.4, and also found reactive exclusively to anti-IgA goat serum in the agar gel precipitation test. The patterns of immunoelectrophoresis of serum Fr. I, whey Fr. I, whey Fr. II-1, whey Fr. II-2, whey Fr. III, stool Fr. I and stool Fr. II against anti-human serum are shown in Figs. 8 and 9.
In ultracentrifugal analysis, serum Fr. I revealed one peak with an $S_{20,w}$ of 6.4 and whey Fr. II-2 formed two bands with $S_{20,w}$ of 11.0 and 16.3. These results confirmed that serum Fr. I contained IgG exclusively and whey Fr. II-2 was composed of secretory IgA and probably its polymer (Tomasi et al., 1965). Since the quantity of stool Fr. II was too small to perform ultracentrifugal analysis, thin layer gel chromatography using Sephadex G-200, super fine, gel was carried out to characterize this fraction. The results indicated that stool Fr. II was very likely to be 11S IgA antibody.

Figure 10 illustrates the results of plaque reduction tests with whey IgA (Fr. II-2) and serum IgG (Fr. I) which gave completely identical results with those obtained with the salting-out fractions as described in the previous experiment.

Whey Fr. II-2 was so diluted as to contain 32 neutralizing units; stool Fr. I and Fr. II 16 neutralizing units. The latter's quantities were too small to concentrate up to 32 units.

The results of kinetic neutralization tests with serum IgG and milk and that with
stool IgA are shown in Figs. 11 and 12, respectively. As shown in Fig. 11, serum IgG and whey IgA gave almost identical kinetic neutralization curves.

Log survival of poliovirus type 2 after incubation with 16 neutralizing units of stool IgA (Fr. II) or serum IgG (Fr. I) decreased linearly, but the inclination of kinetic neutralization curves was not steep and K values calculated were smaller (Table 2) because of a lower antibody concentration (16 neutralizing units).

The curve of stool Fr. I revealed marked deviation from linearity, remaining
32 neutralizing units of each fraction and $1.8 \times 10^6$ PFU of the MEF1 virus were employed.

Fig. 11. Kinetic neutralization curves of poliovirus type 2 (MEF1 strain) with Fr. I (IgG) from pooled serum and Fr. II-2 (IgA) from mother's milk (whey).

a very highly persistent fraction. This can easily be understood by the results of following experiments elucidating the nature of stool Fr. I.

Enhancement of Neutralization with Stool Fr. I by Addition of Anti-humen IgG or IgA Goat Serum

Each of serial dilutions of stool Fr. I and Fr. II was mixed with an equal volume of virus containing approximately 480 PFU/ml and the mixture allowed to stand at 37°C
16 neutralizing units of serum Fr. I, stool Fr. I and stool Fr. II were employed.

The titer of the MEF 1 virus was $1.3 \times 10^6$ PFU.

Fig. 12. Kinetic neutralization curves of poliovirus type 2 (MEF1 strain) with Fr. I from pooled serum (IgG), Fr. I (Fab or the like of stool IgA?) and Fr. II (IgA) from pooled stool extract.

for 1 hr and then at 4 C for 20 hr. Aliquots of each mixture were diluted 1:2 by the addition of anti-human IgA or anti-human IgG goat serum diluted 1:20 beforehand. The control aliquots were diluted 1:2 by addition of PBS. After incubation at 37 C for 1 hr, the surviving virus was titrated by the plaque method.

As shown in Table 3, no enhancement of neutralization was found with stool Fr. II or secretory IgA; on the contrary, addition of anti-IgG or IgA goat serum markedly enhanced neutralization of the virus with stool Fr. I.

The results indicate strongly a possibility that Fr. II represents secretory IgA antibody molecules but stool Fr. I may be composed of fragments of IgA which degraded during processing (Keller, 1968; Keller and Dwyer, 1968).

Characterization of Stool Fr. I by Immunodiffusion and Thin Layer Gel Chromatography

Stool Fr. I was characterized by the double diffusion test using anti-Cohn’s fraction II rabbit serum and fragments of the antibody molecule, Fab, Fc from IgG, \( \kappa \) and \( \lambda \)
Table 3. Enhancement of the neutralization of stool Fr. I and Fr. II with anti IgA or anti IgG serum

<table>
<thead>
<tr>
<th>Dilution of each Fraction</th>
<th>Without Adding Anti IgA or IgG</th>
<th>Anti IgA added</th>
<th>Anti IgG added</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 256</td>
<td>0*</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1: 128</td>
<td>0</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>1: 64</td>
<td>0</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>1: 32</td>
<td>0</td>
<td>67</td>
<td>90</td>
</tr>
<tr>
<td>1: 16</td>
<td>50</td>
<td>95</td>
<td>98</td>
</tr>
<tr>
<td>1: 8</td>
<td>79</td>
<td>97</td>
<td>100</td>
</tr>
<tr>
<td>1: 4</td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1: 2</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Stool Fr. I

<table>
<thead>
<tr>
<th>Dilution of each Fraction</th>
<th>Without Adding Anti IgA or IgG</th>
<th>Anti IgA added</th>
<th>Anti IgG added</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: 256</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1: 128</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1: 64</td>
<td>2</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>1: 32</td>
<td>33</td>
<td>68</td>
<td>55</td>
</tr>
<tr>
<td>1: 16</td>
<td>65</td>
<td>68</td>
<td>84</td>
</tr>
<tr>
<td>1: 8</td>
<td>80</td>
<td>85</td>
<td>95</td>
</tr>
<tr>
<td>1: 4</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1: 2</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Stool Fr. II

* The figures indicate percentage reduction of the plaque in comparison to the control titration of poliovirus type 2.

chains, as reference antigens. The results are shown in Fig. 13 (a) and (b). The precipitation bands between stool Fr. I and Fab, κ or λ chain connected but there was no connection between Fc and stool Fr. I.

Accordingly, it was very likely that stool Fr. I contained fragments of the antibody molecule, mainly Fab or (Fab')2. Since no Fc from serum IgG was demonstrated in stool Fr. I, it was presumed that Fab may have originated from secretory IgA. It was considered further that the above assumption was endorsed by the experiment using thin layer gel chromatography in which the lighter component of stool Fr. I moved faster than serum albumin.

**DISCUSSION**

There have been many papers reporting that the reaction rate of virus neutralization with IgG antibody follows a single hit reaction but the reaction with IgM antibody shows a departure from linearity (Dulbecco et al., 1956; Mandel, 1961; Lafferty, 1963; Svehag et al., 1964; Iwasaki and Ogura, 1968). However, virtually little information is available concerning the mode of neutralizing action of IgA globulin antibodies against viruses. Michaels (1965) reported that the antibody in human colostrum and milk neutralized poliovirus in the first order reaction, but he did not mention its immunoglobulin class. Giovanardi et al., (1965) found that the poliovirus-neutralizing antibody in human colostrum and milk belonged to the β2A class of
Central well: Anti Cohn’s fraction II rabbit serum.
Circumferential wells: Stool Fr. I, the antigen to be tested, and reference antigens, namely, human IgG, Fab, Fc, and chains.

Fig. 13 (a) and (b) Characterization of stool Fr. I by the double diffusion test

Immunoglobulin, but they did not study its mode of action.

On the one hand, it has become evident that the secretory IgA antibody plays an important role in the defence mechanism of virus infection of mucosal surfaces. The authors investigated local resistance of the intestinal tract after oral administration of live attenuated poliovirus vaccine to humans and of attenuated Newcastle disease virus to chickens and found that coproantibody played an important role in the resistance and that the predominant component of the coproantibody was IgA (Kono et al., 1969).

Consequently, we became interested in the mechanism how the secretory IgA antibody works in the virus-neutralization reaction. In this report, first we compared
the mode of neutralization reaction of poliovirus type 2 with the globulin fractions of human serum, mother's milk (whey) and stool extract precipitated by salting-out with half saturation of (NH₄)₂SO₄; no difference was found among them when compared by kinetic and multiplicity neutralization tests.

Then, purified IgA fractions of mother's milk (whey) and of stool extract were tested and a close similarity to serum IgG was shown in kinetic neutralization reaction against the same virus. On the other hand, a marked deviation from linearity was found in other fractions of the stool extract considered to be composed of degraded products of IgA the molecule, probably Fab or the like. It was mentioned by several workers (Freter, 1962; Kawakami et al., 1966) that antibody tends to be degraded in stool by the presence of digestive enzymes and enteric microorganisms. It was endorsed by the fact that an enhanced neutralization reaction of the virus with a stool Fr. I mixture was observed by addition of anti-human globulin goat antibody. Accordingly, we concluded that the neutralization reaction with serum IgG, whey IgA and stool IgA (secretory IgA) was almost identical in respect to their kinetics and avidity. Contrary to Michaels' (1965) findings and ours, Phillipson, Killander and Albertsson (1966); Phillipson (1966) concluded that IgA and IgM showed more pronounced protected fraction of poliovirus than IgG in neutralization reaction by partition in an aqueous polymer phase system and by counter current distribution. However, his data on partition coefficient in the phase system and counter current distribution patterns of IgA should that nature of IgA was closer to that of IgG rather than to that of IgM.

The gross morphology and optical rotatory dispersion data were reported to show a close similarity between IgA and IgG molecules (Svehag and Bloth, 1970). Our findings on secretory IgA in milk and stool are probably justified by these date.

Our data strongly support the concepts that IgA coproantibody neutralizes enteric viruses in intestinal tract effectively and that colostrum or milk IgA ingested by a baby may protect the mucous membrane from infection by invading viruses.

However, it remains to be solved how secretory IgA acts in neutralization of viruses. Does the seretory IgA molecule as it is neutralize the virus? Or dose it react with the virus in a freed form after segregation from the secretory piece? These questions must be answered.

REFERENCES


