

THE FORMATION AND PROPERTIES OF POLIOVIRUS-
NEUTRALIZING ANTIBODY*

III. SEQUENTIAL CHANGES IN ELECTROPHORETIC MOBILITY OF 19S
AND 7S ANTIBODIES SYNTHESIZED BY RABBITS AFTER A SINGLE
VIRUS INJECTION

By SVEN-ERIC SVEHAG,† D.V.M.

(From the Department of Virology, The Public Health Research Institute of the City
of New York, Inc., New York)

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A previous paper in this series (1) described marked differences in the kinetics of the formation of poliovirus (PV)-neutralizing antibody of 19S and 7S type. It was also reported (2) that the antibodies produced in the rabbit after a single PV injection showed progressive changes in their electrophoretic mobility and avidity. The present study analyzes these sequential changes in the mobility of 19S and 7S type PV-neutralizing antibody of rabbit origin. The molecular type of antibody synthesized was identified by zone density gradient ultracentrifugation (1, 3) and/or by its sensitivity to dissociation with sulfhydryl reagents (4, 5).

These studies have demonstrated two antibody populations associated with 7S γ -globulins which differ in their electrophoretic mobility (γ_1 and γ_2), and a systematic sequential change in the mobility ($\beta \rightarrow \gamma_1$) of macroglobulin antibody formed after a single antigenic stimulation. Further, certain antisera were found to contain small amounts of antibody which sedimented at a rate intermediate to that of 19S and 7S antibodies.

Materials and Methods

Virus.—The Brunhilde strain (type 1) of poliovirus and Coxsackie B-4 virus¹ were used. The viruses were cloned and grown on HeLa cell monolayers. Prior to storage at -20° or -70°C virus stocks were partly purified and concentrated by differential centrifugation. Such preparations contained about 1 μg protein per $10^{6.4}$ plaque-forming units (PFU).

Immunization Procedure.—Healthy, $3\frac{1}{2}$ to 5 months old female, randomly inbred Chinchilla-Flemish rabbits weighing $2\frac{1}{2}$ to $3\frac{1}{2}$ kg were injected intravenously into the marginal ear vein with 2 ml diluted stock virus. Two immunization schedules were used. The rabbits were given either a single injection of 4×10^9 PFU of PV or two injections, spaced about 4

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† Fellow of The American-Scandinavian Foundation.

¹ Kindly supplied by Dr. John Fox.

months apart, each containing 4×10^8 PFU. The rabbits were bled by heart puncture, the blood allowed to clot and the sera collected and stored at 3° or -70°C without preservatives.

Rabbit anti-sheep red blood cell sera, collected 5 days after three intravenous injections, at 2 to 3 days intervals, of 0.5 ml boiled red blood cell stromata, were kindly supplied by Dr. Frank Adler.

Assay of Virus-Neutralizing Antibody.—Sera were titrated for neutralizing antibody by mixing equal volumes of virus (150 PFU) and varying dilutions of whole or fractionated serum. The mixtures were held for 18 hours at 3°C plus 3 hours at room temperature and assayed without dilution on HeLa monolayers for virus survivors. When employing very early immune sera, with weak antibody activity, the sensitivity of the assay was increased about 5-fold by extending the time of virus-antibody contact to 1 week at 3°C . Antibody activity is expressed in 50 per cent neutralizing units, defined as the serum dilution at which 50 per cent neutralization occurred. End-point determinations were made by graphic interpolation. Originally, antibody activity was assayed by the kinetic method but, because of non-first order kinetics observed with early antisera (6), this method was abandoned for the 50 per cent end-point method.

Zone Density Gradient Ultracentrifugation, and Reduction of Serum Macroglobulins by Mercaptans.—These methods were the same as described in a previous paper (1) in this series.

Zone Electrophoresis.—Except for minor modifications, the method of Kunkel and Slater (7) was used. Starch block electrophoresis was carried out at 3°C in a barbiturate buffer of pH 8.6 and μ 0.05. Rabbit sera were diluted up to 1:10 in the buffer. A potential of 15 v per cm was applied for 24 to 30 hours, which was the time necessary for the bromphenol blue-labeled albumin to migrate to the anode of the block. Following electrophoresis the block was cut in 0.8 cm segments and the protein eluted with 3 to 6 ml buffered saline solution. The starch eluates were stored at 3°C and assayed within 2 to 3 days for virus-neutralizing activity.

Protein Determination.—Protein analysis of virus stocks, starch eluates, and serum fractions from zone density gradients were carried out by the Lowry modification of the method of Folin-Ciocalteu (8) with crystalline bovine albumin as a standard. Occasionally the ultra-violet absorption of starch eluates at $280\text{ m}\mu$ was determined rather than measuring protein concentration by the Lowry method.

RESULTS

Starch Block Zone Electrophoresis.—Serum samples were collected daily from rabbits given a single intravenous injection of 4×10^9 PFU of virus. Fig. 1 demonstrates the electrophoretic distribution of the neutralizing activity in four serum samples obtained at different times from the same animal and tested in the same starch block. For the 2 day serum the distribution of total protein is included.

The antibody in the 2 day serum migrated as a single, nearly homogeneous component in the β -region. Thereafter a progressive shift of the mobility of the major antibody activity towards the γ_2 -region was observed during the first 2 weeks after immunization. This is indicated by the gradual displacement of the antibody peaks towards the cathode in Fig. 1 and by the skewed antibody distributions in the 4- and 7-day sera. Although the early, highly charged antibody (β -) was found also in 4- and 7-day sera the main activity now ap-

peared in the γ_1 -region. While antibody of γ_2 -mobility was not demonstrable until 10 to 14 days after immunization, antibody of faster mobilities persisted. The higher titer of the 14 day serum permitted electrophoretic separation of a 1:100 dilution of this serum in normal rabbit serum (NRS) with results (Fig. 1, top figure) that confirmed those obtained when undiluted serum was employed. Since the 14 day serum contained more highly charged antibody than could be accounted for by undecayed antibody, synthesized before day 7, this indicated a slight continued synthesis of the β -globulin antibody.

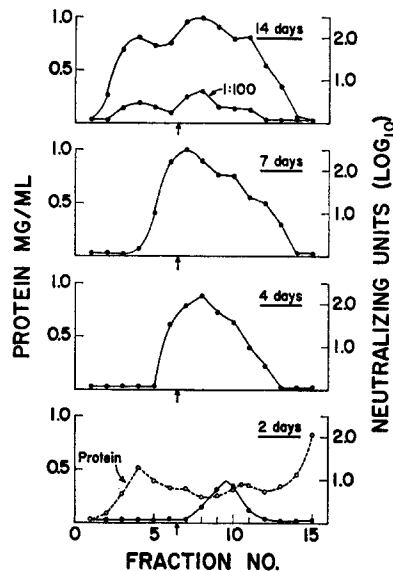


FIG. 1. Electrophoretic distribution of neutralizing activity (●) in serum samples obtained from the same animal following a single injection of poliovirus and tested in the same starch block (barbiturate buffer pH 8.6, μ 0.05). The broken curve (○) represents the distribution of protein.

In rabbit antisera most of the hemagglutinating activity against the Forssman antigen of sheep erythrocytes has been reported to migrate in the fast γ -region (9, 10). Rabbit antiserum against boiled sheep cell stromata was therefore used as a reference reagent by mixing with NRS or 2-day immune sera to PV before subjecting these sera to starch block electrophoresis. The anti-sheep red blood cell serum had earlier been tested by ultracentrifugation in a sucrose gradient and found to contain mainly 19S agglutinating antibody. As seen in Fig. 2 the neutralizing activity to PV had a higher net charge than the fast moving component of Forssman antibody. Also in other experiments the hemagglutinating activity² in Forssman antisera was found mainly or entirely

² Kindly assayed by Dr. Frank L. Adler.

in the γ_1 -region. When weak PV-neutralizing activity, obtained from a 1-day-serum, was concentrated by dialysis against carbowax 20-M (Union Carbide Chemicals Company, New York) and subjected to zone electrophoresis a narrow, symmetric peak of weak activity was recovered in the β -region.

In immune rabbit sera (IRS), collected $4\frac{1}{2}$ months after immunization, the main antibody was associated with slow moving γ_2 -globulins. When a serum collected 3 days after a second virus injection ($4\frac{1}{2}$ months after the first injection) was used undiluted (Fig. 3, bottom figure) the peak of antibody was found in the γ_2 -fraction but activity spread into the faster migrating globulins. The same serum diluted 1:500 in NRS demonstrated activity only in the slow γ -region. Two fractions from the run with undiluted serum, one from the

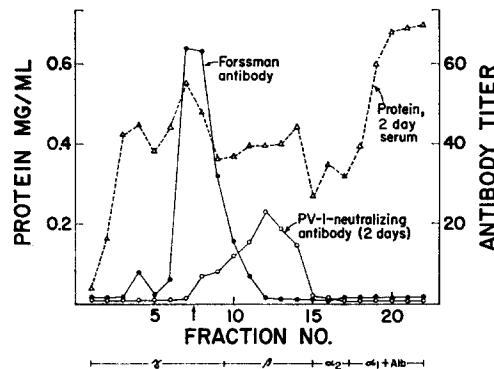


FIG. 2. Electrophoretic distribution of Forssman-agglutinating antibody (●) and poliovirus-neutralizing antibody (○) in starch gel, using barbiturate buffer pH 8.6, μ 0.05. The broken curve represents the distribution of protein. PV-1, poliovirus type 1.

γ_2 -region (Fr. 1) and one from the β -region (Fr. 5) were subjected once more to electrophoresis in starch (Fig. 3, top figure). Fraction 1 gave a single peak of activity in the γ_2 -region. Fraction 5, in contrast, contained antibody associated with β -, γ_1 - and γ_2 -globulins. Thus, minor amounts of the β -globulin antibody were present in this early secondary response serum. Serum collected just prior to re-stimulation also contained 19S antibody (only this antibody was associated with β -globulins) but at levels too low to be detected in the starch eluates. The appearance of antibody in the (γ_1)- β -region was in agreement with the observation (11) that the formation of the highly charged 19S antibody was promptly renewed upon antigenic restimulation.

Zone Density Gradient Ultracentrifugation.—The sequential changes in antibody properties after immunization were characterized further by subjecting whole sera and electrophoretically purified serum fractions to zone density gradient ultracentrifugation in sucrose. Fig. 4 shows the distribution of anti-

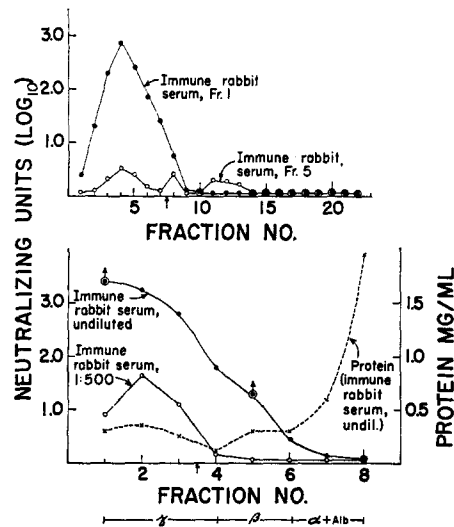


FIG. 3. Electrophoretic distribution of poliovirus-neutralizing antibody in a late immune serum, tested undiluted (●) and diluted 1:500 (○), (bottom figure); and in two eluates from this starch block when tested in another block (top figure). The serum was collected 3 days after a second injection of 4×10^8 PFU of virus; the first injection was given 4½ months earlier.

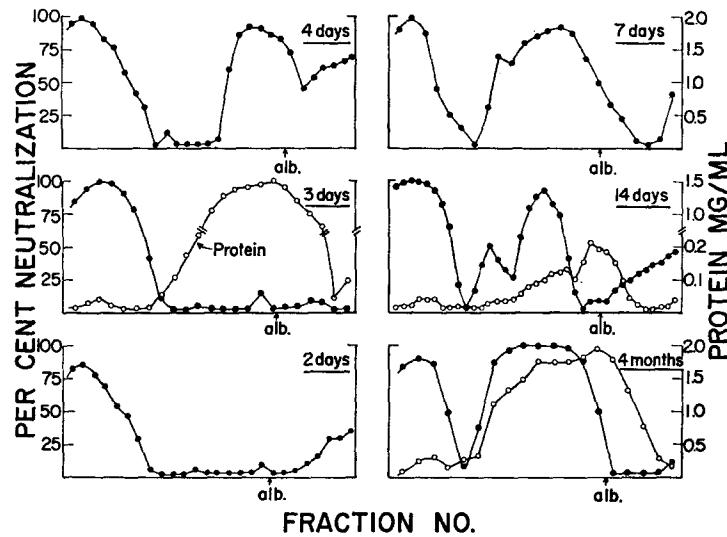


FIG. 4. Distribution of antibody activity (●) and protein (○) in serum samples, obtained from the same animal following a single injection of poliovirus (4×10^9 PFU). The sera were fractionated by zonal density gradient ultracentrifugation in sucrose.

TABLE I
Effect of 2-Mercaptoethanol on the Antibody Activity of Whole Sera or Serum Fractions

2-Mercaptoethanol concentration	Incubated at 3°C for	Type of antiserum	Material treated			Antibody titer	
			Whole serum	Fractions from zone electrophoresis	Fractions from zonal ultracentrifugation	Un-treated	Treated
m	days				S		
0.05	1	3 days, Ag ₂ *			19	24	12
0.05	1	3 " , Ag ₃		γ ₂		400	400
0.05	1	4 " , Ag ₁			19	24	12
0.05	1	14 " , "		γ ₁		95	20
0.05	1	14 " , "		γ ₁ -β		24	6
0.1	1	3 " , "	A †			250	25
0.1	1	4 " , "	B			100	15
0.1	5	2 " , "	A			150	<4
0.1	5	4 " , "	B			400	<4
0.1	5	9 months, "	C			500	500
0.2	1	4 days, "	B			150	<4
0.2	1	4 " , Ag ₃			19	250	<4
0.2	1	7 " , Ag ₁	D			80	<4
0.2	1	7 " , Ag ₃			19	150	<4
0.2	1	14 " , "			19	50	<4
0.2	1	9 months, Ag ₁			7	300	400
0.2	5	4 days, "	B			400	<4
0.2	5	9 months, "	C			500	800
0.3	5	4 days, "	B			400	<4
0.3	5	9 months, "	C			500	600
0.4	1	7 " , "			7	100	100
0.6	1	7 " , "			7	100	100
0.8	1	7 " , "			7	100	180

* Serum collected 3 days after a second injection of poliovirus.

† A, Serum containing only 19S antibody; B, Molecular type of antibody in the serum not determined; C, Serum containing only 7S antibody; D, Serum containing 19S antibody plus minor amounts of 7S antibody.

body in the gradient for serum samples collected from the same animal at various intervals after a single injection of PV (4×10^8 PFU).³ The highly potent 14 day serum was diluted 1:6 prior to density gradient centrifugation, which explains the lower protein levels in this gradient. The position of the bromphenol blue-labeled albumin ($\cong 4.5S$) is indicated in the figure.

³ It can be seen that there is a frequent occurrence of antibody activity of unknown sedimentation properties in the last fractions collected from the sucrose column. By rerunning such top fractions, as well as incubating them with mercaptan, it was demonstrated that the activity was due to both 19S and 7S antibodies. When gradients were collected instead by siphoning fractions from the top of the sucrose column, no activity was observed in the top fractions.

As serum fractions, which neutralized 100 per cent of the virus, were not further diluted to determine their actual activity, the data are semiquantitative and illustrate only the location of the activity. The antibody in the 2 day serum was obtained in the bottom fractions of the tube. When these fractions were rerun in another gradient, the activity was again recovered in the most rapidly sedimenting fractions. In the 3 day serum the activity was still due to rapidly sedimenting antibody, corresponding to the macroglobulins on the

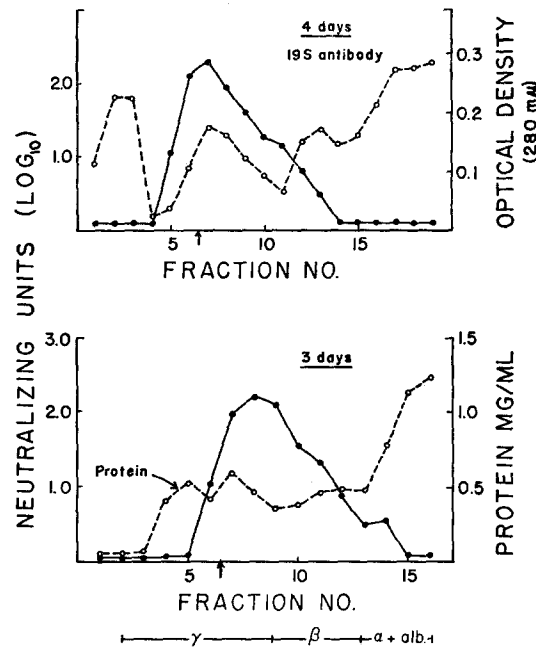


FIG. 5. Electrophoretic distribution of poliovirus-neutralizing antibody (●) of the 19S type, obtained 3 and 4 days following a single intravenous injection of virus. The 19S antibody in the 4 day serum was isolated by density gradient centrifugation in sucrose prior to electrophoresis.

protein curve, but on the 4th day also antibody of low molecular weight (\cong 7S) appeared. The electrophoretic analysis of the same sera (Fig. 1) revealed that this early appearing 7S antibody was a γ_1 -globulin. In 1 and 2 week sera (Fig. 4) high molecular weight antibody was still responsible for the major activity. But 3 to 4 months postimmunization the titer of the 19S type antibody had dropped to a level below that in the 3 day serum, while in contrast, the 7S antibody remained at a moderately high titer (1).

A small intermediate peak of antibody activity was occasionally observed in 7 and 14 day sera. Fig. 4 shows a gradient (the 14 day sample) in which this intermediate peak is more distinct than usual. Treatment of the pooled fractions

from each of the three peaks with 0.2 M 2-mercaptoethanol for 24 hours at 3°C caused no loss in 7S antibody activity but marked reduction in the activity of 19S and intermediately sedimenting antibody. This suggested that the intermediate peak did not consist of 7S aggregates.

Intermediately sedimenting activity was recovered, mixed with an anti-serum to Coxsackie B-4 virus which contained both 19S and 7S antibodies, and rerun in another gradient. The gradient was assayed simultaneously for neutralizing activity to PV and Coxsackie B-4 virus. The activity to PV showed three similar peaks of low activity; two of which corresponded to the 19S and 7S antibody peaks to Coxsackie virus, and a third intermediate (11–15S) peak.

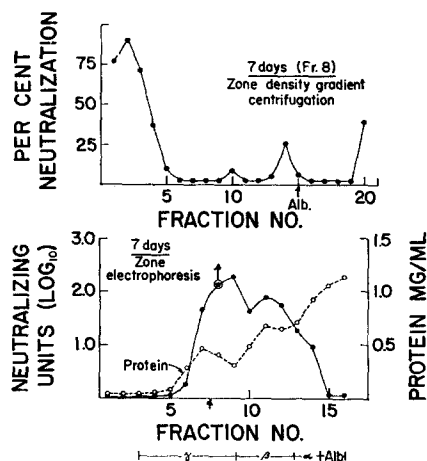


FIG. 6. Electrophoretic distribution of antibody activity (●) and protein (○) in a 7 day serum (bottom figure), and in an eluate from the γ_1 -region of this starch block when subjected to density gradient centrifugation in sucrose (top figure).

Effect of 2-Mercaptoethanol on the Antibody Activity of Whole Sera and Serum Fractions.—Further studies were concerned with determining whether different whole sera and serum fractions showed variations in their lability to sulfhydryl compounds (4, 5). Table I summarizes results obtained with 2-mercaptoethanol at final concentrations of 0.05 to 0.80 M. Incubation with 0.10 M mercaptan for 5 days at 3°C effectively abolished the neutralizing activity of sera or serum fractions in which only macroglobulin antibody was demonstrable by the density gradient centrifugation technique. Using only 1 day incubation at 3°C 0.20 M 2-mercaptoethanol was needed to achieve the same effect. In contrast, antibody titers of late immune sera, found to contain only 7S antibody in the sucrose gradient, were not reduced following incubation with as much as 0.8 M 2-mercaptoethanol. On the contrary, a slight increase in the titers of late 7S antibody was occasionally observed after such mercaptan treatment.

Correlation between Electrophoretic Mobilities and Sedimentation Coefficients of Antibody.—When sera collected prior to 4 days after the single virus injection were subjected to starch block electrophoresis or ultracentrifugation in a sucrose gradient a fairly well defined single peak of antibody activity was observed in both fractionation procedures (Figs. 1 and 4). Fig. 5, bottom figure, shows the electrophoretic distribution of activity obtained with a 3 day serum. Although the activity was no longer located mainly in the β -region, like in 2-day sera, but predominantly in the γ_1 -region, in the sucrose gradient (Fig. 4) activity was still demonstrable only in the bottom fractions. Thus, while one day earlier most 19S antibody was associated with β -globulins (Figs. 1 and 2), the 19S antibody synthesized on day 3 was a γ_1 -globulin.

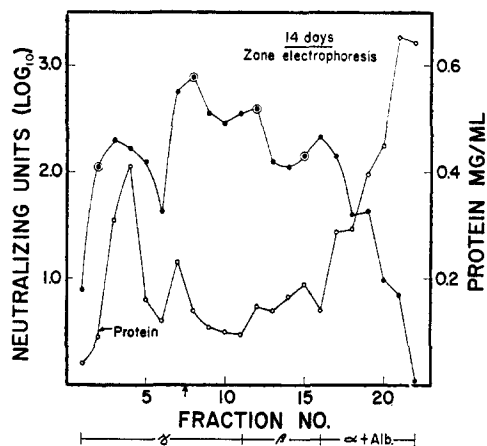


FIG. 7. Electrophoretic distribution of poliovirus-neutralizing antibody (●) and protein (○) in a serum collected 14 days following a single intravenous virus injection (4×10^8 PFU).

It was determined whether the net charge of the 19S type antibody would be still further reduced in the 4 day serum. To exclude the small amounts of 7S γ_1 -antibody present in this serum (Figs. 1 and 4) from the electrophoretic analysis, 19S antibody isolated by density gradient centrifugation was used. The electrophoretic distribution of the 19S antibody activity in the 4 day serum (Fig. 5, top figure) was similar to that in the 3 day serum, indicating a continued synthesis of the 19S γ_1 -antibody. A 7 day serum, earlier found to contain mostly 19S antibody, was subjected to electrophoresis (Fig. 6), and activity from the γ_1 -region (Fr. 8) was then rerun in a sucrose gradient. As seen in Fig. 6, top figure, this γ_1 -activity was associated with macroglobulins and minor amounts of "light" antibody. The low titered 7S antibody in this serum was located in starch eluates 7 and 8. Thus, these results demonstrated that the 19S antibody synthesized between days 3 and 7 (and as will be shown below also on day 14) as well as the 7S antibody produced between days 4 and 7 after antigenic stimulation were both γ_1 -globulins.

Fig. 1 showed that with time after immunization a progressive shift of the mobility of the major antibody activity from the β - to the γ_2 -region occurred. However, although the relative amount of antibody of high net charge greatly decreased, its synthesis continued during the shift. Consequently, the electrophoretic distribution of antibody became increasingly complex with time after

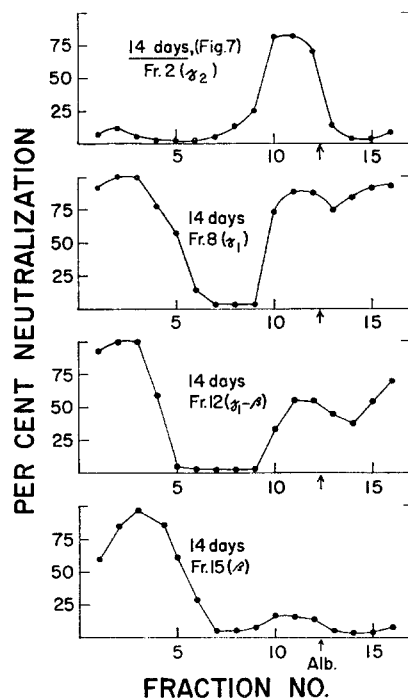


FIG. 8. Relationship between electrophoretic mobility and sedimentation coefficient of antibodies. A 14 day serum was subjected to zone electrophoresis (Fig. 7). The figure above shows the distribution of high and low molecular weight antibody in four eluates recovered from representative regions of this starch block as determined by zonal density gradient centrifugation.

immunization. This is well illustrated by the 14 day serum in Fig. 7 where 3 or 4 peaks of activity are observed. As 14-day sera contained both 7S and 19S antibodies the electrophoretic distribution of these two antibodies in the starch block in Fig. 7 was studied. Four starch eluates, with antibody activity recovered from representative regions of the block, were centrifuged in sucrose gradients and the positions of their neutralizing activity determined. The results obtained with Fr. 15 (Fig. 8, bottom figure) demonstrated that, like in early sera, a macroglobulin antibody was responsible for the activity in the β -region. The γ_1 - β -activity (Fr. 12) was also associated with a macroglobulin

antibody although traces of "light" antibody were found in this fraction. In Fr. 8 from the γ_1 -globulins, the main activity was still due to rapidly sedimenting antibody but this fraction also contained a considerable amount of 7S antibody. Consequently, the γ_1 -peak (Fr. 8) could contain rapidly sedimenting 7S aggregates and to test this the bottom fractions from the sucrose gradient were incubated with 0.10 M 2-mercaptoethanol for 5 days at 3°C. This treatment almost completely abolished their neutralizing activity, indicating that polymers of 7S antibody did not appreciably contribute to the activity in the bottom fractions of the gradient. Finally, when Fr. 2, from the slow γ_2 -region,

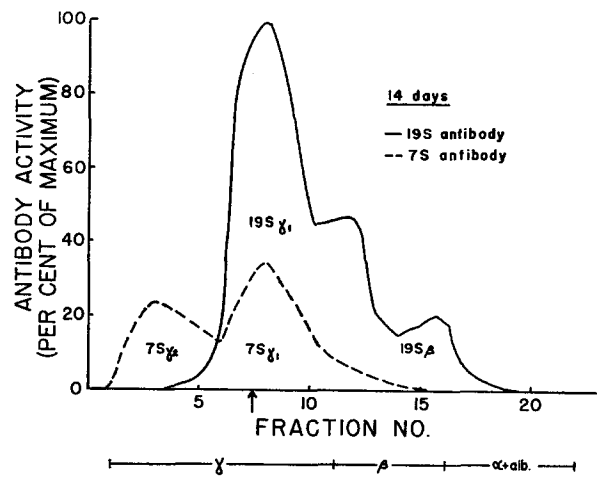


FIG. 9. Electrophoretic distributions of 19S and 7S type poliovirus neutralizing antibodies of rabbit origin. The serum was obtained 14 days after a single intravenous administration of poliovirus (4×10^9 PFU).

was centrifuged in the gradient, its activity was found to be due to 7S antibody. It should be emphasized that the data in Fig. 8 are semiquantitative and that the actual 7S antibody titer in Fr. 12 was very low compared to that of the 19S antibody.

Based on the data from electrophoresis and density gradient centrifugation a quantitative analysis was attempted of the electrophoretic distribution of PV-neutralizing antibody of the 19S and 7S type, obtained by density gradient fractionation of a 14 day serum. The results are shown in Fig. 9. 19S antibody was associated with both γ_1 - and β -globulins. Thus, although shortly after immunization, the major 19S antibody shifted from β - to γ_1 -globulins, the synthesis of the first appearing (days 1 to 2) 19S β -antibody apparently continued. The existence of a third intermediate 19S antibody (γ_1 - β) population was sometimes suggested. 19S antibody was never found to be of the γ_2 type.

7S antibody, in contrast, was associated with both γ_1 - and γ_2 -globulins in 14-day sera (Fig. 9). The 7S γ_1 -antibody, previously observed in 4- and 7-day sera (Figs. 1 and 4), dominated also in 14-day sera. In late immune sera (Fig. 3) 7S antibody was mainly associated with γ_2 -globulins. 7S antibody activity was first observed in the γ_2 -region 10 to 14 days following a single intravenous injection of PV.

DISCUSSION

Previous papers (1, 12) in this series reported a very rapid formation of macroglobulin antibody in the rabbit following a single intravenous injection of PV. In the present study this early appearing (day 1 to 2) 19S type antibody was found to migrate in zone electrophoresis as a nearly homogeneous component in the β -region, while the major 19S antibody subsequently synthesized (from day 3 on) was associated with γ_1 -globulins. Simultaneously to this reduction in its electrophoretic mobility the avidity of the 19S antibody increased greatly (6). Thus, two major 19S antibody populations, differing in their electrophoretic mobility, were observed. It was recently reported that chickens responded to bovine serum albumin with the formation of two kinds of 19S antibodies, which however occurred in fast and slow moving γ -globulin fractions (13).

At this point it should be mentioned that, employing a highly sensitive assay for antibody, sera from non-immunized rabbits often possessed low neutralizing activity against a variety of antigens, among them PV (1). Further, this background activity to PV was associated with mercaptan-sensitive 19S γ_1 - β -globulins which formed weak complexes with the virus (6). Similar findings were made when Coxsackie B-4 virus was used. These observations suggest that the first response to the administration of antigen is the stimulation of the production of a "normal macroglobulin antibody" which is being synthesized at a low rate prior to immunization.

Two types of 7S antibodies have been described in man (14), mouse (15), in horses following prolonged immunization with diphtheria toxin (16), and recently also in guinea pig (17) and chicken (13). Benacerraf *et al.* (17) reported that 7S γ_2 -antibody appeared prior to 7S γ_1 -antibody. This sequence in the response of guinea pigs to protein antigens and hapten conjugates differs from that observed here in the response of rabbits to PV; the synthesis of 7S γ_1 -antibody to PV preceding the formation of 7S γ_2 -antibody.

The recognition of two physically and chemically distinct classes of antibodies (7S and 19S), and the sequence of their appearance after immunization (1, 10, 18-21) has led to the suggestion that different cell types may be responsible for their production (11, 20, 22). A comparison of other parameters (than the kinetics) of the formation of 19S and 7S antibodies to PV (11) also revealed marked differences which are best explained on the assumption that the two

antibodies have different cellular origin. The finding of electrophoretically distinct subclasses of 7S antibody (13–17), and of at least two electrophoretically separate 19S antibody fractions, as reported here and by Benedict *et al.* (13), raises the question whether four or more different cell lines now must be implicated in the production of antibody to a given antigen.

No information is available as yet on possible antigenic differences, or chemical differences (*e.g.* carbohydrate content) between these 19S antibodies of different electrophoretic mobilities. Of some interest is the finding that the 19S PV-neutralizing antibodies appear to be stable for several months at refrigerator temperature, in contrast to the reported lability of rabbit 19S antibody to diphtheria toxoid (10).

19S macroglobulins are known to dissociate into biologically inactive 7S components when properly treated with sulfhydryl compounds (4, 5). This treatment has been used as a means of identifying hemolytic and agglutinating antibody of the 19S type. But the effect of sulfhydryl compounds on neutralizing antibody has not been thoroughly investigated. The activity of PV-neutralizing antibody of the 19S type, whether present in whole serum or serum fractions, was abolished by incubation with ≥ 0.1 M 2-mercaptoethanol for a few days at 3°C. Incubation for only 1 day at 3°C with 0.1 M mercaptan caused incomplete inactivation of the 19S antibody. This suggests that the PV-neutralizing antibody of 19S type may require somewhat longer or more intense treatment with reducing compounds than other rabbit 19S antibodies (10, 23) to achieve complete loss of activity. The titers of 7S antibody to PV (obtained 2 weeks or 2 or 7 months after immunization) were unaffected by treatment with as much as 0.8 M 2-mercaptoethanol.

Depolymerization of 19S antibody to PV of high titer by 2-mercaptoethanol, without subsequent alkylation resulted in a residual antibody fraction (11). When the depolymerization was followed immediately by alkylation no such "mercaptan-resistant" antibody fraction remained. Thus, the fraction was very likely due to restoration of antibody activity by reoxidation following removal of the disulfide bond reducing compound. Jacot-Guillarmod and Isliker recently reported (24) that following dissociation of 19S anti-A and anti-B isoagglutinins with thiols little or no recovery of activity occurred upon reoxidation while the effect of borohydride was highly reversible.

Of particular interest was the finding that serum samples collected 1 week or later following antigenic stimulation often contained small amounts of antibody which sedimented at a rate intermediate to that of 19S and 7S antibodies. The best separation by the density gradient centrifugation technique of the three antibody peaks was obtained when 1- or 2-week sera were used. Since 7S antibody was produced in high titers at 2 weeks the intermediate antibody peak could represent 7S aggregates. But, the marked reduction of the activity of the intermediately sedimenting antibody following mercaptan treatment

made this unlikely. Neither was it likely that the intermediate component represented trailing of classical 19S antibody, as it was well separated from the main 19S peak. Judging from the resolution obtained with the 4.5S albumin and the 7S antibody the intermediate antibody appeared to sediment at a rate of 11S to 15S. When fractions from such intermediate antibody peaks were rerun in other sucrose gradients activity was again associated with an intermediately sedimenting component but also with considerable amounts of contaminating 19S and 7S antibodies. Although the electrophoretic mobility of the intermediately sedimenting antibody was not directly tested, the antibody should be associated with γ_1 - or β -fractions, since these were the only fractions with activity in 7-day sera. Characterization of the intermediately sedimenting activity is difficult, since it is present in very small amounts and only in sera which also contain 19S and 7S antibodies in high titers.

Boyd (25) discussed the possibility that certain human isoagglutinins may have molecular weights between those of serum globulins (about 160,000) and macroglobulin antibodies (about 900,000). However, only very recently Rockey and Kunkel (26) reported experimental evidence for the production of iso-hemagglutinins and a skin-sensitizing antibody of intermediately sedimenting rates (8S to 15S). These authors also discussed the possible antigenic relationship of these antibodies to the β_{2A} (γ_{1A}) class of immunoglobulins, some of which have similar sedimentation properties (27) and are split by mercaptan treatment (28). Mercaptoethanol sensitive, slowly sedimenting antibody has also recently been reported in non-mammalian vertebrates such as ducks (29), frogs, and goldfish (30). The duck antibody, described by Grey (29), was associated with β_2 -globulins in immunoelectrophoresis and resembled, in some ways, the mammalian β_{2A} -immunoglobulins.

SUMMARY

Rabbits injected intravenously with a single high dose of poliovirus (type 1) produced two major 19S and two 7S type neutralizing antibody populations, with different electrophoretic mobilities.

The first 2 to 2½ days after immunization the antibody was of the 19S type and migrated as a single component in the β -region. In contrast, the major 19S antibody synthesized from day 3 on was a γ_1 -globulin. 7S type antibody, appearing on day 4, was contained only in the γ_1 -region, while 10 to 14 days following immunization the 7S antibody was associated with both γ_1 - and γ_2 -globulins. The electrophoretic distribution of antibody in 2-week sera revealed the presence of at least four antibody populations (19S β , 19S γ_1 , 7S γ_1 , and 7S γ_2).

Serum samples collected 1 or 2 weeks following antigenic stimulation often contained small amounts of antibody which sedimented at a rate (11S to 15S) intermediate to that of 7S and 19S antibodies.

The neutralizing activity of the 19S type antibodies and the intermediately sedimenting antibody was destroyed by incubation with 0.1 M (5 days) or 0.2 M (1 day) 2-mercaptoethanol at 3°C. The 7S antibody activity was unaffected by treatment with from 0.1 to 0.8 M mercaptan.

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