Neonatal Immunity. II. Poliocidal Effects of Human Amniotic Fluids.*† (24425)

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The search for antibodies in body fluids and an interest in neonatal immunity has led to a search for antibody to the 3 polioviruses in amniotic fluid of the human subject at term. Although amniotic fluid is of vast importance to development of the fetus, information regarding its composition is scant. Data are available on total protein content and on concentration of various electrolytes(1). Little is known of the amounts of various blood proteins present such as gamma globulin, serum albumin, other globulins and fibrinogen. Work by Brambell and his colleagues(2) has shown that in the rabbit, antibodies appear in amniotic fluid after injection into maternal circulation. They also demonstrated the subsequent passage to and concentration of these antibodies in the stomach of the fetal rabbit, and suggested that it is conceivable that antibodies in the human newborn might come by way of the amniotic fluid. The steps might be as follows: swallowing, and concentration of fluid in the stomach and passage through stomach wall into the peripheral circulation. Concentration of proteins in amniotic fluid taken at birth has been reported(1) to vary from 0 to 1.5 g/100 ml of fluid, average 0.53 g. Quantitative data for gamma globulin content of amniotic fluid taken at term are at present not available. Amniotic fluid has been reported to be bacteriocidal(3). The nature of activity has not been established. This paper is a report on the presence of antibodies to the 3 poliovirus types in amniotic fluids of mothers who delivered normally at term.

Methods. Amniotic fluids (AF) free of blood were collected from women with unruptured membranes who delivered normally at term. One specimen was collected from patient at Caesarean section. Mothers' (MB) and babies' cord bloods (CB) were also collected. Mothers were in general of a low economic status and included both polio vaccinated and non-vaccinated individuals. Most had a high level of antibody to the 3 poliovirus types. The mothers were of various races, ages and previous obstetric experience. The fluids on arrival at laboratory were centrifuged, and those containing sedimented blood cells were excluded from this study. Some fluids were titrated for neutralizing antibody both before and after heat inactivation at 56°C for 30 minutes, others only after inactivation. Neutralizing antibody titers of amniotic fluid specimens and corresponding sera of mother and infant were determined simultaneously by a modification of the suspended cell colorimetric test for neutralizing antibodies, using first passage monkey kidney cells. Amniotic fluids cleared by centrifugation were diluted in 2-fold dilutions starting with undiluted fluid to a dilution of 1:512. Maternal and infant cord sera were titrated by doubling dilutions starting with initial dilution of 1:4. To each dilution was added 100 $TCID_{50}$ of a single type of poliovirus. All specimens were titrated for neutralizing antibody to all 3 poliovirus types. After incubation of amniotic fluid-virus and serum-virus mixtures at room temperature for 1 hour the monkey kidney cells obtained from bottles seeded with trypsinized kidney tissue were added to each tube and incubated 7 days at 37°C. The diluent used was 2% calf serum in Hanks' balanced salt solution with 0.5% lactalbumin hydrolysate. The test was read by microscopic examination for presence or absence of cytopathogenicity. The titer was expressed as the reciprocal of the last dilution of serum or amniotic fluid which showed complete protection. Gamma globulin was determined in amniotic fluids as follows: amniotic fluids cleared of cellular debris were diluted in 4-fold steps

* This investigation was supported by research grant from Nat. Inst. of Allergy and Infect. Dis.

† The authors wish to express their thanks to Dr. Douglas Haynes, Dept. of Obstetrics and Gynecology for assistance in obtaining specimens.

TABLE I. Distribution of Poliomyelitis Antibodies in Amniotic Fluid (AF), Maternal Serum (MB) and Cord Serum (CB) at Term.

Patient	Age	P/G†	Type 1 AF	Type 1 MB	Type 1 CB	Type 2 AF	Type 2 MB	Type 2 CB	Type 3 AF	Type 3 MB	Type 3 CB
						No record of vaccination					
1	16	1/1	64	1024	1024	256	4096	4096	0	32	32
2	16	1/1	0	64	32	0	128	16	0	64	16
3	22	2/2	1	64	64	64	2048	2048	4	256	256
4	24	4/4	4	512	256	2	128	128	2	128	128
5	25	4/5	0	<4	<4	4	512	512	0	64	64
6	26		2	64	128	2	256	256	0	32	32
7	29	6/6	0	32	32	8	1024	512	0	128	128
8	35	3/3	0	128	128	2	256	256	0	64	64
9*	37	2/3	<4	32		2	256		<4	64	
10	39	11/14	0	32	16	1	64	64	0	16	16
11		8/9	0	128	128	16	512	512	1	128	128
						1 ml polio vaccine					
12	15	1/1	4	256	512	2	1024	512	2	512	256
13	19	2/2	4	1024	512	8	1024	512	8	2048	128
14	26	6/7	2	1024	64	<2	1024	16	<2	1024	16
15	29	4/4	0	128	64	8	2048	1024	8	1024	1024
						2 ml polio vaccine					
16	16	1/1	<16	<4	<4	<16	<4	<4	<16	<4	<4
17	18	2/3	16	256	128	8	1024	512	16	512	128
18	22	3/4	16	512	512	2	128	128	0	4	<4
19	25	4/4	4	1024	512						
20	26	6/6	4	2048	1024	2	1024	1024	4	4096	4096
21	30	11/11	0	64	32	2	128	64	1	64	64
22	38	5/5	64	256	256	32	512	256	32	256	256

* Caesarian section.

† P/G = parity/gravidity.

with saline. To 0.6 ml of each dilution was added 0.1 ml of anti-human gamma globulin rabbit serum whose precipitin titer (antigen-dilution method) was 1:50,000. The tubes were incubated at 37°C for ½ hour and over night at 4°C. Precipitation or flocculation was recorded. In a single experiment gamma globulin was concentrated from amniotic fluid using ammonium sulfate. Saturated ammonium sulfate was added to cold amniotic fluid to final concentration of 50% saturation. The precipitate so obtained was dissolved in one-tenth the original amniotic fluid volume, a 10-fold concentration. After dialysis at 4°C for 48 hours this solution was tested for presence of gamma globulin by the method outlined above. This solution was then titrated for presence of neutralizing antibodies against the 3 poliovirus types simultaneously with the original amniotic fluid.

Results. Table I gives data for each of 23 cases. In general, the ratio of neutralizing antibody of amniotic fluid to that of mother's serum, for each virus type is a constant within the limits of experimental error (Table I). An ideal case for demonstrating this is specimen from patient 3 where the ratio of AF to MB is 1:64 for types 1 and 3 and 1:32 for type 2 poliovirus. In instances where the amniotic fluid titer is 0 (less than undiluted) this ratio could not be determined. Disparity between observed antibody levels in matched pairs of cord and maternal blood is occasionally observed (*e.g.* patients 13 and 14), as discussed previously(4).

Twelve amniotic fluids were tested for presence of gamma globulin by the immunological procedure discussed under methods: The 12 were positive for presence of gamma globulin in dilutions ranging from 1:16 to 1:1024.

The polio-neutralizing antibody titers expressed as dilution reciprocals obtained in the single gamma globulin concentration experiment are as follows:

	Type 1	Type 2	Type 3
Original amniotic fluid	4	8	8
10 × concentrated amniotic fluid	16	16	64

The concentrated solution was titrated for

presence of gamma globulin and compared with original fluid as follows: The endpoint of original fluid was between a dilution of 1:16 and 1:64 whereas the endpoint using concentrated material was 1:160. The concentrated solution on this basis was not 10-fold as expected but, closer to the 5-fold as indicated by the polio neutralizing antibody titers.

Discussion. The finding of neutralization of 3 poliovirus serotypes by human amniotic fluid is not unexpected, but the question arises as to whether this poliocidal activity is due to antibody. The demonstration of gamma globulin in the fluid, and the result of the concentration experiment make it likely that this activity is due to an immune mechanism. On the basis of an experiment in which both heated and unheated amniotic fluid was titrated for neutralizing activity it can be said that there is present a heat labile factor besides the heat stable factor which is poliocidal. Whether this is an antibody cannot be determined from our data.

The second question concerns the source of the antibody. The fact that antibodies to the 3 polioviruses are in almost constant ratio to maternal serum, points to transmission of serum gamma globulin from her blood stream to the amniotic fluid. The mechanism by which this occurs has not been elucidated.

It must be borne in mind that the relationship in antibody content of amniotic fluid, cord blood, and maternal blood is not constant for all antibodies. For example, it is well established that complete Rh antibodies do not pass into fetal blood as readily as incomplete Rh antibodies(5) and antibodies against the O antigens of enteric bacteria not as readily as flagellar antibodies(6). In preliminary experiments, unpublished, (Neter) on several specimens described in this paper the presence of antibodies against various O antigens of enteric bacteria was determined. The procedures employed have been described(7,8). The results obtained using selected amniotic fluids are shown in Table II. It was found that antibodies to various enterobacterial antigens used in the test appeared in only 1 of 6 amniotic fluids. This patient #22 also had high poliovirus neutralizing antibodies to the 3 types in her AF. These antibodies were in the ratio of 1:4, 1:16 and 1:8 respectively for the 3 types. On the other hand the ratio of enterobacterial antibodies were 1:2, 1:4 for hemagglutinins and 1:4 and 1:8 for Coombs hemagglutinins.

TABLE II. Enterobacterial Antibodies in Human Amniotic Fluids (Neter, 5).

Patient	Specimens source	Hemagglutinin titers (reciprocal) Sa	Sh	EC	Coombs hemagglutinin titers (reciprocal) Sa	Sh	EC
1	AF	—	—	—	—	—	—
	MB	80	320	80	80	320	80
	CB	—	—	—	40	20	20
3	AF	×	—	—	×	—	—
	MB	40	20	10	40	×	10
	CB	—	—	—	20	—	—
4	AF	—	—	—	—	—	—
	MB	40	80	80	×	160	80
	CB	×	×	×	×	×	×
13	AF	—	—	—	—	—	—
	MB	×	×	×	×	×	×
	CB	—	40	—	80	80	20
20	AF	—	—	—	—	—	—
	MB	160	80	160	160	160	160
	CB	—	—	—	40	40	20
22	AF	40	10	20	40	10	20
	MB	80	40	80	160	×	160
	CB	×	×	×	×	×	×

AF = amniotic fluid; MB = maternal serum; CB = cord serum.
Sa = polyvalent *Salmonella* groups A_1, C_2, D_1, E_1. Sh = polyvalent *Shigella flexneri* 3, *boydii* 1, *sonnei*. EC = polyvalent *Escherichia coli* 111, 55, 26.
— = less than 10; × = not done.

Patient 1, whose poliomyelitis antibody levels in the amniotic fluid were high for types 1 and 2 with a ratio of AF to MB of 1:16, had no detectable antibodies to the enterobacterial antigens. If the passage from maternal circulation to amniotic fluid were due to simple diffusion the ratio of 1:16 should hold and there would have been expected antibodies of a titer 10 to 20 to the polyvalent Shigella antigens by both methods used.

Perhaps the most important question concerns the protective role of amniotic fluid neutralizing substance. Our experiments probably, however, rule out the thought that the antibody in the fetus comes only or to a major degree by way of the amniotic fluid. The good agreement between amounts of polio neutralizing antibody in maternal and infant sera is evidence against the role of amniotic fluid

as a source of infant antibody. If the mechanism involved this fluid the discrepancies between the 2 circulations would be expected to be much greater than the data indicate. Furthermore, many of the amniotic fluids do not have detectable antibody to some of the poliovirus types and the cord serums have appreciable levels of antibody.

Summary. Amniotic fluid of humans at birth has been demonstrated to contain poliocidal substance, active against all 3 serotypes of virus. Evidence has been presented which indicates that the activity is associated with the gamma globulin present in the amniotic fluid. The poliocidal activity is believed to be specific antibody. The presence of these antibodies has no correlation as far as can be determined with the age, color, polio immunization status of the mother nor with her previous obstetrical history. The level of antibody in the amniotic fluid for the 3 polioviruses was in proportion to the levels in the blood serum and in general was demonstrable when the serum level was high. There is also in amniotic fluid an inhibitor to the 3 polioviruses which is heat labile. Its character has not been determined. Enterobacterial hemagglutinins have been demonstrated in 1 of 6 AF tested.

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Received July 14, 1958. P.S.E.B.M., 1958, v99.

Porcine Kidney Cell Cultures for Propagation and Assay of Japanese Encephalitis Virus.* (24426)

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Observations in 1956 at U.S. Army 406th Medical General Laboratory near Tokyo, Japan showed that swine serve as important source of Japanese encephalitis (JE) virus for *Culex tritaeniorhynchus,* Japanese vector mosquito, (unpublished). This finding together with the knowledge that JE virus causes stillbirth in swine(1), stimulated inquiry into possible effects of JE virus upon swine tissues *in vitro.* The initial study employed cultures of swine kidney because the basic principles were known for preparation of primary cultures from renal tissues(2). This article reports that cells in primary cultures from porcine kidney (PK) are rapidly destroyed by each of 5 strains of JE virus and concurrently support virus multiplication. Cultures of swine kidney cells thus furnish a tissue culture system for assay and propagation of JE virus.

Materials and methods. Viruses. Four strains of JE virus were isolated in Japan during 1955 and 1956 from swine blood, Black-crowned Night Heron blood, human brain, and triturated mosquitoes (*Culex tritaeniorhynchus*) (Table I). The fifth virus was Nakayama strain from Dr. C. M. Eklund (USPHS Rocky Mountain Laboratory). Mouse brain suspensions (10% in 10-40% rabbit serum in Hanks' solution) and cultural fluids were stored in sealed glass ampules at −70°C for as long as 2 months before being assayed. Cultural fluids were kept above pH 7.2 by addition of 1.4% aqueous sodium bicarbonate solution to minimize inactivation of JE virus that occurs at lower pH's(3). Kid-

* Sponsored by grants from Office of Surgeon General, Dept. of Army, and N.I.H., and fellowship from Nat. Cancer Inst., N.I.H.