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Received July 22, 1957. P.S.E.B.M., 1957, v96.

# Neonatal Immunity. I. Disparity in Maternal-Infant Poliovirus Antibody.* (23474)

MURRAY M. LIPTON AND ALEX J. STEIGMAN

*Kentucky Child Health Fn. Laboratory, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Ky.*

The studies here reported concern the titers of poliovirus antibodies to the 3 serotypes in the serum of mothers and their newborn infants. It represents part of a study on the local formation and transfer of antibody *in utero*. Since we are dealing exclusively with infants at the moment of birth, the possible role of colostrum will not be discussed nor will the uncertain role of the placenta in production of antibodies. The subject of active and passive immunity of the infant has been reviewed by Edsall(1). Previous reviews include one on placental permeability by Doerr (2). One of the earliest observations of antibody transfer in the human from mother to infant concerned that of diphtheria in 1895 (3). Later studies demonstrated transmission of tetanus antibodies(4). More recently, however, the concern has been with the quantitative aspects of maternal transmission of antibody and, therefore, neonatal immunity.

Although one might expect all antibodies to behave similarly with respect to maternal-infant transmission, this does not appear to be the case. Reagins reportedly do not appear in the umbilical serum whereas blocking antibodies do(5). Viruses may differ among themselves in this respect, thus in Japanese B encephalitis(6) and mumps(7) no disparities have been observed whereas they have been found in vaccinia(8). Employing untyped poliovirus in qualitative tests in monkeys, Aycock and Kramer reported neutralizing antibody in 10 of 12 matched maternal-cord serums tested(9). Among the 3 serotypes of polioviruses we have observed disparities in titer most marked in Type 3. Rather than stress the anticipated agreements we have chosen to consider the possible meaning of the observed disparities among the 3 serotypes.

*Methods.* A series of 48 mothers' and corresponding umbilical cord bloods were collected aseptically generally at term; this includes 4 prematures and 3 sets of twins. The immunization record of each mother in regard to poliomyelitis was recorded but not known at the time of antibody titration. Mothers were of various ages, color, and previous obstetrical history who were seen in pre-natal clinics and delivered at a city hospital. The antibody titrations were performed *in vitro* by a modification of the technic originally published by Salk, Youngner and Ward(10). Monkey kidney cells were obtained by seeding bottles with trypsinized monkey kidney tissue. After 1 week the cells were harvested by use of versene and used in a suspended cell neutralization test using 2% calf serum, 0.5% lactalbumin hydrolysate in Hanks' BBS. Twenty thousand cells were used/tube. The matched maternal and umbilical cord serums were always titrated simultaneously, dilutions of both being made by the same worker. For antibody titrations versus each virus type, 100 $TCD_{50}$ of poliovirus either type 1 (Mahoney), type 2 ($MEF_1$), or type 3 (Saukett)

* This investigation was supported by research grant from Natl. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

TABLE I. Antibody Titer Distribution for Polioviruses in Maternal and Corresponding Cord Serums. No disparities.

| Age | P/G* | Type 1 MB† | Type 1 CB† | Type 2 MB | Type 2 CB | Type 3 MB | Type 3 CB |
|---|---|---|---|---|---|---|---|
| No record of poliomyelitis vaccination | | | | | | | |
| 16 | 1/ 1 | 1024‡ | 1024 | 4096 | 4096 | 32 | 32 |
| 17 | 1/ 1 | 512 | 512 | 512 | 1024 | 256 | 256 |
| 18 | 1/ 1 | 64 | 64 | 128 | 128 | 128 | 128 |
| 20 | 2/ 2 | 32 | 16 | 128 | 128 | 128 | 64 |
| 21 | 1/ 1 | 512 | 512 | 512 | 512 | 512 | 512 |
| 22 | 2/ 2 | 64 | 64 | 2048 | 2048 | 256 | 256 |
| 24 | 4/ 4 | 512 | 256 | 128 | 128 | 128 | 128 |
| 25 | 4/ 5 | <4 | <4 | 512 | 512 | 64 | 64 |
| 26 | — | 64 | 128 | 256 | 256 | 32 | 32 |
| 29 | 6/ 6 | 32 | 32 | 1024 | 512 | 64 | 64 |
| 35 | 3/ 3 | 128 | 128 | 256 | 256 | 64 | 64 |
| 39 | 11/14 | 32 | 16 | 64 | 64 | 16 | 16 |
| 42 | 4/ 4 | 512 | 512 | 2048 | 2048 | 256 | 256 |
| — | 8/ 9 | 128 | 128 | 512 | 512 | 128 | 128 |
| 1 ml of poliomyelitis vaccine | | | | | | | |
| 15 | 1/ 1 | 512 | 512 | 512 | 512 | 512 | 512 |
| 19 | 1/ 1 | 512 | 512 | 16 | 16 | 256 | 256 |
| 22 | 3/ 4 | 2048 | 2048 | 2048 | 2048 | 256 | 256 |
| 27 | — | 256 | 256 | 512 | 256 | 128 | 128 |
| 2 ml of poliomyelitis vaccine | | | | | | | |
| 14 | 1/ 1 | 1024 | 1024 | 1024 | 1024 | 64 | 64 |
| 16 | 1/ 1 | <4 | <4 | 4 | 4 | <4 | <4 |
| 19 | 1/ 1 | 1024 | 2048 | 512 | 512 | 512 | 512 |
| 19 | 3/ 4 | 32 | 32 | 256 | 256 | 8 | 8 |
| 20 | 2/ 2 | 32 | 32 | 1024 | 512 | <4 | <8 |
| §21 | 3/ 3 | 512 | 512 | 2048 | 2048 | 2048 | 2048 |
| 22 | 3/ 3 | 1024 | 1024 | 4096 | 2048 | 512 | 256 |
| 23 | 4/ 5 | 512 | 256 | 2048 | 2048 | 2048 | 2048 |
| 25 | 3/ 3 | 64 | 128 | 256 | 128 | 256 | 128 |
| 25 | 4/ 4 | 1024 | 512 | — | — | — | — |
| 26 | 6/ 6 | 2048 | 1024 | 512 | 512 | 2048 | 2048 |
| 28 | 6/ 6 | 128 | 128 | 128 | 128 | 1024 | 1024 |
| 29 | 7/ 7 | 128 | 128 | 512 | 512 | 512 | 256 |
| 29 | 6/ 8 | 16 | 8 | 512 | 1024 | 128 | 128 |
| 30 | 11/11 | 32 | 32 | 128 | 64 | 256 | 128 |
| 38 | 5/ 5 | 256 | 256 | 512 | 256 | 256 | 256 |

* P/G = Parity-gravid status.
† MB = Maternal blood; CB = Cord blood.
‡ Titer expressed as reciprocal of last serum dilution neutralizing virus.
§ Double ovum twins.

was admixed with doubling dilutions of serum using 2 or 3 tubes per serum dilution per type of virus. After incubation at room temperature for 1 hour, cells were added, the tubes were stoppered and incubated at 36°C. Endpoints were recorded as the last dilution which on microscopic examination at 5 to 7 days did not show degeneration or cytopathogenicity. In instances where large differences were found between mother serum and the corresponding cord serum the titration was repeated. For the purposes of this study a deviation of 1 twofold dilution was considered to be within the realm of experimental error; differences of 4-fold were considered noteworthy especially when observed upon repetition. Standard monkey antisera of the 3 serotypes† were titrated with each test. The type specific viruses were also titrated for each test.

*Results.* In the accompanying Tables I and II are tabulated serum neutralizing antibody titers of the matched maternal and cord sera listed in the order of the patient's poliomyelitis vaccination status and the maternal age. Table I enumerates those matched pairs of sera in which no significant disparities in titer (as described under methods) appeared. Table II enumerates those instances in which differences beyond the realm of experimental error were observed. The disparities do not appear to be related to maternal age, parity or poliomyelitis immunity status of the mother nor to the sex, color or birthweight of the newborn.

Two points may be seen in Tables II and III. In all instances where differences are noted the maternal serum has a higher titer than the corresponding cord serum. Secondly, the largest number of disparities is to be found in the antibody to the type 3 poliovirus with differences up to 64-fold. Type 2 antibody disparities appear less frequently. In only one instance it is the lone disparity (mother MC). Otherwise type 2 disparities are accompanied by type 3 differences and types 1 and 3 differences (Table III). In 1 instance (mother GS) the difference between maternal and cord serum is 64-fold for type 2 while only 4-fold for type 3. In 2 cases type 1 antibodies are higher in the maternal circulation than in the cord serum in the absence of antibody differences between these sera to the other poliovirus types. In both of these cases the disparity is only 4-fold. The other instances of a type 1 antibody disparity are in the presence of type 2 and 3 differences which in one instance (mother MB) are much greater for types 2 and 3 (64-fold) than for type 1 (16-fold).

One case is of special interest and may have

† Prepared by Dr. Herbert A. Wenner, University of Kansas.

TABLE II. Antibody Titers *vs* Polioviruses in Maternal and Corresponding Cord Serums. Disparities.

| Mother | Age | P/G | Type 1 | | Type 2 | | Type 3 | |
|---|---|---|---|---|---|---|---|---|
| | | | MB | CB | MB | CB | MB | CB |
| | | | No record of poliomyelitis vaccination | | | | | |
| CB | 14 | 1/ 1 | 128 | 128 | <4 | <4 | 16 | 4 |
| GS | 16 | 1/ 1 | 128 | 64 | 256 | <4 | 128 | 32 |
| MS* | 32 | 11/12 | 256 | 64 | 32 | 8 | 16 | <4 |
| | | | 1 ml of poliomyelitis vaccine | | | | | |
| AF | 19 | 2/ 2 | 512 | 512 | 1024 | 512 | 2048 | 64 |
| MB† | 26 | 7/ 7 | 1024 | 64 | 1024 | 16 | 1024 | 16 |
| AN‡ | 28 | 5/ 5 | 256 | 256 | 512 | 512 | 16 | <2 |
| DL | 29 | 4/ 4 | 128 | 32 | 2048 | 1024 | 1024 | 1024 |
| | | | 2 ml of poliomyelitis vaccine | | | | | |
| MP | 18 | 5/ 5 | 256 | 128 | 1024 | 256 | 512 | 128 |
| RG | 22 | 4/ 4 | 512 | 512 | 128 | 128 | 4 | 0 |
| JH | 22 | 2/ 2 | 256 | 256 | 512 | 512 | 128 | 32 |
| CN | 27 | 8/ 8 | 32 | 16 | 64 | 8 | 128 | 4 |
| EB | 31 | 6/ 6 | 256 | 256 | 256 | 64 | 256 | 32 |
| MC | 33 | 7/ 7 | 64 | 64 | 512 | 128 | 128 | 128 |
| | | | 3 ml of poliomyelitis vaccine | | | | | |
| MG§ | 30 | 5/ 5 | 512 | 128 | 1024 | 1024 | 512 | 512 |

For abbreviations see footnotes Table 1.

* 30 wk gestation. † 1 ml vaccine 1 wk before delivery. ‡ Double-ovum twins.
§ Single-ovum twins.

clinical significance. In Table II it can be seen that mother MB was given 1 ml of poliomyelitis vaccine 1 week prior to delivery. The significance of the striking differences observed between maternal and cord serum will be covered in the discussion.

In Table IV are the results obtained with sera from 3 sets of twins, 2 sets of which were heterozygous (mothers AN and AW) and the last uniovular (mother MG). In the AW twins antibody titers are approximately the same in all 3 specimens against the 3 poliovirus types. In the AN family types 1 and 2 antibodies are in equal titer for all 3 specimens. For type 3 poliovirus, however, the maternal serum antibody is 8-fold higher than the cord serums, both of which are the same. In the uniovular twins (mother MG) the type 1 antibodies are of 4-fold lower titer in the cord serum of each of the twins than in the maternal serum. Antibodies to the other poliovirus types are the same for all 3 specimens.

*Discussion.* The reason for the observed disparities in titer between maternal and umbilical cord serum is not known, nor for the disparity to be more frequent (Table III) for types 3, 2, and 1 polio antibody, in that order. The possible role of a bio-physical explanation is suggested by the work of Timasheff(11) who utilized electrophoresis convection to partition the antibodies of type 1 and 2 poliovirus; no observations were reported for the type 3. He observed human type 2 antibody in highest concentration (37-fold) in a slow-

TABLE III. Tabulation of Observed Titer Disparities by Serotype of Virus.

| Mother | Type 1 | Type 2 | Type 3 |
|---|---|---|---|
| DL | 4*† | | |
| MG‡ | 4 | | |
| MC | | 4 | |
| RG | | | >4 |
| AF | | | 32 |
| JH | | | 4 |
| CB | | | 4 |
| AN‡ | | | >8 |
| MP | | 4 | 4 |
| EB | | 4 | 8 |
| CN | | 8 | 32 |
| GS | | 64 | 4 |
| MB§ | 16 | 64 | 64 |
| MS | 4 | 4 | >4 |

* In all instances maternal higher than corresponding cord serum.
† Indicates fold difference in titer between maternal and cord serums.
‡ Twins—both twins had same titers.
§ Received 1 ml poliomyelitis vaccine 1 wk prior to delivery.

TABLE IV. Poliomyelitis Antibody Titers of Maternal and Cord Serums of Twins.

| Mother | Age | P/G* | ml polio vaccine | Spec. source | Sex of newborn | Type of twin | Poliovirus antibody Type 1 | Type 2 | Type 3 |
|---|---|---|---|---|---|---|---|---|---|
| AN | 28 | 5/5 | 1 | Mother | | | 256 | 512 | 16 |
| | | | | Twin 1 | ♂ | Double ovum | 256 | 512 | <2 |
| | | | | " 2 | ♀ | | 128 | 512 | <2 |
| AW | 21 | 3/3 | 2 | Mother | | | 512 | 2048 | 2048 |
| | | | | Twin 1 | ♂ | *Idem* | 512 | 2048 | 2048 |
| | | | | " 2 | ♀ | | 512 | 1024 | 2048 |
| MG | 30 | 5/5 | 3 | Mother | | | 256 | 1024 | 256 |
| | | | | Twin 1 | ♀ | Single ovum | 64 | 1024 | 256 |
| | | | | " 2 | ♀ | | 64 | 1024 | 256 |

* P/G = Parity-gravid status.

moving gamma globulin fraction T 1. T 2 fraction of slightly greater mobility revealed a concentration of 17x while the T 3 fraction revealed antibody only 5x as active as the original serum. On the other hand the type 1 antibodies revealed maximum concentration (43x) in the T 4 gamma globulin fraction of intermediate mobility. In the monkey hyperimmunized with type 2 live attenuated virus, specific antibodies appear principally in a slow-migrating $\beta$ globulin fraction rather than in the gamma globulin component(11).

It would appear that the configuration and mobility of the several specific types of antibodies varies in complexity. Our data suggest that Type 1 antibody was most easily transferred from mother to offspring; the fact that the Type 1 poliovirus is the poorest antigen of the three, may be related to its yielding antibody particles which are smaller and less complex, hence easily transferred. Such physical factors might explain failure of antibody (hemagglutinins) to the pathogenic *E. coli* being transferred to the human newborn while antibody to the non-pathogenic *E. coli* are transferred(12).

How and when do the various antibodies find their way from the maternal circulation to that of the newborn? Patient MB (Table III) injected with 1 ml of poliomyelitis vaccine (Salk type) had serum titers of 1:1024 against all 3 types of virus when delivered a week later. This apparent booster response in her did not affect the infant's titers, whose cord serum revealed 1:64, 1:16 and 1:16 against types 1, 2, and 3 viruses respectively. Brambell's work(13) raises the question of several independent mechanisms being involved in the transfer of antibody from mother to offspring.

It would appear that further investigation regarding human neonatal immunity will be required in order to clarify when and by which mechanisms the transfer of antibody occurs.

*Summary.* Neutralizing antibody titrations for the 3 poliovirus serotypes performed on maternal and umbilical cord bloods at 48 deliveries revealed disparities of 4 to 64-fold in 14 individuals, the maternal titer always being higher. Double ovum twins had identical titers with each other, though not necessarily with their mother. The least frequent disparity was for the Type 1 antibody. The possible role of physical factors affecting the transfer of the different serotype antibody molecules is discussed.

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Received July 22, 1957. P.S.E.B.M., 1957, v96.

## Effect of Aureomycin on Hepatic Utilization of Labeled Body Fat in Rats.* (23475)

JAMES E. ANDERSON† AND JOHN G. CONIGLIO (Introduced by Frank R. Blood)
*Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tenn.*

Rats fed Aureomycin and given a tracer dose of $CH_3C^{14}OONa$ orally or parenterally 24 hours later have more $C^{14}$ incorporated in hepatic fatty acids than control rats not treated with the antibiotic(1). In order to investigate the mechanism responsible for the increased radioactivity, we have studied the effect of administration of Aureomycin on mobilization of labeled body fat to the liver and on its subsequent accumulation in this organ. As part of this investigation we have made a 4-day balance study of orally administered $C^{14}$-tripalmitin in normal rats.

*Materials and methods.* Adult, male Sprague-Dawley rats were paired by weight and kept on Purina Laboratory Chow *ad libitum*. In order to label the body fat, a daily dose of 25 mg of carboxyl-labeled $C^{14}$-tripalmitin, contained in 1 ml of olive oil, was fed by stomach tube to each rat for 4 days. During this period and the subsequent day food was allowed *ad libitum*. The radioactive tripalmitin was not fed on the fifth day so that disappearance of absorbed labeled fat from the blood stream would be complete. On the sixth day 5 mg of Aureomycin (Lederle) were given by stomach tube to a member of each pair of rats. After administration of antibiotic the rats were fasted 24 hours and killed by decapitation. Control rats were treated similarly except that water was given instead of Aureomycin. Feces excreted during the first 5 days were pooled for each rat. Fecal samples of the sixth day were combined with the contents of the gastrointestinal tract of each animal. Immediately after decapitation of the animals the organs and tissues to be analyzed were placed in alcoholic KOH. After digestion and saponification of the samples chemical and radioactivity determinations of total fatty acids were made by procedures described previously(2).

*Results.* The livers of rats treated with Aureomycin contained fatty acids in amounts similar to those in the control group. The specific activity and total activity of the hepatic fatty acids of both groups also were similar. These data are given in Table I in the form of an average and standard deviation for each of the 2 groups. Examination of the data on the basis of each pair of rats also proved that there was no consistent significant difference between experimental and control animals.

In a similar manner, analyses of fatty acids of other tissues revealed no significant differences in amount or radioactivity between the 2 groups. Carcass total fatty acids of antibiotic-treated animals contained 32.5% (S.D. = 3.9) of the absorbed activity; those of controls contained 31.3% (S.D. = 5.8). Results of analyses of intestinal fatty acids were more variable and ranged from 0.78 to 2.04% of the absorbed activity in the experimental group and from 0.81 to 1.60% in the control group.

A 4-day balance period in all rats before administration of antibiotic resulted in an average absorption value of 88.1% (S.D. = 3.2) of the tripalmitin fed.

* This paper is based on work performed partly under contract with U. S. Atomic Energy Com.

† Work done while on a medical student research fellowship from National Fn. for Infantile Paralysis.