PERMEABILITY OF THE HUMAN PLACENTA TO ANTIBODIES*†

A Quantitative Study

By ALEXANDER S. WIENER, M.D., and I. JEROME SILVERMAN, M.D.

(From the Serological and Bacteriological Laboratory of the Office of the Chief Medical Examiner, and the Bellevue Hospital Out-Patient Department Laboratory of the Department of Hospitals of New York City, New York)

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A problem of some importance and one which soon attracted the attention of immunologists is the source of serum antibodies during the neonatal period. In early experiments on various animal species, some investigators (1–3) noted a passage of antibodies from mother to newborn through the placenta, while others (4, 5) reported the placenta to be impermeable to antibodies. In cattle it was found that the antibodies of recently born calves are derived mainly from the colostrum (5). For example, Smith and Little (6) showed that as many as 75 to 80 per cent of calves that failed to obtain colostrum by nursing succumbed to a generalized *Bacillus coli* septicemia.

To explain these contradictory findings, Kuttner and Ratner (7) suggested that there was a correlation between the anatomical structure of the placenta and its permeability to antibodies. According to their idea, the more dense the barrier between the circulations of mother and fetus the less transmission of antibodies occurs. In instances where there is no significant placental transfer of antibodies, as in cattle, the colostrum plays the major rôle. In general, the importance of the colostrum is inversely proportional to that of the placenta under the theory of Kuttner and Ratner. For a more complete discussion of the passage of antibodies and heterologous proteins through the placenta, colostrum and milk, see Ratner *et al.* (8).

In man, since the chorionic villi bathe in the maternal blood, it would be expected under the theory just enunciated that the placental transfer of antibodies would be the major source of antibodies for the newborn. At

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any rate, the common observation that infants artificially fed from birth thrive as well as breast-fed infants proves that the colostrum is not essential for their well being. In this connection may be cited the observations of Kuttner and Ratner (7) on the antitoxin content of colostrum and cord blood.

For the investigation of the placental transfer of immune bodies in human beings various antibodies have been used. In 1895, only a few years after the announcement of the discovery of diphtheria antitoxin by von Behring and his associates, Fischl and von Wundschheim (9) demonstrated the presence of diphtheria antitoxin in a large proportion of cord bloods. Shortly thereafter, Schumacher (10) presented evidence for the passage through the human placenta of agglutinins for typhoid bacilli. Halban and Landsteiner (11) made a comparative study of heterohemolysins, heterohemagglutinins and antitoxins in maternal and umbilical cord sera and found them always to exist in considerably smaller quantities in the cord sera. Evidence has been adduced more recently for the passage of isoagglutinins through the placenta (12, 13). An unexplained observation, however, was that frequently isoagglutinins were lacking from cord blood even when the infant belonged to the same group as the mother. It occurred to the present authors that the reason for this behavior might be found by making quantitative studies on the isoagglutinins in maternal and cord bloods. Moreover, by comparing the titers of a variety of antibodies it might be possible to establish an "index of permeability" for the human placenta; or what might perhaps more properly be called a "coefficient of distribution" of antibodies between mother and fetus.

Methods and Materials

For the present study the following antibodies were selected: the isoagglutinins α and β, heteroagglutinins for rabbit and sheep blood and syphilitic reagin. The titrations for the hemagglutinins were carried out in the usual way in small test tubes by mixing progressively doubled dilutions of the serum being examined with equal volumes of the dilute test blood suspensions (14). The titrations of syphilitic reagin were made in a similar way except that the tests were set up on paraffin-ringed slides (15). The test antigens used for titrating the syphilitic reagin were the so called Kline diagnostic and exclusion antigens for heated serums. Sample protocols are given in Table I. For the sake of uniformity, in this table the strengths of the agglutination reactions both for red cells and Kline antigen have been rated up to maximum of four plus, depending on the size of the clumps. The titers were taken equal to the reciprocal of the highest dilution of serum still giving a reaction visible to the naked eye (two plus or more).

RESULTS

In Table I it will be seen that the ratios (designated for convenience by the letter R) of the titers of the various antibodies in the maternal blood

TABLE I

Titration of Antibodies in Maternal Blood and Umbilical Cord Blood

Case No.	Test antigens	Dilutions of maternal serum									Dilutions of cord serum						Ratio (R) of titers
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	Titer	1:1	1:2	1:4	1:8	1:16	Titer	
1	B cells	++++	++++	+++	++	−	−	−	−	8	++	−	−	−	−	1	8
Mother	Rabbit cells	++++	++++	++++	++++	+++	++	−	−	32	+++	++	−	−	−	2	16
group A	Sheep cells	+++	−	−	−	−	−	−	−	1	−	−	−	−	−	0	—
Child	D*	++++	+++	++	−	−	−	−	−	4	−	−	−	−	−	0	—
group O	E	++++	++++	+++	++	−	−	−	−	8	+++	−	−	−	−	1	8
2	A_1 cells	++++	++++	++++	++++	+++	++	−	−	32	+++	−	−	−	−	1	32
Mother	B cells	++++	++++	++++	+++	++	−	−	−	16	+++	+++	−	−	−	2	8
group O	Rabbit cells	++++	++++	++++	++++	+++	++	−	−	32	++++	++++	+++	−	−	4	8
Child	Sheep cells	+++	+	−	−	−	−	−	−	1	−	−	−	−	−	0	—
group O																	
3	A_1 cells	++++	++++	++++	++++	+++	+++	++	−	64	++++	++++	++++	+++	−	8	8
Mother	B cells	++++	++++	++++	++++	++++	+++	++	−	64	++++	++++	++++	+++	−	8	8
group O	Rabbit cells	++	+++	++++	++++	++++	++++	++	−	64	++++	++++	++++	++	−	8	8
Child																	
group O																	

* D = Kline diagnostic antigen. E = Kline exclusion antigen.

to the titers of the corresponding antibodies in the fetal blood vary between 8 and 32 in the nine instances where a value for R could be obtained. The experiments completed to date have yielded a total of 56 ratios including the nine listed in Table I, and in this entire series the value for R has never fallen below 4 nor exceeded 32. One thing of interest which became evident early in the study was that the variation of the value of R from one individual to another was not significantly greater than the variation of R for different antibodies in the same individual.

It is important to bear in mind that the observed or apparent variation in the value of R must be considerably greater than the actual one on account of the limitations of the technique. Since the dilutions of serum are progressively doubled, it is clear that the error must be close to 50 per cent, assuming that the results are as accurate as they possibly can be. As a matter of fact the error is often greater, since it is not unusual to find on repetition of titrations a difference of one tube.

It is possible by making certain assumptions to calculate the actual variations in the values of R from those observed. Let us assume for example, that R has the constant value 12, and the error of the titration technique used is $\frac{1}{2}$ of a serial dilution. If the true titer of a particular antibody in the mother be set equal to $12x$, then the titer of the corresponding antibody in the cord blood would be x. In the tests, the titer of the maternal antibody might be found to lie anywhere between $9x$ and $18x$, whereas the observed antibody titer for cord blood would fall between $\frac{3}{4}x$ and $\frac{3}{2}x$. The observed value of R instead of being 12 could therefore have any value between $9x \div \frac{3}{2}x$ and $18x \div \frac{3}{4}x$, that is, between 6 and 24. Conversely, if R is found to vary between 6 and 24 and it is known that the method has an error of $\frac{1}{2}$ dilution, one would have to conclude that R actually does not vary at all, its true value being probably equal to 12.

In the present study, as already mentioned, the calculated R's ranged from 4 to 32, which closely approximates the range in the hypothetical case just described. It is not unreasonable to conclude, therefore, that in human beings antibodies, at any rate those investigated here, are distributed at the time of birth so that the ratios of their titers in maternal and fetal bloods are relatively constant. The true value of R lies somewhere between 8 and 16. As additional evidence in support of these conclusions can be mentioned some of our experiments which revealed that the observed variations in the value of R in different individuals, or between different antibodies in the same individual, were no greater than the variable results obtained for the value of R for a particular antibody in a particular mother and child on repeating the titrations several times.

It now becomes clear why in certain cases in which the mother and child belong to the same group isoagglutinins are not demonstrable in the cord

blood. If the titer of the isoagglutinin in the mother's blood is much less than 8, there will not be enough isoagglutinin present in the cord blood to be detectable with the standard technique used. However, that does not indicate the failure of placental transfer of the isoagglutinin. For example, in one of our cases where the mother belonged to group A and child to group O, the titrations set up in the usual way (readings after 2 hours at room temperature) showed the titer of the β agglutinins in the maternal blood to be 4 and β agglutinins were apparently absent from the cord blood; but when the readings were taken after standing in the ice box overnight the respective titers were 8 and 1. The claims made by some writers that the natural heteroagglutinins for sheep cells do not pass through the placenta can easily be answered in a similar way, since as indicated in Table I the titer of the agglutinins in the mother is usually considerably below 8.[1]

COMMENT

It is of interest to contrast the results obtained in a previous study by Wiener and Derby (17) with those given above. In attempting to ascertain whether syphilitic reagin in the spinal fluid of patients with neurosyphilis is formed locally or derived from the blood by filtration, parallel titrations were made of isoagglutinins and syphilitic reagin in the blood and spinal fluid of such patients. It was found that even when the titer of reagin in the spinal fluid equalled that of the blood and the titer of the isoagglutinin in the blood was much higher than that of the reagin, no isoagglutinins could be demonstrated in the spinal fluid. This indicated that the reagin in spinal fluid is at least in large part formed locally. On the other hand, the fact that the ratio of the reagin titers of maternal to cord blood is equal to the value of the ratio, R, for other antibodies such as the isoagglutinins, indicates that syphilitic reagin in umbilical cord blood, like other antibodies, is derived from the maternal blood by filtration through the placenta.

A question of special interest which is related to the present study is whether or not sensitizing bodies (the so called reagins of Coca) pass through the placenta in a similar fashion. According to Coca (18), reagins differ

[1] In the present study the behavior of bacterial agglutinins with regard to the placenta was not studied, but it is reasonable to assume that it would be similar to that of hemagglutinins. No direct evidence in support of this assumption is available, but it is of interest to cite a remark found in a report by Toomey (16) on agglutinins for *B. coli* in maternal and cord serum. This author remarks that the maximum titer of these bacterial agglutinins in the maternal blood was 640, whereas for cord blood the maximum was 80. The value for R for agglutinins for *B. coli* calculated from these maximum values would be 8, which agrees with that obtained for the hemagglutinins.

from other antibodies in their great affinity for tissue cells, so that they become fixed in the placenta and do not reach the fetal circulation. On the other hand, many writers (in particular, Ratner (19)) maintain that reagins in human beings behave like anaphylactic antibodies, which have been shown to pass through the placenta in guinea pigs (20). Ratner's view, as well as the recent report by Cohen *et al.* (21) on *Macacus rhesus* monkeys, is open to the objection that conditions in man and animal may not be the same. One way of settling this question is to make quantitative studies of the reagins in maternal and cord blood of human beings in order to see whether reagins obey the same rules as other antibodies. The recent report by Zohn (22) who actively sensitized mothers to ascaris but failed to find reagins for ascaris in the newborn has the weakness that he did not titrate the reagins in the mother. On the other hand, this objection cannot be raised against the study by Bell and Eriksson (23) which was carried out quantitatively. In their study, reagins were not found in the cord blood even when the titers of the maternal reagins were exceedingly high. This work has never been repeated, but if confirmed would support Coca's view.

SUMMARY AND CONCLUSIONS

The ratio of the titers of various antibodies, namely, hemagglutinins and syphilitic reagin, in the maternal blood to that of the corresponding antibody in the cord blood was found to be relatively constant, falling somewhere between 8 and 16. This figure may be considered the "index of permeability" of human placenta to antibodies, or the coefficient of distribution of antibodies between maternal and cord blood. The possible application of these findings to the study of the placental permeability to sensitizing antibodies (or reagins) is discussed.

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