TRANSMISSION OF ANTIBODIES AGAINST INFLUENZA VIRUS A ACROSS THE PLACENTA

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The transmission of specific immunity from mother to offspring is related to placental permeability. Thus in the human and in those animal species with a *placenta haemochorialis*, antibodies can be demonstrated in the new-born offspring; this, however, is not the case in animals where the placental barrier consists of more tissue layers, which do not become eroded and which continue to separate the maternal from the foetal circulation.

A number of workers have shown that in many instances the serum antibody levels of the newborn were higher than those found in their respective mothers. Liebling and Schmitz (1943) investigated diphtheria antitoxin, Pryce and Burnet (1932) staphylococcal antitoxin, Barr *et al.* (1949) diphtheria antitoxin, Murray *et al.* (1950) staphylococcal antitoxin, Murray and Calman (1953) anti-streptolysin. These findings suggest that transplacental transmission of antibodies does not take place by a simple process of diffusion and osmosis.

In an extensive review of the literature, Calman and Murray (1951) have evolved a hypothesis to explain the higher foetal antibody concentration. They postulate a break down of the complex antibody molecule and the passage of its constituent parts across the placenta to be later re-formed into antibody in the foetus. This hypothesis, they state, would also explain certain selective transmissions of proteins across the placenta and they quote work done by Lochead on the transmission of egg and ox albumin in rabbits, and experiments carried out by Timmerman on the differential passage of typhoid O and H agglutinins in the human.

In a series of experiments on pregnant rabbits Brambell *et al.* (1949 and 1950) have shown that agglutinin to *Brucella abortus* are transmitted by way of the yolk-sac splanchnopleur. They have also

shown that the selectivity of this membrane to the passage of various antibodies is a property developed from the 20th to the 24th day of pregnancy. It has been suggested that since the human foetus is known to swallow amniotic fluid the concentration of antibody may take place in the stomach or the primitive fore-gut, derived from the splanchnopleur.

Most of our knowledge about the concentration of foetal antibodies has thus been derived from studying experimental immunisation. As regards natural infection Perkins *et al.* (1950) have investigated the transmission of antibody to polio virus but no appreciable difference in the level of neutralizing antibody was found in the sera from mothers and from cord bloods.

Present Investigation

The 1957/58 outbreak of influenza due to the Asian strain (influenza virus A2) offered an excellent opportunity of studying this problem under natural conditions in general practice. Women of child-bearing age might be expected to have had no previous immunity, since the Asian strain of influenza virus was a new variant, which had probably not been epidemic at least since the early 1890's.

Conditions were thus favourable for a study of the transplacental passage of antibody after a recent natural infection, in contrast to previous work which dealt mainly with artificial immunisation or long past, natural infection.

Method. In the majority of mothers, the diagnosis of influenza was made on clinical and epidemiological grounds during the epidemic; no serological proof was obtained at the time of the attack. At confinement 10 ml. of blood was obtained from the placental end of the umbilical cord and the same amount from mothers by venepuncture within three days of confinement. The specimens were collected in dry, sterile containers or vacuum venules and forwarded by hand—usually within 12 hours—to the local public health laboratory. The sera were titrated for their content of complement-fixing antibody to the soluble antigen of influenza A virus. The method used was that described by Mayer *et al.* (1946).

Similar specimens were obtained from mothers who had no illness but who gave a history of family contact with influenza, and also from mothers who had had neither illness nor a history of contact with influenza at home, but who were pregnant during the period of the epidemic. Thus comparable postnatal specimens

were obtained from three different groups of mothers and babies with three different degrees of home contact with influenza.

Results

Twenty-three mothers had influenza during pregnancy, and the results of the serological investigations on maternal and cord bloods are shown in table I. A positive antibody titre at a dilution of 1 in 8 was accepted as significant in this investigation, since there has been no major outbreak due to any strain of influenza A in this country for several years. Table I shows that in all but three cases (Nos. 5, 36, and 40) a positive result was obtained in one or both of the tested sera from mother and baby (84 per cent). In the three exceptions the interval between infection and the test was 36 weeks, which could account for the negative results.

In 13 cases antibody titres in cord blood were higher than those in the maternal bloods. These mothers had influenza during the first

TABLE I.

ANTIBODY TITRES AGAINST INFLUENZA VIRUS A IN BLOOD OF MOTHERS AND INFANTS AT CONFINEMENT

In mothers who had been ill with Asian influenza during pregnancy.

Case No.	*Duration of pregnancy in weeks at time of infection*	*Antibody titres: Influenza virus A*			
		Maternal		*Foetal*	
		Neg. at $\frac{1}{8}$	*Positive*	*Neg. at* $\frac{1}{8}$	*Positive*
32	2		$\frac{1}{8}$		$\frac{1}{16}$
5	4	neg		neg	
36	4	neg		neg	
40	4	neg		neg	
1	6	neg			$\frac{1}{16}$
68	6		$\frac{1}{64}$		$\frac{1}{64}$
47	9	neg			$\frac{1}{16}$
21	10	neg			$\frac{1}{16}$
77	10	neg			$\frac{1}{16}$
45	11	neg			$\frac{1}{16}$
69	11	neg			$\frac{1}{16}$
30	12	neg			$\frac{1}{16}$
57	14		$\frac{1}{8}$		$\frac{1}{16}$
79	16		$\frac{1}{40}$		$\frac{1}{64}$
41	19		$\frac{1}{16}$		$\frac{1}{32}$
78	19		$\frac{1}{20}$		$\frac{1}{20}$
80	19		$\frac{1}{64}$		$\frac{1}{64}$
28	20	neg			$\frac{1}{16}$
25	25		$\frac{1}{16}$		$\frac{1}{32}$
76	31		$\frac{1}{32}$		$\frac{1}{16}$
50	34		$\frac{1}{8}$		$\frac{1}{8}$
81	34		$\frac{1}{40}$		$\frac{1}{40}$
18	38		$\frac{1}{16}$		$\frac{1}{16}$

25 weeks of pregnancy—in all but one case, No. 25, the infection occurred before the 20th week. In nine cases, where infection occurred in the second half of pregnancy, maternal titres were either equal to or higher than foetal titres.

Cases Nos. 68 and 80 showed unusually high antibody titres and in both cases continuous illness in the mother was recorded. Case No. 68 suffered from an exacerbation of chronic bronchitis which persisted throughout pregnancy. Case No. 80 had appendicectomy performed in the first and cholecystectomy in the last trimester of pregnancy.

Nine mothers, who were not taken ill, had a recorded contact with influenza in their own homes during pregnancy; in four of the babies (44 per cent) and three of the mothers a raised titre of antibody was found (table II). In two of these cases, where contact occurred in the 22nd and 24th week of pregnancy, the foetal titre was higher than the maternal; in the third, contact occurred in the 26th week and the foetal titre was equal to the maternal; and in the fourth case, where infection occurred in the 38th week, the maternal titre was higher than foetal.

TABLE II.

ANTIBODY TITRES AGAINST INFLUENZA VIRUS A IN BLOOD OF MOTHERS AND INFANTS AT CONFINEMENT

In healthy mothers with a home contact of influenza during pregnancy.

Case No.	*Duration of pregnancy in weeks at time of contact*	*Antibody titres: Influenza virus A*			
		Maternal		*Foetal*	
		Neg. at $\frac{1}{8}$	*Positive*	*Neg. at* $\frac{1}{8}$	*Positive*
70	7	neg		neg	
67	15	neg		neg	
59	18	neg		neg	
10	22		$\frac{1}{8}$		$\frac{1}{16}$
8	24	neg at $\frac{1}{4}$			$\frac{1}{8}$
34	25	neg		neg	
17	26		$\frac{1}{16}$		$\frac{1}{16}$
62	27	neg		neg	
24	28	neg		neg	
52	38		$\frac{1}{16}$		$\frac{1}{8}$

Forty-eight mothers acted as controls. They gave no history of illness or home contact with influenza during pregnancy. In twelve of this group (25 per cent), positive serological results were obtained and these are listed in table III. The duration of pregnancy is shown but the dates of infection cannot be ascertained. Assuming subclinical infection to have occurred during the height of the

epidemic in the middle of October 1957, high foetal antibody titres were again found where infection was assumed to have taken place in the first half of pregnancy—with the exception of case No. 44. No tests were done on maternal serum in case No. 16. The high titres in case No. 37 cannot be explained.

TABLE III.

ANTIBODY TITRES AGAINST INFLUENZA VIRUS A IN BLOOD OF MOTHERS AND INFANTS AT CONFINEMENT

In healthy mothers who had no home contact with influenza.

Case No.	*Duration of pregnancy in weeks at peak of epidemic*	*Antibody titres: Influenza virus A*			
		Maternal		*Foetal*	
		Neg. at $\frac{1}{8}$	*Positive*	*Neg. at* $\frac{1}{8}$	*Positive*
53	5	neg			$\frac{1}{8}$
51	7	neg			$\frac{1}{8}$
49	7	neg			$\frac{1}{8}$
37	8		$\frac{1}{64}$		$\frac{1}{64}$
27	8	neg			$\frac{1}{16}$
19	8		$\frac{1}{16}$		$\frac{1}{32}$
66	10	neg			$\frac{1}{8}$
9	11	neg			$\frac{1}{8}$
48	17		$\frac{1}{16}$		$\frac{1}{16}$
46	17		$\frac{1}{16}$		$\frac{1}{32}$
44	28	neg			$\frac{1}{8}$
16	30				$\frac{1}{8}$

Discussion

The present study confirms the findings of previous workers that foetal antibody concentrations higher than maternal are often found. However, by studying active rather than passive immunisation, after a natural infection with influenza, which stimulates a relatively short-lasting immune reaction to the soluble complement-fixation antigen, it has been possible to show that in a majority of cases the relationship between foetal and maternal antibody titres depends upon the age of the pregnancy at the time of infection.

Thus, in the majority of cases where infection occurred in the first half of pregnancy, foetal titres were higher than maternal titres. This is in agreement with the findings of Barr and Glenny (1949), using diphtheria antitoxin, in the eleven cases where foetal antibody concentration was higher than maternal, and where the boosting by Schick testing and the two injections of alum precipitated toxoid were carried out before or during the fourth month of pregnancy (M. Barr, 1958), it would also be reasonable to assume that the cells forming the placental barrier, or those in the foetus derived from the splanchnopleur, are able to carry out these con-

centrations during some period of time before the 25th week of pregnancy. This assumption is supported by the findings of Brambel *et al.* (1949 and 1950) who have shown that the selective property of the splanchnopleur is strictly confined to a limited period of time during pregnancy.

The consistency with which foetal antibody titres were higher than the maternal, in cases where infection occurred in the early half of pregnancy, might in fact be dependent upon another mechanism, namely the time which had lapsed between infection and antibody determination. A raised antibody titre following a natural infection with influenza virus A may fall to a level of 1/8 or below in about three months (Watson, 1960). The relatively higher foetal antibody concentrations after that period might solely depend upon the rate of fall of antibody level in foetal blood being slower than in the mother's blood.

Thus, during the rising phase of the maternal titre, which might last for only two to six weeks, one occasionally found a maternal titre higher than in cord blood (table I, case 76; table II, case 52). During the following weeks when the maternal titre might stay fairly constant before starting to drop, one could expect to find high maternal and foetal titres equal to each other (cases 17, 18, 50, 78, 80, and 81). If the maternal titre stayed persistently high, then one would expect the foetal titre to have remained equally high (cases 37 and 68). Once the maternal titre began to drop, the foetal titre appeared to lag behind (cases 25, 41, 79). Eventually but only after an interval of about six months, the maternal titres showed a low positive figure or absence of detectable antibody while the foetal titre, still lagging, shewed a higher positive value (cases 1, 21, 28, 30, 32, 45, 47, 57, 69 and 77). At a still later date both foetal and maternal bloods had lost their antibody content (cases 5, 36 and 40).

Conclusion

1. Using a naturally occurring infection of Asian influenza as the challenge, it was possible to show that maternal complement-fixing antibody appeared in the normal time during pregnancy but that titres only reverted to negative by about 28 weeks after infection, or about twice as long as in some non-pregnant patients.

2. Foetal antibody titres, measured in cord blood, also rose comparably; but, if more than 15 weeks had elapsed from the time of infection, the values remained higher than in maternal blood, and were still consistently positive up to 34 weeks, once up to 38 weeks, after the attack of influenza in the mother.

3. The mechanism whereby this rise in antibody titres in foetal blood is brought about may be that suggested by others already,

namely, the concentration of maternal antibody elements by foetal tissues.

4. The mechanism whereby foetal antibody titres are maintained at a level higher than in maternal blood after the 15th week from infection is not known.

Summary

Eighty-one maternal and cord blood samples were titrated for their content of complement-fixing antibody to influenza virus A antigen during the 1957/58 epidemic of " Asian " influenza.

Out of the total of 81 tests, 30 pairs of maternal and foetal sera showed a positive result.

The time taken for positive maternal titres to revert to negative during pregnancy was about 28 weeks or longer, and for foetal titres about 36 weeks or longer.

In 17 cases where infection had occurred in the first 25 weeks of pregnancy, or more than 15 weeks before testing the cord blood, foetal titres were found to be higher than maternal titres.

Where infection occurred during the last 10 weeks of pregnancy, maternal titres were equal to or higher than those in cord blood.

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