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Maternal Antibodies in Human Foetal Sera at Different Stages of Gestation

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Summary. Complement-fixing antibodies to ten viral antigens and/or mercaptoethanol treated anti-streptolysin-O and anti-staphylolysin-α were determined in thirty human sera from foetuses at different stages of gestation, in fifty-seven full-term cord sera, and in the corresponding maternal sera. In the smallest foetuses (crown–heel length of 125–150 mm), no antibody was found. In those of length 155–215 mm, antibody was detectable if the corresponding maternal value was high. In foetuses of 230–380 mm, antibody was detected occasionally even when the maternal value was low. Sera of foetuses of 390 mm or more contained the same antibodies as the maternal sample, and foetal titres sometimes exceeded those of the mother. Titres in the full-term cord sera were significantly higher than the maternal titres for seven of the twelve antibodies studied.

INTRODUCTION

It has been demonstrated by Buffe and Burtin (1967), using the immunofluorescence technique, that the human foetus is able to synthesize immunoglobulins from the 12th week of gestation onwards. Most of the immunoglobulin in the foetal serum consists, however, of maternal γG derived through the placenta. Transfer of other immunoglobulins has not been demonstrated. γG has been detected in a foetus of 11 weeks, whereafter the level rises evenly, until just before birth when the rise is more rapid (de Muralt, 1962). The ready placental transport of γG might be expected to apply equally to all γG antibodies, and indeed numerous studies have revealed that various antibodies, known generally to be of γG type, are found in full-term cord blood at an equal, or even a higher level, than in the maternal blood (de Muralt, 1962; Schultze and Heremans, 1966). Reports on the antibody levels in human foetuses during pregnancy are, however, very few, and they concern only anti-staphylolysin, streptococcal antibodies, diphtheria antitoxin and Rh antibodies Vahlquist, Lagercrantz and Nordbring, 1950; Mollison, 1951; (Osborn, Dancis and Rosenberg, 1952; Hanson and Holm, 1961). From these it is known that foetal antibody titres rise progressively from the 4th month of pregnancy to near term and correspond to the γG transport from mother to foetus. These studies were made before the molecular classification of immunoglobulins was established.

The aim of the present work was to compare the serum levels of some viral antibodies and anti-streptolysin and anti-staphylolysin in human foetuses at various stages of development with the levels of the corresponding γG antibodies in the maternal serum.

TABLE 1

ANTIBODIES IN FOETAL AND MATERNAL SERA

No.	Foetus (F) or mother (M)	Crown–heel length (mm)	Weight (g)	Titre of complement-fixing antibody to:										Mercaptoethanol treated	
				Influenza A	Parainfluenza 1	Parainfluenza 3	Adeno	Respiratory syncytial	Mumps	Cytomegalo	Ornithosis	Herpes simplex	Measles	Antistreptolysin (i.u./ml)	Antistaphylolysin (i u./ml)
1	F	125	40	—	—	—	—	—	—	—	—	—	—	—	—
	M													400	0·9
2	F	135	60	—	—	—	—	—	—	—	—	—	—		
	M			128	32	8	32	—	4	—	4	—	4		
3	F	140	75	—	—	—	—	—	—	—	—	—	—	—	—
	M			32	16	16	16	—	—	8	—	8	—	100	1·25
4	F	145	52											—	—
	M													80	0·28
5	F	150	73	—	—	—	—	—	—	—	—	—	—	—	—
	M			8	8	8	16	—	8	64	—	8	16	80	0·40
6	F	155	76											—	—
	M													220	0·56
7	F	155	80	—	—	—	—	—	—	—	—	—	4	—	—
	M			16	8	16	16	8	16	—	—	32	128	220	0·56
8	F	160	85	—	—	—	—	—	—	—	—	—	—	—	—
	M			8	8	64	8	16	16	64	4	16	16	160	0·20
9	F	165	90	—	8	8	—	—	4	—	—	—	—	—	—
	M			4	128	128	8	8	64	64	—	32	16	80	0·56
10	F	165	90											—	—
	M													100	1·25
11	F	170	105	—	—	—	—	—	—	—	—	—	—		
	M			16	8	—	16	8	16	32	16	32	8		
12	F	190	140	—	—	—	—	—	—	—	—	—	—	—	—
	M			—	—	4	8	8	—	64	—	—	4	110	0·56
13	F	190	162	4	—	—	4	—	—	—	4	—	—		
	M			64	4	4	16	4	—	—	16	—	—		
14	F	195	161											—	—
	M													110	0·56

15	F	215	165											—	—
	M													220	0·40
16	F	215	180											100	—
	M													1100	0.64
17	F	230	170											56	—
	M													110	—
18	F	235	220	—	—	—	—	—	—	—	—	—	—	—	—
	M			—	4	—	4	—	4	—	—	4	—	110	0·28
19	F	245	275	—	—	—	8	—	—	—	—	—	—	50	—
	M			8	8	4	64	—	—	16	—	4	—	200	—
20	F	290	520	—	—	—	—	—	—	—	—	4	—	50	0·32
	M			8	16	8	8	—	—	4	4	16	16	200	1·25
21	F	320	680	8	—	—	—	—	4	32	—	32	—		
	M			32	8	—	—	—	32	64	—	128	—		
22	F	380	840	—	—	—	16	4	4	4	4	16	16		
	M			16	8	8	32	16	16	64	32	16	32		
23	F	380	840	64	16	8	16	4	8	4	—	—	8	25	0·18
	M			32	8	8	16	4	8	4	—	—	8	25	0·18
24	F	380	1440	8	8	—	8	—	8	16	—	16	—		
	M			16	16	—	8	—	16	8	—	16	—		
25	F	390	1330	4	—	4	—	16	—	8	—	16	8	**80**	—
	M			8	4	8	4	16	—	64	8	32	16	—	0·40
26	F	400	1610	**128**	32	—	32	4	64	**16**	**32**	—	—		
	M			**32**	16	—	16	—	32	—	—	—	—		
27	F	430	1740	32	8	—	32	—	4	—	—	8	32	100	
	M			32	8	—	16	—	64	—	—	4	16	200	
28	F	450	2320	32	4	—	16	—	—	—	—	—	8	110	0·36
	M			32	16	—	8	—	—	—	—	—	4	160	0·7
29	F	460	2100	32	4	—	8	4	32	16	—	32	8		
	M			64	4	—	4	—	64	8	—	32	8		
30	F	470	2450	**128**	8	—	—	—	32	**16**	—	—	—	50	
	M			**32**	64	—	32	—	32	—	—	—	—	400	

Values below detectable level (in viral antibodies <4, in anti-streptolysin $\leqslant 18$ i.u./ml and in anti-staphylolysin $\leqslant 0{\cdot}16$ i.u./ml) are referred by —. Pairs with foetal value significantly higher than the maternal one are printed in bold.

In the foetuses from the later part of gestation, higher titres than in the mother were occasionally observed, and antibody levels were therefore determined in the cord bloods of full-term neonates and their mothers.

MATERIALS AND METHODS

Sera

Umbilical cord sera were obtained from twenty legal abortions, from ten prematures and from fifty-seven full-term newborns. Care was taken to collect cord blood without contamination by Wharton's jelly. Corresponding maternal sera were taken on the same day as the foetal samples. The sera were stored at −40° until examined, and before antibody determinations they were heated at 56° for 30 minutes.

Antibody assays

The complement-fixation micromethod of Sever (1962) as modified by Mäntyjärvi (1966) was used to determine antibodies to influenza A (obtained from Professor K. Cantell, State Serum Institute, Helsinki), parainfluenza 1 (obtained from Professor K. Cantell, State Serum Institute, Helsinki), parainfluenza 3, adenovirus, respiratory syncytial virus, mumps (obtained from Professor K. Cantell, State Serum Institute, Helsinki), cytomegalovirus, ornithosis (obtained from Burroughs Wellcome and Co., London), herpes simplex and measles. The titres are expressed as reciprocals. Anti-streptolysin-O was determined as described by Ipsen (1944) and anti-staphylolysin-α as described by Packalén and Bergqvist (1947). Corresponding foetal and maternal specimens were tested simultaneously.

Differentiation of antibodies of γG class

There is good evidence that viral antibodies of γM class fix practically no complement (Pike, 1967; Wiedermann, Denk and Kunz, 1967; Toivanen, Salmi and Mäntyjärvi, unpublished). The same is known of various γA antibodies (Cohen and Porter, 1964; Pike, 1967). Thus the complement-fixation titres in our material can be considered to represent γG antibodies. For determination of γG anti-streptolysin and anti-staphylolysin, sera diluted 1:4 were treated with a final concentration of 0·1 M 2-mercaptoethanol at 37° for 60 minutes, and were then dialysed for 48 hours against phosphate-buffered saline, pH 7·2, containing 0·02 M sodium iodoacetate.

Statistics

To compare the difference of antibody levels in the newborns and mothers, the sign-test (Siegel, 1956) was used.

RESULTS

Results of antibody determinations from foetuses and their mothers are shown in Table 1. Results of anti-streptolysin and anti-staphylolysin are reported only for mercaptoethanol treated samples: the results for untreated maternal samples were very close (±one tube) to those observed after mercaptoethanol treatment, indicating these antibodies to be mainly of γG class. From the results of the antibody tests in the foetal sera, the material can be divided into four groups. The first one comprises the smallest foetuses (pairs 1–5), in which no antibodies were found. In the second group (pairs 6–16) some

low foetal antibody titres were found in cases with a high maternal titre. The third group (pairs 17–24) comprises foetuses with detectable antibodies to several antigens; in many cases the maternal titre was not unusually high. In the three largest foetuses of this group

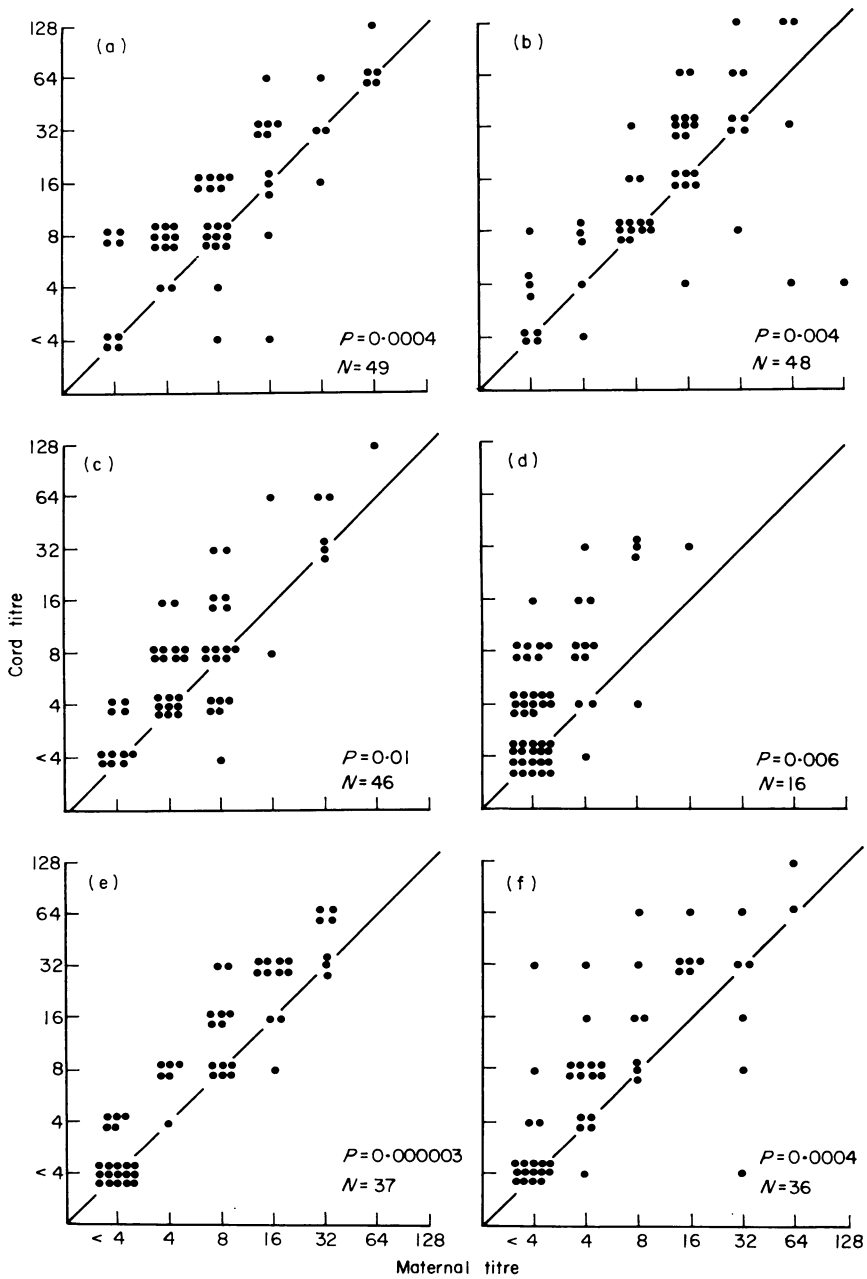

FIG. 1. Complement-fixing viral antibodies in full-term cord and maternal sera. N = number of pairs with maternal value exceeding the minimal detectable level. (a) Influenza A, (b) adenovirus, (c) parainfluenza 1, (d) parainfluenza 3, (e) herpes simplex, and (f) measles.

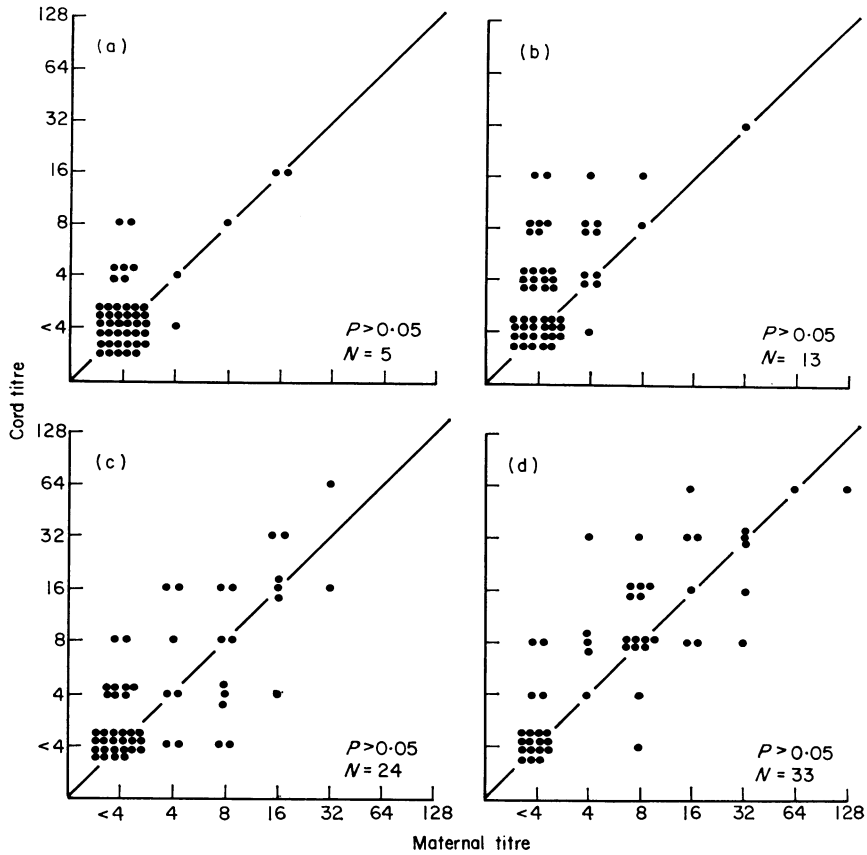

FIG. 2. Complement-fixing viral antibodies in full-term cord and maternal sera. N = Number of pairs with maternal value exceeding the minimal detectable level. (a) Ornithosis, (b) respiratory syncytial, (c) mumps, and (d) cytomegalo.

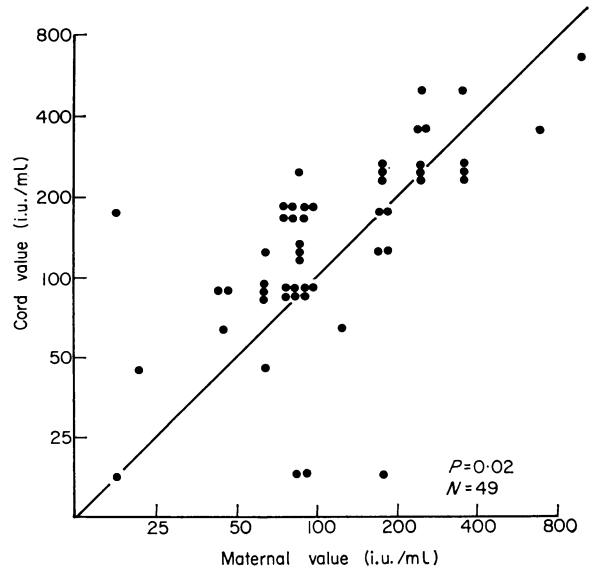

FIG. 3. Mercaptoethanol treated anti-streptolysin-O in full-term cord and maternal sera. N = Number of pairs with maternal value exceeding the minimal detectable level.

(numbers 22–24), each with the crown–heel length of 380 mm, the titres of most foetal antibodies were equal to those of the mother. Finally, in pairs 25–30, the foetal serum contained, with few exceptions, the same antibodies as the maternal sample: in six instances, as indicated in Table 1, the foetal titre was significantly (four-fold) higher than the corresponding value for the mother.

Antibody determinations in the fifty-seven full-term foetuses and their mothers are presented in Figs. 1–4. The cord blood titres were significantly higher than the corresponding maternal titres for seven of the twelve antibodies studied. In calculation of the

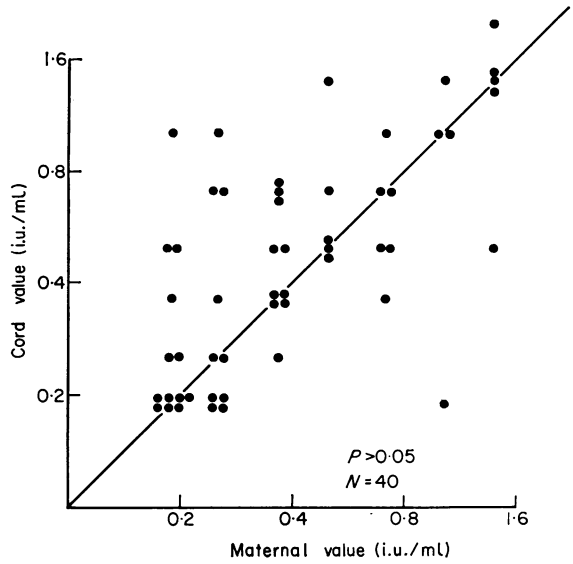

FIG. 4. Mercaptoethanol treated anti-staphylolysin-α in cord and maternal sera. N = Number of pairs with maternal value exceeding the minimal detectable level.

significance, only those pairs could be taken into consideration which had the maternal value at or above the lowest level detectable (for viral antibodies, titre of 4; anti-streptolysin, 18 i.u./ml; anti-staphylolysin, 0·16 i.u./ml).

For the viral antibodies showing a significant difference between the maternal and newborn values, the geometric means of the maternal titres were as follows (all pairs studied included, titre<4 considered as 2):

Antibodies to influenza A	11·2
adenovirus	8·7
parainfluenza 1	5·4
parainfluenza 3	2·6
herpes simplex	6·3
measles	4·8

The corresponding values for the remaining four viral antibodies were somewhat lower:

Antibodies to respiratory syncytial	2·5
ornithosis	2·3
mumps	3·8
cytomegalo	6·1

DISCUSSION

It has been known since the observations of Bryce and Burnet (1932) on staphylococcal antitoxin that antibody levels in the neonatal cord blood may be higher than in the mother: this phenomenon, which applies to other antibodies (de Muralt, 1962; Schultze and Heremans, 1966), has often been disregarded or attributed to technical error (Wiener, 1951). Recently it has been demonstrated, using accurate methods, that the γG concentration in the cord blood is significantly higher than in the mother (Kohler and Farr, 1966; Michaux, Heremans and Hitzig, 1966). These observations are supported by the present report, which demonstrates that the levels of numerous antibodies in the newborn are significantly higher than the concentrations of the corresponding antibodies of γG class in the mother. Such a situation was observed in foetuses with crown–heel length $\geqslant 390$ mm, aged 31 weeks or more. This was not established for all antibodies in the same serum; indeed it was sometimes found that the titre of one antibody was higher in the foetus than in the mother while the converse held for another antibody.

Our observations indicate that foetal antibody titres exceeding those of the mother are reached during the last few weeks before birth. At this time the foetal serum contains all the antibodies of γG class detectable in the serum of the mother. Before this, the transfer of antibodies to the foetus is irregular. In foetuses with crown–heel length of 155–215 mm they could be demonstrated only if the corresponding maternal titre was high. In foetuses of 150 mm or less no antibodies were found by our techniques irrespective of high maternal values.

In our material, the smallest foetuses with detectable antibodies were 155 and 165 mm in crown–heel length and aged 13–15 weeks. The antibodies found in these cases were to measles, parainfluenza 1 and 3, and mumps. Similar results have been presented previously for anti-streptolysin (Vahlquist *et al.*, 1950) and diphtheria antitoxin (Osborn *et al.*, 1952). Rh antibodies have been demonstrated in a foetus of 10 weeks (Mollison, 1951).

In four of the ten viral antibodies studied no significant difference was demonstrated between the newborn and maternal values. The geometric means of maternal titres of these four antibodies were somewhat lower than for the others. However, low maternal values do not necessarily predict equally low values in the cord blood. Thus Michaux *et al.* (1966) found in a Swiss population significantly higher γG concentrations in the cord blood than in the mother, while an opposite finding was made in their samples from Bantu Negroes. The white mothers had an average γG level of $10{\cdot}67 \pm 0{\cdot}28$ mg/ml and negro mothers $19{\cdot}86 \pm 0{\cdot}41$ mg/ml. The discrepancy in placental transmission between these two populations was revealed when the distribution of the foetal values was investigated as a function of the maternal concentration and a regression line calculated. The slope of the line made it clear that the foetal value exceeded that of the mother when the maternal concentration was less than 15 mg/ml. When the maternal γG concentration exceeded 15 mg/ml, the foetal value did not reach the maternal level. Naturally this does not apply in the absence of maternal γG. If in our material (viral antibodies with maternal titre $\geqslant 4$, $N = 307$) a regression line with the aid of $\log_2$ of the reciprocals is calculated, it is found that:

$$y = 0{\cdot}956 + 0{\cdot}825x,$$

where y equals the foetal and x the maternal value. This line is in principle similar to that of Michaux *et al.* (1966). The regression coefficient 0·825 is significantly different ($P =$

0·001) from unity, indicating that the foetal and maternal titres are not directly correlated. When $y = x$, a value of 5·48 is obtained for them. This is equal to a titre of 45. In other words, when the maternal titre is less than 45, the foetal titre should exceed that of the mother; when the maternal titre exceeds 45, the foetal titre should be less than the maternal one. Unfortunately our material includes only fifteen maternal values over this limit; they do not confirm this expectation.

From the evidence outlined above, it may be assumed that the absence of any demonstrable difference between maternal and newborn titres in the four viral antibodies in our material is due to the absence of the maternal antibody in many cases.

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