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Fetal infection resulting from maternal rubella after the first trimester of pregnancy

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SUMMARY

We have tried to measure the incidence of prenatal infection in 304 infants whose mothers had had rubella at various times after the first 12 weeks of pregnancy. Two methods of assessment were used: first, serum obtained soon after birth was tested for specific IgM antibody; secondly, serum obtained after the age of eight months was tested for specific IgG. When maternal rubella occurred 12–16 weeks after the last menstrual period specific IgM antibody was detected in 28 out of 50 infants (56%). The proportion fell progressively to 12% after maternal rubella at 24–28 weeks, rose to 19% after rubella at 28–36 weeks and then to 58% when the illness occurred during the last month of pregnancy. In all, IgM antibody was detected in 77 out of 260 infants (29%). The fetus can thus be infected at any time during the second and third trimesters of pregnancy, but the risk varies at different stages.

The figures for the prevalence of IgG antibody were greater throughout, because some infants had IgG who had previously lacked specific IgM. After maternal rubella at 12–16 weeks IgG antibody persisted in 22 out of 31 infants (71%). The proportion fell to 28% after rubella at 24–28 weeks and then increased progressively to 94% after rubella during the last month. In all, IgG antibody persisted in 94 out of 190 infants (49%). The true rate of fetal infection probably lies between the rates estimated from the presence of IgM antibody and the subsequent prevalence of IgG.

Infants whose mothers had rubella at any time during pregnancy should be examined regularly for possible evidence of damage.

INTRODUCTION

Maternal rubella during the first trimester of pregnancy probably causes fetal infection in the majority of cases. Isolation rates of virus from the products of conception and from fetuses have ranged from 47% to 73% in studies in which maternal rubella has been diagnosed clinically (Alford, Neva & Weller, 1964; Horstmann *et al.* 1965; Monif *et al.* 1965). Even higher figures have been obtained in studies restricted to cases in which rubella has been confirmed serologically: Rawls, Desmyter & Melnick (1968) recovered virus from 9 out of 10 fetuses and from one infant who had been exposed to infection at 32 days gestation, an overall rate of 91%. Thompson & Tobin (1970) isolated virus from 15 out of 17 fetuses (88%).

Fetal infection does not invariably cause major defects, and the incidence of the latter has been estimated in various prospective surveys as 10–45% (Manson, Logan & Loy, 1960; Lundström, 1962; Pitt & Keir, 1965; Siegel, Fuerst & Guince, 1971). This wide range of results probably reflects differences in the methods of conducting the surveys and of assessing abnormalities. Most, if not all, of the figures are probably underestimates, since they were obtained from studies that necessarily depended on clinical, rather than serological, diagnosis of the maternal illness. Although the degree of risk is not known precisely it is generally regarded as unacceptably high; in the United Kingdom nearly all such pregnancies are now terminated and prospective studies are no longer possible.

After the first trimester, and particularly during the fourth month of pregnancy, the risk of serious fetal damage declines sharply. Malformations of the heart and eye are seldom seen, but the later appearance of more subtle abnormalities suggests that chronic infection may still occur. Hardy *et al.* (1969) found that only 7 out of 22 infants whose mothers had had rubella at 31–33 weeks of gestation were completely normal; the others had handicaps such as deafness, retarded development and problems of communication, and ten had persistent rubella antibody after the age of six months.

Several factors could modify the hazards of maternal rubella after the first trimester. First, if the placenta becomes a barrier the fetus may be less often infected. Secondly, malformation is unlikely after the critical stage of organogenesis have passed. Thirdly, the maturing immune system may become able to limit the infection and perhaps even effect a cure by the same mechanisms that operate postnatally.

Serological evidence of fetal infection following maternal rubella during the second and third trimesters has been found in two previous studies. Vesikari (1971, 1972) followed up 23 infants and detected persistent haemagglutination-inhibition (HI) antibody after the age of seven months in 12 (52%), but was unable to detect specific IgM antibody at birth. Vejtorp & Mansa (1980) examined sera from 209 infants within two months of birth: using sucrose density gradient centrifugation and testing the fractions by HI they detected IgM antibody in 35 cases (17%).

We describe here a serological study of 304 infants whose mothers had had confirmed rubella at various times after the twelfth week of pregnancy. We have tried

to measure the frequency of fetal infection in two ways, using methods described in our previous work. First, we have used immunofluorescence (IF) and radioimmunoassay (RIA) to detect specific IgM antibody, using IF for fractions obtained from sucrose gradients and RIA to test whole serum (Cradock-Watson *et al.* 1979). Secondly, we have used IF and RIA to detect persistent IgG antibody in whole serum after the age of eight months, when maternal antibody should have disappeared (Cradock-Watson, Ridehalgh & Chantler, 1976). Specimens to be tested for IgM were received from numerous different laboratories in which maternal rubella had been confirmed serologically. Later specimens, to be tested for IgG, were received either from the same laboratories or from paediatricians and family doctors. The infants thus formed part of a prospective study and, unlike most cases of congenital rubella, were not selected retrospectively on account of any abnormalities; indeed, almost all of them were normal and healthy at birth.

MATERIALS AND METHODS

Infants whose mothers had had rubella during pregnancy

The main study group comprised 304 infants whose mothers had had serologically confirmed rubella at various times after the first 12 weeks of pregnancy (calculated from the first day of the last menstrual period) up to one day before delivery. Subclinical maternal infections were excluded. Single specimens of serum collected from 266 of these infants at the following ages were tested for specific IgM antibody: 0–4 weeks, 245 sera; 5–13 weeks, 19 sera; 20 weeks, 1 serum; 7 months, 1 serum. Two methods were used: (1) 256 sera were fractionated on sucrose density gradients and the peak IgM fraction (fraction 2 or 3) was tested by IF; (2) 204 sera were tested by RIA without prior fractionation.

Total IgM concentrations in 189 sera, taken within four weeks of birth, were measured by single radial diffusion in commercial immuno-plates (Hyland Laboratories). For comparison we measured the total IgM concentrations in 25 infants with the congenital rubella syndrome (infected during the first trimester) and 50 normal infants whose mothers had not had rubella during pregnancy. We have expressed the results in international units per ml (i.u./ml) (Rowe, Grab & Anderson, 1972) and have regarded concentrations of less than 30 i.u./ml as being within normal limits. For conversion to mg/dl the figures should be multiplied by 0·82.

Serum specimens from 190 infants aged between eight months and three years were tested for specific IgG antibody by IF. Of these, 111 were additionally tested by RIA.

Infants not at risk from maternal rubella

In order to measure the prevalence of rubella IgG antibody in infants with no known maternal history of rubella during pregnancy, we tested sera from 200 children aged between ten months and three years by IF. These children had an age distribution similar to those in the main study group who were at risk from maternal rubella and who were tested for persistence of IgG antibody.

Sucrose density gradient centrifugation

Serum was diluted 1/2 and centrifuged on a 12·5–37·5 % (w/v) sucrose gradient at 33000 rev./min for 17 h. Eleven or 12 fractions were collected dropwise after piercing the bottom of the tube (Cradock-Watson *et al.* 1976, 1979).

Immunofluorescence

Staining was carried out as previously described (Cradock-Watson, Bourne & Vandervelde, 1972). Briefly, cover-slip cultures of BHK21 cells infected with rubella virus were treated with dilutions of serum (from 1/16) or serum fractions (from 1/2), and were then stained with fluorescein-labelled immunoglobulins prepared against human IgG or IgM (Wellcome Reagents Limited). The cover-slips were finally mounted in glycerol and examined by dark-ground illumination from a quartz-halogen lamp.

Radioimmunassay

RIA was carried out as described by Kangro, Pattison & Heath (1978). Briefly, rubella haemagglutinating antigen (Wellcome Reagents Limited) or control antigen prepared from uninfected cells was fixed to the wells of polyvinyl chloride microtitre trays (Dynatech Laboratories Limited). Wells were treated first with serum (for 2 h in IgG assays and 3 h IgM assays) and then with ^{125}I-labelled anti-human IgG (1 h) or IgM (2 h). After washing, individual wells were clipped from the trays and the bound radioactivity was measured in a gamma counter. Results were calculated as previously described, and the titre was determined as the highest dilution with a binding index of 2. Sera that appeared initially to be IgM-positive were titrated after absorption with IgG-coated latex beads* in order to remove any IgM with anti-IgG activity (Chantler *et al.* 1976; Cradock-Watson *et al.* 1979).

Radial haemolysis

Radial haemolysis (RH) was carried out by the method in current use in the Public Health Laboratory Service (Kurtz *et al.* 1980). Briefly, sheep erythrocytes treated with rubella haemagglutinin were incorporated with guinea-pig complement in 1 % agarose in 100 mm × 100 mm square Petri dishes to a depth of about 2 mm. Holes of 3 mm diameter were punched in the gel and filled with 10 μl of inactivated undiluted serum. A standard serum containing 15 international units of rubella HI antibody per ml was included in each dish (Bradstreet *et al.* 1978). The diameters of the zones of haemolysis were measured after incubation at 37 °C overnight.

* Kindly supplied by Dr Shireen Chantler, Wellcome Research Laboratories.

RESULTS

Specific IgM antibody in infants whose mothers had had rubella after the first 12 weeks of pregnancy

We compared IF and RIA by using both methods to test sera from 194 infants. The RIA titres of these sera, and the IF titres of their peak IgM fractions are shown in the figure. In 55 out of 58 sera whose fractions were positive by IF (titres 2–512) RIA was also positive (titres 400–70000). Three sera were IF-positive but RIA-negative, and one was IF-negative but RIA-positive. These four sera with discrepant results came from children who subsequently had persistent IgG antibody at ages between 10 and 13 months and who were, therefore, probably infected *in utero*. The remaining 135 sera were negative by both methods. Agreement was sufficiently good for us to accept positive results obtained by either method and to conclude that the 59 sera shown individually in Fig. 1 contained specific IgM antibody. We tested sera from a further 72 infants by either IF or RIA and found rubella IgM in 18 (15 by IF and 3 by RIA). Thus, in all, we detected IgM antibody in 77 out of 266 infants (29 %).

In 50 IgM-positive cases we also measured the HI titre of the peak IgM fraction, using overnight incubation of antigen and antibody at 4 °C. The titre was $\geqslant 4$ in 24 fractions, $=2$ in 11, and <2 in 15. Thus, in 26 cases (52 %) we would not have been able to detect specific IgM antibody with confidence if we had relied on the HI test alone.

Specific IgG antibody in infants whose mothers had had rubella after the first 12 weeks of pregnancy

Ninety-four out of 190 sera (49 %), taken at ages between eight months and three years, were positive when tested for IgG antibody by IF (Table 1). When 57 of these were additionally tested by RIA 54 were positive ($\geqslant 100$) and three were negative.

Ninety-six sera were negative when tested for IgG antibody, 54 by both methods and 42 by IF alone. No serum was IF-negative but RIA-positive. Agreement between the two methods was sufficiently good for us to conclude that all the IF-positive sera contained IgG antibody.

IF-positive sera of sufficient volume were additionally tested by RH and HI. The RH test was positive with 30 out of 38 sera, although in five specimens the zone diameter indicated an antibody titre of less than 15 international units. Of the RH-positive sera, 24 out of 28 were also positive by HI. In some specimens, therefore, antibody would not have been detected if the RH or HI tests alone had been used.

Frequency of fetal infection

The numbers of infants with IgM and/or persistent IgG antibody are given in Table 2. All those with IgM antibody who were followed up were found to have persistent IgG. A minimum figure for the fetal infection rate can therefore be obtained from the proportion of infants who were tested for IgM and were found to be positive. For the whole period this figure was 77 out of 266 (29 %).

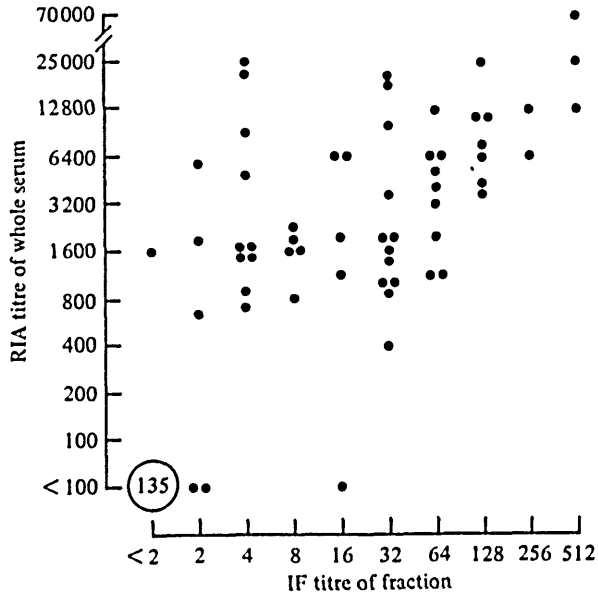

Fig. 1. Rubella IgM antibody titres in sera from 194 infants whose mothers had had rubella after the first 12 weeks of pregnancy. Abscissa = titre of peak IgM fraction determined by immunofluorescence (IF). Ordinate = titre of whole serum determined by radioimmunoassay (RIA).

Table 1. *Comparison of immunofluorescence (IF) and radioimmunoassay (RIA) for detecting rubella IgG antibody in* 190 *children aged between eight months and three years whose mothers had had rubella after the first* 12 *weeks of pregnancy*

	RIA titre			
IF titre	⩾100	<100	n.t.	Total
⩾16	54*	3†	37*	94
<16	0	54	42	96
Total	54	57	79	190

n.t. = not tested.
* IF titres 32–4096 (median = 512).
† IF titres 16, 64, 1024.

Persistent IgG antibody, however, was not confined to those who had previously had specific IgM but was also found in 29 infants who had been IgM-negative and 14 who had never been tested. If these were all infected *in utero* then the fetal infection rate, estimated from the prevalence of IgG antibody, was 94 out of 190 (49 %).

Table 3 gives the fetal infection rates, estimated by both methods, after maternal rubella at different stages of pregnancy. The rate estimated from the presence of IgM antibody was 56 % when rubella occurred 12–16 weeks after the last menstrual period. It fell progressively to 12 % after maternal rubella at 24–28 weeks, rose to 19 % after rubella at 28–36 weeks and then to 58 % when the illness occurred

Table 2. *Number of infants with IgM and/or persistent IgG antibody following maternal rubella after the first 12 weeks of pregnancy*

IgM antibody	Persistent IgG antibody +	−	n.t.	Total
+	51	0	26	77
−	29	72	88	189
n.t.	14	24	—	38
Total	94	96	114	304

n.t. = not tested.

Table 3. *Number of infants with IgM, and/or persistent IgG, antibody following maternal rubella at different times after the first 12 weeks of pregnancy*

Time of maternal rubella after LMP	No. of cases	No. of infants with antibody/no. tested (%) IgM	Persistent IgG
⩾12, <16 weeks	51	28/50 (56)	22/31 (71)
⩾16, <20 weeks	78	19/71 (27)	21/47 (45)
⩾20, <24 weeks	53	6/45 (13)	10/35 (29)
⩾24, <28 weeks	42	4/33 (12)	7/25 (28)
⩾28, <32 weeks	31	5/27 (19)	8/19 (42)
⩾32, <36 weeks	25	4/21 (19)	11/17 (65)
⩾36 weeks	24	11/19 (58)	15/16 (94)
Whole period	304	77/266 (29)	94/190 (49)

during the last month of pregnancy. The rates estimated from the subsequent prevalence of IgG antibody were higher at each stage, falling from 71% to 28% and then rising during the last trimester to 94% in the last month.

Fewer infants were born after maternal rubella at 12–16 weeks than after rubella at 16–20 weeks, possibly because some pregnancies in the former group were terminated (although we have no record of the number). After 20 weeks the number of infants in each four-week period fell progressively. It is doubtful if this indicates any reduction in the attack rate of acute rubella; it may merely reflect diminishing anxiety with a consequent reduction in the number of cases referred to the laboratory.

Total IgM concentrations in different groups of infants

The total IgM concentration was less than 30 i.u./ml in 92% of normal infants (Table 4). The proportion with normal values was only slightly less (82%) in infants who were at risk from maternal rubella but who lacked specific IgM. When specific IgM was present, however, the proportion with normal total concentrations was only 9%; the remainder, with specific IgM, had total concentrations ranging from 30 to 395 i.u./ml. These latter values overlapped considerably with those from infants with the congenital rubella syndrome (infected during the first trimester), who all had raised IgM concentrations ranging from 62 to 440 i.u./ml.

Table 4. *Total IgM concentrations (i.u./ml*) in infants with the congenital rubella syndrome, infants whose mothers had had rubella after the first 12 weeks of pregnancy, and normal infants*

Total IgM concentration (i.u./ml)	Congenital rubella syndrome	Maternal rubella after first 12 weeks of pregnancy: Specific IgM present	Maternal rubella after first 12 weeks of pregnancy: Specific IgM not detected	Normal infants
⩾400	2	0	0	0
⩾200, <400	14	5 (2 deaf)	2	0
⩾100, <200	6	6 (1 deaf)	2	1
⩾50, <100	3	16 (3 deaf)	7	2
⩾30, <50	0	14 (1 deaf)	13	1
<30	0	5 (9%)	111 (82%)	46 (92%)
Number of cases studied	25	53	135	50

* For conversion to mg/dl the figures should be multiplied by 0·82.

In infants who were at risk from maternal rubella but who lacked specific IgM the subsequent prevalence of persistent IgG antibody was 25%, whether or not the total IgM was raised. In this group, therefore, the presence or absence of infection could not have been inferred from the total IgM concentration.

Clinical status of infants whose mothers had had rubella after the first 12 weeks of pregnancy

We do not know the number of abortions, either natural or therapeutic. One infant was stillborn (IgM-negative) after maternal rubella at 18 weeks; another died at the age of 17 days (IgM-positive) after maternal rubella at 13 weeks, but had no malformations. Of the other 302 infants only 5 had obvious abnormalities at birth: two twins had talipes (1 IgM-positive, 1 negative) after maternal rubella at 15 weeks; 2 infants had abnormalities of the digits and 1 had pulmonary stenosis. None of these last three was infected. Twelve infants, infected at times ranging from 12 to 18½ weeks, have so far shown signs of deafness during the first year of life: all have persistent IgG antibody, 10 out of 11 had specific IgM, and all of 7 who were tested during the first month of life had raised total IgM concentrations (Table 4). Our investigation was serological rather than clinical, and there may well be other defects that have not come to our attention.

Rubella IgG antibody in unselected children aged between ten months and three years, not at risk from maternal rubella

Rubella IgG antibody was detected by IF in 12 out of 200 children (6%). One, aged 19 months, had congenital heart disease and may have been a case of congenital rubella, although the mother gave no history of rubella during pregnancy.

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DISCUSSION

In this study and in our previous work (Cradock-Watson *et al.* 1979) we obtained good correlation between IF and RIA for detecting IgM antibody in infants, and found very little evidence of false positive results. Because of this, and because our cases accumulated prospectively, our figures for the fetal infection rates (Table 3), based on the presence of IgM antibody, are unlikely to be overestimates.

The figures for the prevalence of IgG antibody are greater because many IgM-negative infants subsequently had IgG at the age of eight months or more. This could have occurred in three ways. First, some of them may have had traces of maternal IgG. This was possibly true of two babies who had titres of 16 and 32 (by IF) at eight months, but is unlikely to have occurred in the others, who all had higher titres and were mostly tested at the age of ten months or more. Secondly, some of the children may have had postnatal acute rubella. Assuming that 88 (29 %) of the 304 infants were IgM-positive and 216 (71 %) were IgM-negative, and that postnatal rubella occurred in 13 of the latter (6 %, as in unselected controls), then we would expect 88 + 13 = 101 (33 %) of the children to have IgG antibody. In fact, the observed prevalence of IgG was greater – namely 49 % – corresponding to 149 children. It is unlikely that the excess (149 – 88) was due solely to postnatal infection, as this would have required an incidence of acute rubella of 28 %. There remains a third possibility, namely that some infants may have been infected pre- or peri-natally but had no detectable IgM antibody when they were tested. Assuming no lack of IgM production, a negative result could occur in two ways. First, if maternal rubella occurs shortly before delivery, then blood collected at, or soon after, birth may be taken too soon. We observed this phenomenon in one infant whose mother had had rubella a week before delivery: cord serum was IgM-negative, but a second specimen at the age of 26 days was positive. The same thing could also have occurred in two others who were IgM-negative at birth but were not retested until the age of one year, when they were found to have persistent IgG. Secondly, the fetal IgM response after infection in mid-pregnancy may be over before birth. If so, it is briefer than after first trimester rubella. This, in turn, suggests that the developing fetus, maturing towards the postnatal type of immune response, may be better able to curtail the infection and eliminate infectious virus. In this connexion further studies of the duration of IgM production after birth would be of interest.

The true rate of fetal infection probably lies between the rates calculated from the presence of IgM and from the subsequent prevalence of IgG. Its decline between 12 and 28 weeks shows that the placenta increasingly prevents the transfer of virus but never becomes a complete barrier. Events during the last week of pregnancy are complicated by the possibility that the infant might be infected in the birth canal during parturition, or exogenously from its mother after birth. This could have occurred in seven cases in our series. Nevertheless, the increase in fetal infection during the last trimester shows that the previous decline is reversed and suggests that the placenta may be as permeable to rubella virus during the last month as during the first 12 weeks.

Our results show that the fetus can be infected at any time during the second

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and third trimesters, although the risk varies at different stages. The long-term effects of such infection are not yet known, but the observations of Hardy and her colleagues suggest that deafness and mental impairment may not be uncommon. These defects may be generally mild, and their detection may require sophisticated techniques of audiometry and mental assessment, but clearly all infected children should be examined regularly since they may benefit from remedial measures. Assessment will be most efficient and informative if infected children can be identified and compared with those who escaped infection. To do this accurately, however, requires serological methods more sensitive than those in current use. For example, if we had relied solely on the HI test for examining gradient fractions we would have detected with certainty only about half the IgM-positive cases. The total concentration of IgM would not have been a reliable guide in any individual case. We would also have had some failures if we had relied on HI or RH for detecting IgG; moreover, we would have needed venous blood, whereas the more sensitive IF and RIA techniques can be performed on capillary samples.

Infants with the congenital rubella syndrome commonly excrete large amounts of virus for several months after birth, and may infect non-immune nurses (Cooper *et al.* 1965; Schiff & Dine, 1965). Their IgM response lasts for about the same length of time (Cradock-Watson *et al.* 1976, 1979). Virus has also been recovered from a minority of infants infected during the second and third trimesters (Hardy *et al.* 1969), but the duration of excretion and its relation, if any, to the presence of specific IgM antibody is not known. Further studies are needed to show whether these infants are likely to infect susceptible contacts.

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