Papers and Originals

Studies on Rubella in Pregnancy

Report of the Public Health Laboratory Service Working Party on Rubella*

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Summary: In a further survey of women of childbearing age neutralizing antibody to rubella virus was found in 80 to 96% of those sampled from different parts of the country.

When assessment was restricted to susceptible pregnant contacts and rubella in the index case was confirmed by virus isolation immunoglobulin in the dosage used did not appear to give any appreciable protection.

One instance of a confirmed second attack after known exposure to rubella among more than 100 immune contacts is recorded.

The implications of all these findings are discussed.

Introduction

Rubella virus causes alarm because of its teratogenic properties. In susceptible persons infected during early pregnancy the virus reaches the embryonic tissues during the viraemic stage of the disease. In persons with immunity as a result of past infection this is unlikely to occur because of the presence of specific neutralizing antibody in the blood, though the possibility of reinfection cannot be entirely ruled out. Arising from this, an endeavour to give passive protection to women exposed to rubella in early pregnancy by the inoculation of immune (gamma) globulin and thereby to prevent congenital defects has been a continuing duty of the Public Health Laboratory Service since 1954. McDonald (1963) and McDonald and Peckham (1967) have analysed the results of the very extensive data from the clinical and epidemiological investigations. They came to the conclusion that the moderate dosage of globulin which has been used over a number of years reduced not only the incidence of clinical rubella but also the appearance of rubella-type defects in the infants of women who escaped the disease.

Though very valuable, these investigations were hampered by the lack of any parallel laboratory studies. The issue of immunoglobulin has been based solely on the clinical diagnosis of rubella in an index case with whom the pregnant woman had been in contact; even though not all fleeting maculopapular rashes are due to this disease. A history of rubella from the pregnant woman was the only evidence of past infection and this was considered an inadequate basis on which to distinguish those susceptible from those immune. The occurrence of subclinical infection could not be determined, nor could the amount of specific neutralizing antibody in the different batches of immunoglobulin be estimated.

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Since the investigations of McDonald and Peckham techniques for virus isolation and for serological studies by neutralization, complement-fixation, and haemagglutination-inhibition tests have become available. Already in a number of areas in England the presence of rubella neutralizing antibody has been found in from 79 to 95% of the adult female populations tested (see *Brit med. J.*, 1967; P.H.L.S., 1967; Field, 1967; Field *et al.*, 1967). The need therefore for a fresh approach which would include the results of laboratory investigations on a wide scale formed the basis for instituting the present Working Party. This interim report gives an account of some of the problems which are being studied by it.

Surveys

(1) *Serological Survey of Women Not Recently in Contact With Rubella.*—For this, members of the Working Party arranged locally to obtain sera for rubella antibody titration from patients less than 20 to greater than 40 years of age who were attending antenatal clinics or in hospital for other reasons. Results were recorded on a standard card.

(2) *Inquiry Into Prophylactic Value of 750 mg. of Immunoglobulin Given to Women in First Trimester of Pregnancy Who Have Been in Contact With Rubella.*—For this, in addition to members of the Working Party, directors of many public health laboratories participated. When immunoglobulin was requested because of exposure to rubella the patient's doctor was asked first to obtain from her a blood sample before giving the globulin intramuscularly. When possible, and particularly when the source of rubella was a member of the family, the doctor was asked also to take nasal and throat swabs from this index case in order to confirm the diagnosis and permit more accurate assessment of the globulin's effectiveness. Because many such patients were young children blood samples were not routinely collected from them. Specimens were returned to the laboratory by the quickest available route and the results of tests were recorded on standard cards. A second blood sample was obtained six weeks later from many of the contacts given the immunoglobulin. Particular attention was paid to those whose first serum lacked rubella-neutralizing antibody. After this interval passively circulating antibody would have disappeared but, including the incubation period, intercurrent infection in a susceptible person would be serologically demonstrable. In those already immune an increase of antibody titre could indicate a reinfection. By October 1967, when the number of paired samples of serum examined was considered sufficiently large relative to the number of second attacks of rubella, which had been found to be negligible, it was decided to restrict the collection of second blood samples to those initially found susceptible. When clinical or subclinical rubella was demonstrated during the pregnancy and therapeutic abortion had not been done the patient's doctor was again contacted towards the end of pregnancy and asked to record whether the infant was normal or showed any abnormalities at birth. He was also asked to obtain a sample of cord blood and throat and nasal swabs from the infant. The information on this aspect is, however, insufficient to be included in this report.

(3) *Survey of Women Recently in Contact With Rubella but Not Pregnant and Not Given Immunoglobulin.*—A need was recognized for an additional control group in assessing the value of immunoglobulin. To provide this a number of doctors in practice in various

parts of the country were approached. They were invited to submit to the appropriate laboratory nasal and throat swabs from any young patients with suspected rubella, particularly when the household contained one or more women of child-bearing age who were not pregnant. From these women samples of blood were requested as soon as possible after contact with the disease and again in six weeks, particularly from any found susceptible initially. The development of rubella in any of the adults was also to be reported. At the time of analysis the numbers in this survey were comparatively small but the survey is being continued.

(4) *Immunoglobulin.*—All globulin which has been issued for the prophylaxis of rubella in these investigations has been checked for its specific antibody content. Problems relating to the tests are the subject of a separate report (P.H.L.S., 1968).

Epidemiological Organization

Correlation of the investigations was done by the Epidemiological Research Laboratory. This included:

(1) The preparation of suitable explanatory protocols for the studies. These were distributed to doctors and other staff taking part.

(2) The design of a series of suitable standard record cards for patients to provide information on such relevant aspects as age, nationality, past history of rubella, number of pregnancies and miscarriages, history of recent contact with rubella or of the appearance of clinical rubella, details of any specimens collected, and the results obtained.

(3) The provision and issue of immunoglobulin with a high rubella antibody titre for use in the investigation.

(4) The distribution to doctors of specimen sets for taking nasal and throat swabs and blood from patients. The doctors then returned these sets to laboratories by post when local delivery was not possible.

(5) The return of all completed cards for analysis of the results.

(6) Personal liaison (by D. R.) with doctors, both in laboratories and in practice, who co-operated in the investigations. This was found to be of great value.

Laboratory Methods

This being a co-operative study uniform though not necessarily completely identical methods were used. In the different laboratories the R.K.$_{13}$ continuous line of rabbit kidney cells (Beale *et al.*, 1963) was available. The sensitivity of these cells was checked by repeated titration of known strains of rubella virus and for comparison of the results of neutralization tests a group of sera was also circulated among laboratories for titration.

Virus isolation was based on the method of Hutchinson and Thompson (1965). Swab extracts were cultured for up to 14 days in R.K.$_{13}$ cells, being examined at intervals for cytopathic effects. Subculture into further R.K.$_{13}$ cells for a similar period, and in some laboratories into vervet or patas monkey kidney cells also, was done before specimens were discarded as negative.

Serum neutralization tests were also based on the methods of Hutchinson and Thompson (1965) and Field (1967). They were regarded as screen tests in depth rather than full titrations and were done at initial serum dilutions of 1/4, 1/16, and 1/64. For uniformity and clarity all titres refer to complete inhibition of cytopathic effect at the initial serum dilutions used, intermediate titres being interpolations. By this means, though titres were lower than by a proportional plaque reduction there was less doubt in the interpretation of low positive titres.

In this report all the serological results were obtained by neutralization test. Field *et al.* (1967) have shown the haemagglutination-inhibition test to be as reliable and more rapid than the neutralization test. It is likely to be the main serological method in future reports.

Results

Control Sera

By way of introduction the results of titrations on some of the control sera distributed to laboratories are given in Table I. These show good correlation, the methods adopted in the various laboratories being sufficiently uniform.

TABLE I.—*Rubella-neutralizing Antibody in the Control Sera: Results of Tests by Members of the Working Party*

Serum	Laboratories						
	A	B	C	D	E	F	G
1	4	12	4	6	24	4	8
2	16	12	4	12	12	16	8
3	16	24	16	12	48	24	32
4	<4	<4	<4	<4	<4	<4	<4
5	4	6	4	6	8	4	8
6	16	24	16	24	16	32	32
7	64	48	32	24	24	32	32
8	16	12	16	12	16	12	32
9	<4	<4	<4	<4	<4	<4	<4

The figures are reciprocals of the initial serum dilution.

Survey No. 1

In this survey of women not recent contacts of rubella 2,007 samples of serum collected in different parts of the country were tested. Table II gives the antibody titres of sera according to area. Of the total, 184 (9%) with titres of less than 1/4 were susceptible and 1,779 (89%) with titres of 1/8 or greater were regarded as immune owing to past infection. In the small number, 44 (2%), with titres of 1/4 it is doubtful whether this is real evidence of past infection. It is more likely to represent non-specific viral inhibition in the neutralization test. With such an initial titre a person may, when exposed to rubella, develop the disease and show an adequate rise in antibody titre just as a primary infection. When titres of 1/8 or more are found clinical rubella has been observed only rarely and antibody titres remain virtually unchanged.

TABLE II.—*Rubella Antibody Titres Found in Different Areas of Survey*

Area	Reciprocal Titres of Initial Serum Dilutions					Total
	<4	4	8–16	32–64	>64	
Bedford ..	12 (8)	6 (4)	41	54	36	149
Bristol ..	18 (18)	0	38	42	0	98
Carmarthen ..	9 (14)	11 (17)	18	22	5	65
Coventry ..	8 (5)	1 (1)	29	80	29	147
Leeds/Keighley	26 (4)	6 (1)	213	380	10	635
Liverpool ..	5 (17)	1 (3)	12	8	3	29
London ..	38 (20)	0	77	71	6	192
Manchester ..	61 (9)	15 (2)	297	220	57	650
Newcastle ..	7 (17)	4 (9)	18	13	0	42
Total ..	184 (9)	44 (2)	743 (37)	890 (44)	146 (7)	2,007

In this and the following tables the figures in parentheses are percentages.

The proportion of women susceptible to rubella varied in the different areas, ranging from 20% in London to 4% in Leeds. Whatever the reasons for this, including problems of sampling and the natural spread of the disease, the results confirm that a high proportion of women of child-bearing age in this country are already immune to rubella. This is again shown in Table III, in which the sera have been grouped according to age. The proportion of those susceptible falls from 16% in women under 20 years old to less than 10% in other groups.

TABLE III.—*Rubella Immunity at Different Ages*

Age in Years	Rubella Antibody Titres			Total
	Reciprocals of Initial Serum Dilutions			
	Susceptible <4	Equivocal 4	Immune 8–8+	
<20	58 (16)	8	302 (82)	368
20–24	43 (8)	19	500 (88)	562
25–29	44 (9)	6	417 (90)	467
30–34	20 (7)	5	276 (92)	301
35–39	18 (9)	4	171 (89)	193
40+	1 (1)	2	113 (97)	116

Because the population of the United Kingdom includes many immigrants the sera have been arranged in Table IV according to country of origin. In this the duration of stay here is not taken into account. The proportion of susceptible women varies, but apart perhaps from the African group, which is small, the risk among immigrant populations appears to be similar to the risk to those born in this country.

TABLE IV.—*Rubella Immunity and Country of Origin*

Origin	Rubella Antibody Titres: Reciprocals of Initial Serum Dilutions		
	Susceptible <4	Equivocal 4	Immune 8–8+
British	153 (9)	41	1,593 (89)
European	2 (7)	2	26 (85)
Indian/Pakistan	2 (4)	0	43 (95)
West Indian	16 (21)	0	61 (78)
African	5 (33)	1	9 (60)
Other	6 (11)	0	47 (89)

It was of interest to see whether a reported history of rubella agreed with the state of immunity. Table V shows that 5% of those who stated that they had had the disease were still susceptible. Of those who gave no history of infection and those who did not know only 12% and 9% respectively lacked antibody. The remainder were immune, having a similar range of antibody titres to those who had been ill.

TABLE V.—*Rubella Immunity and History of Disease*

Past History of Rubella	Rubella Antibody Titres: Reciprocals of Initial Serum Dilutions					Total
	<4	4	8–16	32–64	>64	
Yes	26 (5)	6	190	278	45	545
No	127 (12)	30	426	445	72	1,100
Not known	31 (9)	8	127	167	29	362

Inquiry No. 2

The inquiry being made into the prophylactic value of a 750-mg. dose of immunoglobulin is recorded in Table VI. Of 1,747 persons who were given globulin because of presumed exposure to rubella 1,483 (85%) already had antibody, a figure similar to that in the survey (Table II). As with the survey the sera came from different parts of the country. Among the 264 found susceptible, rubella developed in 46 (17%) despite the immunoglobulin given to them, 24 having an illness with rash and 22 a subclinical infection confirmed serologically. In 28 instances the index case was a member of the household and this led to 14 clinical and 14 subclinical infections. In the 18 instances when the index case was not a household member there were 10 clinical and 8 subclinical infections.

TABLE VI.—*Rubella Contacts Given 750 mg. of Immunoglobulin*

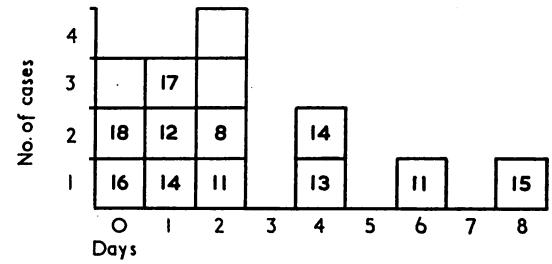
Total No. of contacts		1,747
No. possessing antibody		1,483 (85)
No. susceptible		264 (15)
No. developing rubella	Clinical 24, Subclinical 22	46

Material for attempted virus isolation was not available from all the index cases, but the results in a subgroup in which rubella was confirmed by virus isolation from them are shown in Table VII. Of 126 pregnant women who received immunoglobulin 25 were shown to be susceptible and 14 developed rubella, 11 having an illness with rash. With a proved index case in the same household the infection rate was 80%, but when contact was outside the household the rate was 20%. Despite the small numbers this difference is significant at the 5% level ($P \leq 0.05$).

In Fig. 1 the number of days between the appearance of a rash in the index child and inoculation of the 14 mothers with immunoglobulin is shown. The days after inoculation when the maternal rash appeared in 11 of them is also given.

TABLE VII.—*Pregnant Contacts of Rubella Given Immunoglobulin: Rubella Virus Isolated from Index Case*

No. of pregnant persons in contact with rubella		126
No. susceptible	With index case in household 15; With index case not in household 10	25
No. who developed rubella		14
Index case in household	Clinical rubella 9; Subclinical rubella 3	12
Index case not in household—clinical rubella		2



Interval between dates of onset of rash in index case, inoculation with immunoglobulin, and development of maternal rash. Numbers in boxes represent interval in days between inoculation and date of rash; subclinical cases are shown by blank boxes.

Among a further subgroup of 62 susceptible women in which six cases of rubella developed—one clinical and five subclinical—virus isolation from the index case, though attempted, was unsuccessful. This was at least partly explicable by the fact that the nasal and throat swabs were not collected until 4 to 10 days after the onset of illness.

Control Group

The control group (No. 3) of women of child-bearing age, household contacts of a case of rubella but not pregnant at the time and therefore not given immunoglobulin, is at this stage much smaller than the inquiry group. However, Table VIII (I) shows the total number tested to be 107 with 15 still susceptible, eight of whom then developed rubella—six clinically and two subclinically; and (II) a subgroup of 41 in which there was an index case in the same household proved by virus isolation. Among these, nine were still susceptible and seven developed rubella—five clinically and two subclinically—an infection rate of 78% and therefore similar to the rate among susceptible household contacts who received immunoglobulin.

TABLE VIII.—*Control Group of Rubella Contacts Not Given Immunoglobulin*

		I	II
No. of contacts		107	41
No. with antibody		92	32
No. susceptible		15	9
No. developing rubella	Clinical	6	5
	Subclinical	2	2
		8	7

In a search for evidence of second attacks 106 women exposed to rubella were studied. In each instance rubella in the index case was proved by virus isolation. There were 84 who were pregnant and who had received the standard amount of immunoglobulin and 22 who were not pregnant. All had neutralizing antibody in the initial sample of serum tested. Of these, one pregnant mother developed an illness with rash and from her a strain of rubella virus was isolated. Neither in her case nor in the remainder did a clear increase in antibody titre occur, though 14 showed a minor equivocal variation.

Conclusions

That foetal abnormalities do result from infection of the mother with rubella during early pregnancy is accepted. What is less clear is the extent to which maternal infection is likely to occur or, if there is exposure, the extent to which immunoglobulin can give protection. The results so far available from

a co-operative study in many areas and given here are interim and do not necessarily represent final conclusions. On the proportion of those immune to rubella because of past infection this has averaged 89% in our survey of 2,007 sera, ranging between 80 and 96% in different parts of the country. In the quite separate investigation of 1,747 pregnant contacts of rubella, serum from whom was obtained before immunoglobulin was given, 85% had immunity. These figures agree generally with previous reports from this and other countries and are applicable whether or not there has been a past history of rubella. In any event, as shown separately (P.H.L.S., 1968) immunoglobulin in susceptible volunteers has only a marginal effect in terms of circulating antibody. Because the risk of a second attack of rubella appears slight it would seem that most of the contacts received immunoglobulin unnecessarily. With the serological tests available it should now be practicable to test for rubella antibody serum from any woman before or as soon as possible after the start of pregnancy.

Previous estimates of the protective effect of immunoglobulin against rubella have been obscured by the high proportion already immune. Protection can only be accurately assessed in the small proportion who are susceptible. On this one criterion, irrespective of whether the illness in the index case was always true rubella, 46 out of 264 (17%) of those in our inquiry given the standard amount of globulin developed serologically proved rubella. With the further restriction of confirmation of rubella in the index case by virus isolation the number of susceptible persons fell to 25 and 14 of these developed serologically proved rubella. It was also found that in 12 of the 14, the index case was a member of the same household, a pointer to the high risk from close contact. The same criterion of virus isolation from the index case in the, at present, small control group of household contacts not pregnant and not given immunoglobulin showed that seven out of nine susceptible contacts developed the disease, again pointing to the high risk from close contact.

When only susceptible women in contact with proved rubella are considered the figures admittedly are small, but they do not suggest any protective effect against rubella by immunoglobulin in the dosage used. Larger amounts already tried (P.H.L.S., 1967) seem to have been no more effective, but the problem is to select and treat rapidly those specially at risk. Further studies will be done, but it is probable that ultimately an active vaccination programme will be more successful. From the findings rubella is as likely to be inapparent as it is to be an illness with a rash, so that it is difficult to decide on clinical grounds whether immunoglobulin has any suppressive effect on the illness. It did not do so in the one second attack recorded.

Because of the opportunities for therapeutic abortion and other factors follow-up information on infants born of mothers who had rubella during pregnancy is not at present available for this series.

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Measurement of Rubella Antibody in Immunoglobulin: its Disappearance from the Blood after Injection

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Summary: Rubella antibody titrations were done on samples of human immunoglobulin by neutralization and haemagglutination-inhibition methods. No significant variation was found in the antibody content of different batches. The specificity of the methods was confirmed by tests on a batch of human globulin specially prepared from plasma samples lacking rubella antibody.

Divided doses of immunoglobulin were given to volunteers who had no rubella antibody. Low titres were then detected in the blood for a limited period and the disappearance of this antibody was followed.

Introduction

Because of the chance of congenital abnormality after maternal rubella in early pregnancy human immunoglobulin is given prophylactically to pregnant women at risk.

Recently it has become possible to estimate the rubella antibody in human immunoglobulin, but titres appear to vary widely. Generally the higher range of titres, 1/256 to 1/2,048, were obtained with low virus dosage, 25 TCD_{50} or less, in neutralization tests (Schiff, Sever, and Huebner, 1963; Neva and Weller, 1964; Oxford, 1966; Murphy and Reid, 1967). Lower antibody titres, 1/64 to 1/512, were reported when a virus dose of 100 TCD_{50} was used (Parkman, Mundon, McCown, and Buescher, 1964; Givan, Rozee, and Rhodes, 1965; Green, Balsamo, Giles, Krugman, and Mirick, 1965; Picciotto, 1965; Hull and Butorac, 1966). Stewart, Parkman, Hopps, Douglas, Hamilton, and Meyer (1967) found rubella haemagglutination-inhibiting antibody titres of immunoglobulin to be in the range 1/1,024 to 1/4,096.

In the investigation now reported the rubella antibody content of a number of batches of human immunoglobulin used for rubella prophylaxis was assayed by neutralization and haemagglutination-inhibition methods. Specificity of the tests was checked by means of a specially prepared batch of human globulin devoid of rubella antibody. The decline and disappear-

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