J. Hyg., Camb. (1967), **65**, 409
Printed in Great Britain

The occurrence of neutralizing and complement fixing antibodies in rubella

By ANNE M. FIELD

Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London, N.W. 9

(*Received* 3 *May* 1967)

Since rubella virus was isolated in 1962 neutralization tests in tissue culture have been used widely to detect antibody in human sera. Complement-fixing antigens have also been developed (Sever *et al.* 1965; Stern, 1965; Schell & Wong, 1966; Schmidt & Lennette, 1966*a*; 1966*b*) and are commercially available.

As in other virus diseases, rubella neutralizing and complement-fixing antibodies are not identical, the former being more persistant. For surveys therefore the neutralization test is more useful as an indicator of past infection, whereas the complement-fixation test is of value in studies of acute and of congenital rubella infection.

MATERIALS AND METHODS

RK 13 *cell cultures*

These cultures (Beale, Christofinis & Furminger, 1963) were grown at 37° C. in Pyrex Roux bottles using synthetic medium 199 (S.M. 199) containing 0·088% sodium bicarbonate and 5% calf serum. A mixture of 0·05% trypsin and a 1/2500 dilution of versene in phosphate buffered saline solution A (Dulbecco & Vogt, 1954) was used to remove cells from the bottles. Pyrex tubes (16 × 150 mm.) were each seeded with 100,000 cells in 1 ml. growth medium and confluent cell monolayers were obtained after 3 days. At this stage the growth medium was replaced by maintenance medium consisting of S.M. 199 containing 0·176% sodium bicarbonate and 1% calf serum. The tubes were then rolled at 36·5° C. for 3 days before inoculation but no further medium changes were made. Calf serum was not inactivated at any time.

Virus for use in neutralization tests

The 'West Point' strain of rubella virus adapted to grow in RK 13 cells was used. Virus pools for the neutralization tests were prepared in RK 13 bottle cultures and stored in 1 or 2 ml. volumes at −70° C. Titres of these pools ranged from $10^{3\cdot0}$ to $10^{5\cdot0}$ TCD 50 per 0·1 ml.

Neutralization test

The diluent was S.M. 199 containing 0·088% sodium bicarbonate. Sera were not inactivated before testing. Serial twofold or fourfold dilutions of serum in 0·3 ml. volumes were mixed with equal volumes of 'West Point' virus diluted to contain

about 100 TCD50 in 0·1 ml. After incubation in a warm air incubator at 36° C for $1\frac{1}{4}$ hr. the virus-serum mixtures were placed at 4° C. for 1–2 hr. Each virus-serum mixture in 0·2 ml. volumes was inoculated into each of two RK 13 tubes. A positive human serum of known titre was included in each test.

Tubes were read on the fourth and the sixth or seventh days after inoculation. Microfoci were not counted, but the degree of cytopathic effect was estimated by direct microscopy. The end-point in the antibody titration was taken as the highest dilution causing complete or almost complete inhibition of the cytopathic effect.

Titres are expressed as the initial serum dilution before mixing with virus.

Complement-fixing antigen

This was bought as a lyophilized 20 % cell pack of rubella-infected LLC-MK2 cell cultures.

One ampoule of the antigen was reconstituted with 2 ml. veronal buffered saline pH 7·2 as a 1/2 dilution. This was stored in 0·1 ml. volumes at −70° C. until required for use when 0·1 ml. was thawed and diluted. On titration against a high-titre positive human serum the optimal dilution of the available batch was 1/24 but to ensure sensitivity in tests with unknown sera a 1/16 dilution was used, i.e. a further 1/8 of the stock dilution.

Complement-fixation test

A microtest five volume method adapted from Sever (1962) and Pereira, Pereira & Law (1964) was used, each unit volume being 0·025 ml. delivered with a calibrated dropping pipette. Sera were inactivated at 56° C. for 30 min. before testing. Serial twofold dilutions of serum were titrated against the 1/16 dilution of antigen in W.H.O. plastic plates. Complement, 1·8 to 2·0 MHD in 1 vol., was added to each virus–serum mixture. The plates were sealed with Sellotape, kept overnight at 4° C. and then held at room temperature for 10 min. before the sensitized cell suspension was added. The haemolytic system consisted of equal volumes of a 2 % suspension of sheep erythrocytes (standardized with the aid of a spectrophotometer) and a 1/100 dilution of haemolysin. The system was allowed to react for 15 min. at 37° C. before overnight storage at 4° C. Before they were added to the test the sensitized cells were warmed at 37° C. for 10 min. Two volumes of sensitized cells were added to each virus–serum-complement mixture and to all controls. The plates were again sealed with Sellotape and incubated at 36·5° C. for 1 hr.; for the first half hour they were shaken by hand at 10 min. intervals. Before the test was read the cells were left to settle for 3 hr. at 4° C. A known positive control human serum was included in each test.

Titres are expressed as the highest dilution of serum giving 50 % or more fixation with the antigen.

RESULTS

Neutralizing antibody in normal adult populations

In the early stages of these studies sera were tested at a range of dilutions: 1/4, 1/8, 1/16, 1/32 and 1/64. Later the range, 1/4, 1/16 and 1/64 was tested. Those containing antibody at dilutions of 1/4 or greater were regarded as having immunity.

Sera obtained from 24 members of the laboratory staff were tested for neutralizing antibody. Twenty sera (83 %) contained antibody (see Table 1).

Table 1. *Rubella neutralizing antibody in normal human sera*

Source of sera	No. tested	No. (percentage) with antibody (titre ⩾ 1/4)
Laboratory staff	24	20 (83 %)
Patients with glandular fever	10	8 (80 %)
Pregnant women (Portsmouth)	92	79 (86 %)
Pregnant women (England and Wales)	108	93 (86 %)
Totals	234	200 (86 %)

Table 2. *Rubella neutralizing antibody in the adult population of a Welsh mining village*

Sex	No. tested	No. (percentage) with antibody (titre ⩾ 1/4)
Female	114	105 (92 %)
Male	111	108 (97 %)
Both sexes	225	213 (95 %)

Sera from ten patients with glandular fever were tested and eight (80 %) had antibody.

Pregnant women from the Portsmouth area were bled soon after contact with suspected rubella. Seventy-nine of the 92 sera tested (86 %) had neutralizing antibody. Pregnant women from other parts of England and Wales were also bled soon after contact with suspected rubella. Ninety-three of 108 sera tested (86 %) had neutralizing antibody.

Sera collected for other purposes from adults living in a South Wales mining village were tested for rubella antibody (Table 2). Of 225 sera 213 had neutralizing antibody (95 %) consisting of 92 % of the females and 97 % of the males. The proportion of immune persons in the different groups rose with increasing age (Table 3) from 77 % in the 15–20-year age group to 98–100 % in the age groups over 40 years.

Neutralizing antibody titres were mainly within the range 1/16 to 1/48 (Table 4); titres of 1/4 to 1/12 were met less frequently and high titres, 1/64 or more, were comparatively rare. Titres between 1/16 and 1/48 were the most common in all the

age groups of the Welsh mining village inhabitants (Table 5). Thus there was little if any indication that antibody titres were likely to be lower in older persons, although this has been suggested (Givan, Rozee & Rhodes, 1965; Oxford, 1966).

Table 3. *Rubella neutralizing antibody and age*

(Sera from the population of a Welsh mining village.)

Age (years)	No. tested	No. with antibody	Percentage with antibody
15–20	22	17	77
21–25	24	21	87
26–30	16	15	94
31–40	28	27	97
41–50	44	43	98
51–60	54	53	98
61–70	37	37	100
Total	225	213	95

Table 4. *Titres of rubella neutralizing antibody in normal adult sera*

Source of sera	No. with antibody	Percentage of sera in each titre range*		
		Low titres	Medium titres	High titres
Laboratory staff	20	15	75	10
Patients with glandular fever	8	37½	37½	25
Pregnant women (Portsmouth)	79	27	67	6
Pregnant women (England and Wales)	93	12	83	5
Welsh mining village	213	25	60	15
Total	413	22	67	11

* Low titres: 1/4, 1/6, 1/8, 1/12; medium titres: 1/16, 1/24, 1/32, 1/48; high titres: 1/64, > 1/64.

Table 5. *Titres of rubella neutralizing antibody according to age*

(Sera from the population of a Welsh mining village.)

Age in years	No. with antibody	No. of sera in each range of titres*		
		Low titres	Medium titres	High titres
15–20	17	6	10	1
21–25	21	3	14	4
26–30	15	4	11	0
31–40	27	5	15	7
41–50	43	11	26	6
51–60	53	19	26	8
61–70	37	5	27	5
Total	213	53	129	31

*Low, medium and high titres: see footnote to Table 4.

https://doi.org/10.1017/S0022172400045927 Published online by Cambridge University Press

Table 6. *Rubella complement-fixing and neutralizing antibody in normal sera*

No.	Age (years)	Antibody titres: Complement-fixing	Antibody titres: Neutralizing
1	18	< 4	32
2	19	> 8	32
3	21	< 4	32
4	21	< 4	32
5	23	< 4	< 4
6	24	< 4	32
7	24	> 8	32
8	30	< 4	12
9	33	< 4	8
10	34	< 4	16
11	38	< 4	32
12	43	< 4	32
13	55	< 4	8
14	61	< 4	32
15	66	< 4	8

Table 7. *Neutralizing and complement-fixing antibody response to infection with rubella virus**

Time after onset of infection†	Neutralizing antibody titres: No. tested	Neutralizing antibody titres: Titres observed	Complement-fixing antibody titres: No. tested	Complement-fixing antibody titres: Titres observed
0 day	7	< 4, < 4, < 4, 4, 4, 4, 6	7	< 4, < 4, < 4, < 4, < 4, < 4, < 8
1 day	6	< 4, < 4, < 4, 6, 6, 16	5	< 4, < 4, < 4, < 4, 8
2 days	4	< 4, 6, 6, 16	4	< 4, < 4, < 4, < 4
3 days	2	< 4, 4	2	< 4, < 4
5 days	1	12	1	< 4
6 days	1	< 4	1	< 4
7 days	1	48	1	32
10 days	1	32	1	> 32
14 days	2	48, 48	2	> 32, > 32
18 days	1	> 64	1	8
19 days	1	24	1	16
24 days	2	16, 32	2	< 4, > 32
4–6 weeks	5	24, 24, 24, 32, 48	5	8, 8, 16, > 32, > 32
7–9 weeks	3	24, 32, 48	3	8, 8, 16
10–12 weeks	2	24, 48	2	> 32, > 32
3–6 months	3	24, 32, 32	4	< 4, 8, 16, > 32
7–12 months	0		0	
12–18 months	3	32, 32, 64	2	16, 16
> 18 months	1	64 (2 yr.), 64 (3 yr)	0	

* Rubella virus was isolated from each of these patients.
† Day 0 = day of onset of rash.

https://doi.org/10.1017/S0022172400045927 Published online by Cambridge University Press

Complement-fixing antibody in normal adult populations

Fifteen sera from the adults of the Welsh mining village were tested for complement-fixing as well as neutralizing antibody (Table 6). Although 14 had neutralizing antibody only two had complement-fixing antibody, these being from persons aged 19 and 24 years respectively. This would indicate fairly recent infection and it substantiates the evidence (Table 3) of the build up of immunity between the ages of 15 and 25 years.

Table 8. *Neutralizing and complement-fixing antibody response to infection with rubella virus**

Time after onset of infection†	Neutralizing antibody titres		Complement-fixing antibody titres	
	No. tested	Titres observed	No. tested	Titres observed
0 day	2	< 4, < 4	2	< 4, < 4
1 day	3	< 4, 6, 12	3	< 4, < 4, < 4
2 days	4	< 4, < 4, 4, 8	3	< 4, < 4, < 4
3 days	3	< 4, 4, 6	3	< 4, < 4, < 4
4 days	5	< 4, < 4, < 10, 4, 4	5	< 4, < 4, < 4, < 8, 4
5 days	1	< 4	1	< 4
6 days	1	< 4	1	< 4
7 days	2	16, 24	2	< 4, 16
8 days	1	< 4	0	
9 days	3	6, 12, 16	3	< 4, 4, > 32
10 days	1	16	1	16
12 days	1	16	1	> 32
13 days	3	12, 24, 32	3	16, > 32, > 32
15 days	2	8, > 64	2	4, > 32
16 days	1	64	1	> 32
17 days	1	24	1	> 32
20 days	1	48	1	> 32
28 days	2	12, 24	2	8, > 32
4–6 weeks	4	12, 12, 24, 24	4	8, 16, 16, 16
7–9 weeks	5	16, 32, 32, 32, 48	5	16, > 32, > 32, > 32, > 32
10–12 weeks	2	12, 24	2	8, 16
3–6 months	2	24, 48	2	8, > 32

* No rubella virus was isolated but each patient showed a diagnostic rise in antibody titre.
† Day 0 = day of onset of rash.

Antibody following rubella infection

Samples of serum from many patients with rubella were examined and the antibody results are shown in Tables 7 and 8 and graphically in Fig. 1. Those on Table 7 are serological results from patients from whom virus was isolated during the acute stage of illness. The results on Table 8 are from patients in whom fourfold or greater rises in antibody titre occurred although virus was not isolated, usually because the necessary specimens were not received.

Neutralizing antibody was found to develop rapidly after the onset of illness

https://doi.org/10.1017/S0022172400045927 Published online by Cambridge University Press

often being present even on the day of appearance of the rash. Of 40 sera taken during the first 6 days post-rash 20 (50 %) contained neutralizing antibody.

Complement-fixing antibody appeared later than did neutralizing antibody and was rarely present in the first 6 days of illness. Of 38 sera taken during this period only two had complement-fixing antibody.

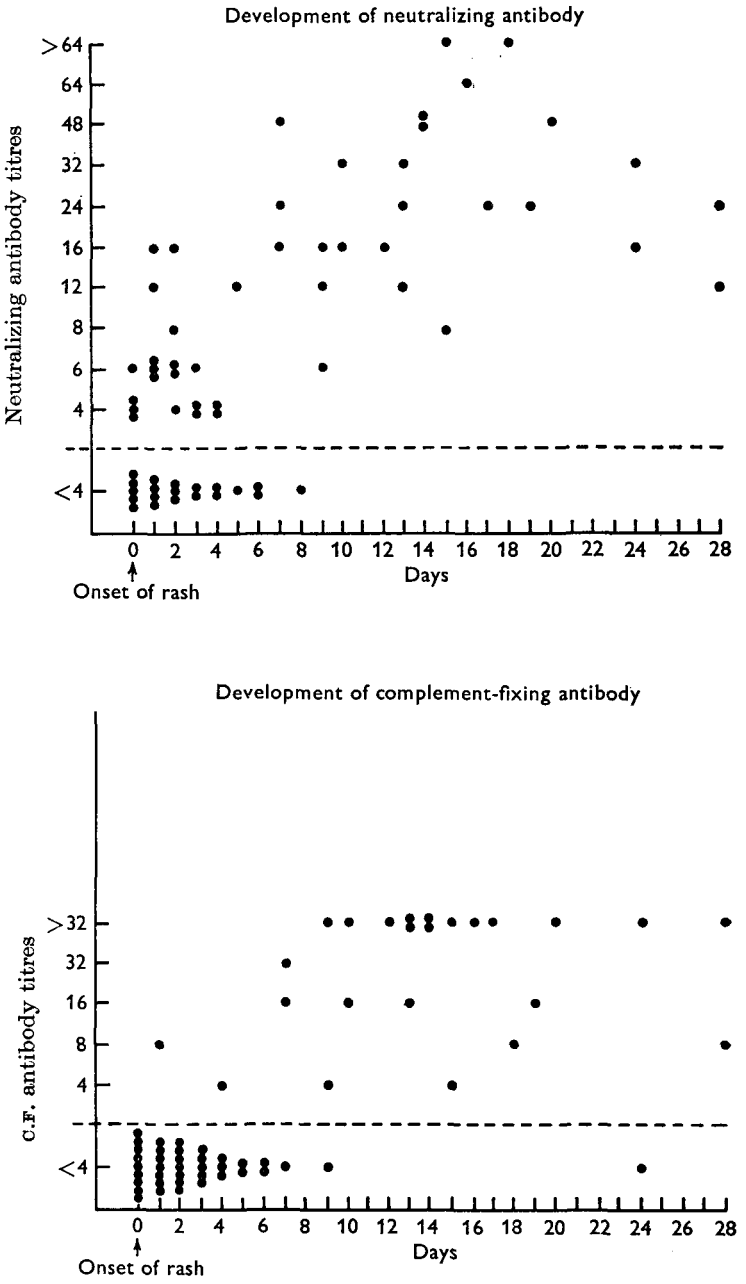

Fig. 1. The development of rubella neutralizing and complement-fixing antibodies.

https://doi.org/10.1017/S0022172400045927 Published online by Cambridge University Press

High titres of neutralizing and complement-fixing antibody were attained 1–3 weeks after the onset of illness.

In every patient from whom virus was isolated a rise in neutralizing antibody titre was shown, such rises being usually sixfold or eightfold in suitably spaced serum samples. A fourfold rise in neutralizing antibody titre was considered to be of diagnostic significance. With one exception complement-fixing antibody was demonstrated in convalescent serum samples. Blood specimens were taken from

Table 9. *Rubella antibody and virus isolation in congenital rubella*

Case no.	Age	Rubella antibody: Neut.	Rubella antibody: C.F.	Virus isolation	Criteria for inclusion in the group
1	1 day	40	n.t.	+ T/S	Clinically congenital rubella
2	1 day	24	< 4	+ T/S, Blood clot	Clinically congenital rubella
3	1 day	64	4	n.t.	Healthy at birth. Mother had virologically-confirmed rubella in early pregnancy
4	1 day	48	8	+ T/S, Urine	Clinically congenital rubella
5	2 days	64	4	+ T/S	Healthy at birth but virus isolated
6	3 days	48	n.t.	+ N/S	Clinically congenital rubella
7	3 days	64	> 32	−	
8	6 days	48	16	n.t.	History of maternal rubella. No clinical details
9	6 days	> 64	n.t.	+ N/S, Urine	Clinically congenital rubella
10	6 days	64	4	+ T/S	
11	2 weeks	48	< 4	n.t.	Unchanged titres in 4 weeks.
	6 weeks	48	< 4	n.t.	Clinically congenital rubella
12	6 weeks	48	< 4	+ Urine	Clinically congenital rubella
13	3 months	> 64	< 4	+ N/S.T/S, Lens fluid	
14	3 months	> 64	> 32	n.t.	Unchanged titres in 5 months
	8 months	> 64	> 32	n.t.	Clinically congenital rubella
15	4 months	> 64	> 32	n.t.	Clinically congenital rubella
16	5 months	32	< 4	n.t.	Clinically suspected congenital rubella
17	6 months	> 64	> 32	n.t.	Clinically congenital rubella
18	11 months	48	n.t.	+ N/S.T/S, Urine	
19	14 months	> 64	< 4	−	
20	14 months	64	n.t.	n.t.	
21	18 months	> 64	< 4	−	
22	22 months	48	n.t.	n.t.	
23	24 months	16	n.t.	n.t.	
24	24 months	16	< 4	n.t.	

n.t. = not tested + = virus isolated − = virus not isolated

https://doi.org/10.1017/S0022172400045927 Published online by Cambridge University Press

this patient on the day of onset and 24 and 110 days later. Virus was isolated on the day of onset and a neutralizing antibody response to infection was shown, but in neither of the two convalescent serum samples did complement-fixing antibody appear.

Neutralizing antibody in congenital rubella

Passively acquired maternal antibody disappears from the infant's circulation 3–6 months after birth so that infants not infected by rubella virus *in utero* do not possess rubella antibody by this time. The persistence of neutralizing antibody after the age of 3–6 months indicates infection *in utero* (Dudgeon, Butler & Plotkin, 1964; Butler *et al.* 1965; Alford, 1965; Sever *et al.* 1966).

Table 10. *Rubella antibody in infants with congenital rubella and their mothers*

Case no.	Infant's antibody titres		Mother's antibody titres		Age of infant
	Neut	C.F.	Neut	C.F.	
1	40	n.t.	24	< 4	1 day
4*	48	8	48	4	1 day
6	48	n.t.	48	4	3 days
7	64	> 32	> 64	> 32	3 days
8	48	16	48	> 32	6 days
9	> 64	n.t.	> 64	n.t.	6 days
11	48	< 4	–	–	2 weeks
	48	< 4	48	< 4	6 weeks
14	> 64	> 32	48	< 4	3 months
	> 64	> 32	32	< 4	8 months
15	> 64	> 32	> 64	< 4	4 months
16	32	< 4	32	< 4	5 months
18	48	n.t.	48	> 32	11 months
19	> 64	< 4	> 64	< 4	14 months
24	16	< 4	> 64	< 4	24 months

* Case 4: mother's serum 5 weeks later than infant's.

Table 9 shows antibody and virus isolation results on 24 infants of whom 21 had signs of congenital rubella. Two infants were healthy at birth, but rubella virus was isolated from one indicating infection *in utero*. The mother of the second healthy child had virologically confirmed rubella in early pregnancy. No clinical details were available on one infant, but there was a history of maternal rubella.

All 24 infants, who were aged 1 day to 2 years, had neutralizing antibody. Titres in the infants were very similar to those of the maternal sera taken at the same time (Table 10).

Table 11 shows neutralizing antibody titres of sera from a control group of 15 children with congenital heart disease but no history of maternal rubella or other signs of congenital rubella. Only three had rubella neutralizing antibody and they were under 4 months of age.

Titres of neutralizing antibody in infants with congenital rubella and their mothers were usually higher than the antibody titres found in the general popula-

tion or in sera taken from 1 to 18 months after rubella unconnected with pregnancy (Table 12). Low titres were not found in the congenital rubella group; 50% of the affected infants and 36% of their mothers had titres of 1/64 or more. Such high titres were uncommon in the general population even after a recent infection.

Complement-fixing antibody in congenital rubella

Most infants with congenital rubella had complement-fixing antibody, generally to a low titre, during their first week of life. Similar titres were demonstrated in the maternal sera taken at this time (Tables 9 and 10).

Table 11. *Rubella neutralizing antibody in infants with congenital heart disease*

No.	Age	Neutralizing antibody titre
1	2 weeks	< 8
2	3 weeks	< 4
3	4 weeks	12
4	4 weeks	< 4
5	2 months	< 4
6	3 months	< 8
7	3 months	< 8
8	3 months	12
9	3 months	16
10	8 months	< 4
11	10 months	< 4
12	11 months	< 8
13	14 months	< 4
14	3 years	< 4
15	4 years	< 4

Table 12. *Titres of neutralizing antibody in congenital rubella*

Source of sera	No. with anti-body	Percentage at each range of titres*		
		Low titres	Medium titres	High titres
Normal adults	413	22	67	11
Sera taken 1–18 months after infection	32	0	88	12
Mothers of infants with congenital rubella	14	0	64	36
Infants with congenital rubella	26	0	50	50

* Low, medium and high titres: see footnote to Table 4.

Complement-fixing antibody was also present to a high titre in sera from three older infants, i.e. one child aged 4 months whose mother had no complement-fixing antibody at the time in her serum, one child aged 6 months and the third one at both 3 and 8 months of age (case 14), although this mother also was negative at the relevant times.

It is likely that complement-fixing antibody present early in the infant's life is maternal in origin and that the higher titres in older infants are developed by the child's own immune mechanism in response to the virus infection which continues after birth (Sever *et al.* 1966; Monif, Hardy & Sever, 1967). Mothers generally lose complement-fixing antibody by the time the child is 3 months old but in one instance (case 18) the mother's serum gave a positive reaction in the complement-fixation test when the child was 11 months old.

DISCUSSION

Work with experimental rubella in volunteers has shown that the presence of rubella neutralizing antibody at a titre of 1/4 or more prevents re-infection with the virus (Green *et al.* 1964, 1965; Schiff, Sever & Huebner, 1965). This indicates that detectable antibody and immunity are synonymous. It may be concluded from the surveys detailed here and from other published surveys (Givan *et al.* 1965; Sever, Schiff & Huebner 1964; Hutchinson & Thompson, 1965) that 80–95 % of women in an industrial society are immune to re-infection with rubella virus. Local variations in the prevalence of immunity occur, as shown by the high degree of immunity in the Welsh mining community compared with the population of England and Wales as a whole. Similar variations have already been described (Sever *et al.* 1964; Report, 1967).

Unfortunately neutralization tests are laborious and expensive. The complement fixation test reveals antibody shortly after infection with rubella virus but is not suitable for detecting a rubella infection which occurred some years previously. Thus the complement-fixation test cannot replace the neutralization test in measuring immunity. The newly described haemagglutination inhibition test (Stewart *et al.*, 1967) should prove extremely useful for this purpose however since the findings tend to be similar to those of neutralization tests.

Because the positive complement-fixation test indicates recent rubella infection its occurrence in a mother and child shortly after the birth of the child will suggest rubella infection at least of the mother during pregnancy but not always infection of the foetus *in utero* because the antibody in the child at this early stage of life is largely maternal (Alford, 1965). Antibody in the child may either disappear in the first few weeks of life, in which case there was no intrauterine infection, or it may persist. If it persists this is interpreted by Alford (1965) as a gradual loss of maternal antibody (IgG) accompanied by an increase of the infant's own antibody, initially IgM then IgG, which remains indefinitely. The low titre of complement-fixing antibody found in very young infants, which correlates well with the mother's antibody titre, is residual maternal antibody. The high titres of complement-fixing antibody found in older children represent antibody synthesized by the child in response to rubella infection *in utero*.

The comparatively high neutralizing antibody titres found in mothers of children with congenital rubella may reflect a continuing antigenic stimulus resulting from persistence of infectious virus in the foetus during the last 6 months of pregnancy. Infants with congenital rubella commonly excrete virus in large

quantities even when there is neutralizing antibody in the circulation. This persistence of virus may account for the high antibody titres found in this group.

SUMMARY

Neutralization and complement-fixation tests to detect rubella antibody are described. Results of such tests show that between 80 and 95 % of adults in England and Wales have neutralizing antibody. The presence of complement-fixing antibody indicates recent infection with rubella virus.

Results are presented showing the development of neutralizing and complement-fixing antibody after rubella.

Antibody tests on children with congenital rubella and their mothers are detailed. It is noted that higher neutralizing antibody titres are found in this group than in the general population.

I wish to express my thanks to Dr J. V. T. Gostling of the Public Health Laboratory, Portsmouth, for sera from pregnant women; to Dr P. C. Elwood of the Medical Research Council Epidemiological Research Unit (South Wales) for the sera from the Welsh mining village; to Dr J. A. Dudgeon of Hospital for Sick Children, Great Ormond Street, London, for providing the 'West Point' strain of rubella virus, and to Miss Marjorie Bennett, Mrs Marion Woodroof and Mr Colin Whitehouse for their assistance with the neutralization tests.

REFERENCES

Alford, C. A. (1965). Studies on antibody in congenital rubella infections. I. Physicochemical and immunologic investigations of rubella neutralizing antibody. *Am. J. Dis. Child.* **110**, 455.

Beale, A. J., Christofinis, G. C. & Furminger, I. G. S. (1963). Rabbit cells susceptible to rubella virus. *Lancet* ii, 640.

Butler, N. R., Dudgeon, J. A., Hayes, K., Peckham, C. S. & Wybar, K. (1965). Persistence of rubella antibody with and without embryopathy. A follow-up study of children exposed to maternal rubella. *Br. med. J.* ii, 1027.

Dudgeon, J. A., Butler, N. R. & Plotkin, S. A. (1964). Further serological studies on the rubella syndrome. *Br. med. J.* ii, 155.

Dulbecco, R. & Vogt, M. (1954). Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. exp. Med.* **99**, 167.

Givan, K. F., Rozee, K. R. & Rhodes, A. J. (1965). Incidence of rubella antibodies in female subjects. *Can. med. Ass. J.* **92**, 126.

Green, R. H., Balsamo, M. R., Giles, J. P., Krugman, S. & Mirick, G. S. (1964). Studies on the experimental transmission, clinical course, epidemiology and prevention of rubella. *Trans. Ass. Am. Physns.* **77**, 118.

Green, R. H., Balsamo, M. R., Giles, J. P., Krugman, S. & Mirick, G. S. (1965). Studies of the natural history and prevention of rubella. *Am. J. Dis. Child.* **110**, 348.

Hutchinson, D. N. & Thompson, K. M. (1965). Continuous rabbit kidney cell cultures in the diagnosis of rubella infections. *Mon. Bull. Minist. Hlth* **24**, 385.

Monif, G. R. G., Hardy, J. H. & Sever, J. L. (1967). The lack of association between the appearance of complement fixing antibodies and the recovery of virus in a child with congenital rubella. *Pediatrics, Springfield* **39**, 289.

Oxford, J. (1966). Rubella virus—1966. *Publ. Hlth, Lond.* **80**, 270.

Pereira, H. G., Pereira, M. S. & Law, V. G. (1964). Antigenic variants of influenza A_2 virus. *Bull. Wld Hlth Org.* **31**, 129.

Report to Public Health Laboratory Service Rubella Working Party, 1967. (1967). *Br. med. J.* (In the Press).

Schell, K. & Wong, K. T. (1966). Stability and storage of rubella complement fixing antigen. *Nature, Lond.* **212**, 621.

Schiff, G. M., Sever, J. L. & Huebner, R. J. (1965). Experimental rubella. Clinical and laboratory findings. *Archs. intern. Med.* **116**, 537.

Schmidt, N. J. & Lennette, E. H. (1966*a*). Rubella complement-fixing antigens derived from the fluid and cellular phases of infected BHK-21 cells: extraction of cell-associated antigen with alkaline buffers. *J. Immun.* **97**, 815.

Schmidt, N. J. & Lennette, E. H. (1966*b*). The complement-fixing antigen of rubella virus. *Proc. Soc. exp. Biol. Med.* **121**, 243.

Sever, J. L. (1962). Application of a microtechnique to viral serological investigations. *J. Immun.* **88**, 320.

Sever, J. L., Huebner, R. J., Castellano, G. A., Sarma, P. S., Fabiyi, A., Schiff, G. M. & Cusumano, C. L. (1965). Rubella complement fixation test. *Science, N.Y.* **148**, 385.

Sever, J. L., Huebner, R. J., Fabiyi, A., Monif, G. R., Castellano, G., Cusumano, C. L., Traub, R. J., Ley, A. C., Gilkeson, M. R. & Roberts, J. M. (1966). Antibody responses in acute and chronic rubella. *Proc. Soc. exp. Biol. Med.* **122**, 513.

Sever, J. L., Schiff, G. M. & Huebner, R. J. (1964). Frequency of rubella antibody among pregnant women and other human and animal populations. *Obstet. Gynec., N.Y.* **23**, 153.

Stewart, G. L., Parkman, P. D., Hopps, H. E., Douglas, R. D., Hamilton, J. P. & Meyer, H. M. (1967). Rubella virus hemagglutination-inhibition test. *New Engl. J. Med.* **276**, 554.

Stern, H. (1965). Rubella virus complement-fixation test. *Nature, Lond.* **208**, 200.