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Specific immunoglobulin responses after varicella and herpes zoster

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SUMMARY

The indirect immunofluorescence technique has been used to titrate the specific immunoglobulins in 200 sera from 64 patients with varicella, and 195 sera from 67 patients with herpes zoster. IgG and IgM antibodies were detected in all patients with varicella, and IgA in 59 (92%). All three classes of antibody appeared 2–5 days after the onset of the rash, increased virtually simultaneously and reached maximum titres during the second and third weeks. IgG then declined slowly, but never became undetectable and was still present in five subjects who were retested after 2–4 years; it was present in 88 out of 100 healthy young adults and probably persists indefinitely after varicella. IgA and IgM antibodies declined more rapidly and were not detected in specimens taken more than a year after the illness. IgA, however, may possibly persist in some cases since low titres were found in 8 out of 88 young adults who possessed IgG antibody and had presumably had varicella in the past. IgA responses were significantly weaker in children under the age of 6 years than in older children and adults.

Six out of 67 patients with zoster were tested at various times before the onset of the rash: IgG antibody was detected in all. IgG was present in all sera taken after the onset of the rash, increased rapidly after 2–5 days, reached maximum titres during the second and third weeks and then declined slowly. IgA antibody was detected in 66 patients (99%) and IgM in 52 (78%); both types of antibody followed transient courses, as in varicella.

Maximum titres of IgG and complement-fixing antibodies were greater after zoster than after varicella, but the differences were not significant. IgA and IgM titres in young adults with zoster were significantly lower than in older patients, and also lower than in young adults with varicella.

Increases in varicella-zoster antibody in patients with herpes simplex virus infections consisted mainly of IgG, sometimes IgA, but never IgM.

INTRODUCTION

The circulating antibodies that appear in man after primary viral infection have been shown, in the case of several different viruses, to include both IgG

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and IgM (Svehag & Mandel, 1964; Schluederberg, 1965; Ohta-Hatano & Hinuma, 1966). The IgM response is usually transient, and in rubella in particular the presence of specific IgM has been found to indicate recent infection (Best, Banatvala & Watson, 1969; Cradock-Watson, Bourne & Vandervelde, 1972; Pattison & Dane, 1975). IgG antibody is more permanent: although titres decline after the initial response they tend to persist indefinitely at lower values, especially after those infections that are normally followed by lifelong immunity. IgA antibody, too, appears in the blood after primary viral infection but there is less agreement about its duration. In rubella, for example, Cradock-Watson *et al.* (1972) found the IgA response to be transient, but other workers, using different methods, have reported persistence of IgA for at least a year (Ogra *et al.* 1971; Al-Nakib, Best & Banatvala, 1975).

The specific immunoglobulin responses after secondary infection in man have received less study, probably because most systemic viral infections are followed by immunity which is solid enough to make reinfection uncommon and subclinical. Rises in the titre of pre-existing antibody have, however, been observed in symptomless patients in contact with cases of measles and rubella and in subjects challenged with attenuated strains of these viruses (Krugman *et al.* 1965; Schluederberg & Karelitz, 1965; Horstmann *et al.* 1970; Boué, Nicolas & Montagnon, 1971; MacDonald *et al.* 1978). Studies of some of these subclinical reinfections have shown that the antibody consists of IgG, but not IgM, and have supported the generally held view, based on studies with diverse antigens in a variety of species, that IgM antibody is more characteristic of primary than of secondary immunization (Pike, 1967).

After varicella, clinically recognizable secondary infection may occur in the form of herpes zoster. This is thought to be due to reactivation of varicella-zoster (V-Z) virus which has remained latent, probably in the posterior root ganglia, following an attack of chickenpox many years previously (Hope-Simpson, 1965). Attempts to compare IgM responses in the two diseases, however, have given conflicting results. Leonard, Schmidt & Lennette (1970), who tested serum fractions obtained by DEAE-cellulose chromatography, detected IgM antibody by both neutralization and immunofluorescence after varicella but not after zoster. In later work in which they examined sucrose density gradient fractions Schmidt & Lennette (1975) again detected IgM with neutralizing activity in all patients with varicella (5 cases) but in only 1 out of 22 patients with zoster. Ross & McDaid (1972) did not test patients with varicella, but detected IgM antibody by immunofluorescence in 20 out of 40 patients with zoster. Brunell *et al.* (1975), using immunofluorescence to demonstrate cell membrane antigen, detected IgG, IgA and IgM antibodies in all patients with varicella and zoster (3 and 63 cases, respectively).

The conflicting results obtained by previous workers, although mainly attributable to technical differences, may also be partly due to differences in the ages of the patients studied. Varicella is a disease of childhood, but zoster occurs mainly in adults and becomes commoner with increasing age (McGregor, 1957; Hope-Simpson, 1965). Cases of zoster therefore cover an extensive range of ages and the

period of latency presumably also varies widely. We have compared the IgG, IgA and IgM responses in patients of similar ages with varicella and zoster by using the immunofluorescence technique to titrate these antibodies in serial specimens from young adults. We have also compared the peak titres of these antibodies in patients of widely different ages by testing sera submitted for routine serological diagnosis. Because of the suggestion by Hope-Simpson (1965) that a rise in the titre of pre-existing antibody may be provoked by contact with a case of varicella we have also examined paired sera from persons in close contact with cases of this disease. Finally, we have used immunofluorescence to study the V-Z-specific immunoglobulins in patients with primary herpes simplex virus (HSV) infections, since rises in the titre of V-Z complement-fixing (CF) antibody in such cases have been described and are commonly observed in diagnostic work (Kapsenberg, 1965; Svedmyr, 1965; Schmidt, Lennette & Magoffin, 1969).

Previous workers who have studied V-Z antibodies by immunofluorescence have used various techniques to prepare antigens for staining. Weller & Coons (1954) used acetone-fixed infected monolayers on coverslips. Schmidt *et al.* (1965) and Ross & McDaid (1972) made suspensions of infected cells which were then spotted on to slides, dried, and fixed with acetone. Williams, Gershon & Brunell (1974) stained infected cells in suspension, not dried or fixed, in order to detect surface membrane antigens; their method was evidently relatively free from non-specific staining since sera were tested at a dilution of 1/2, but it requires a supply of freshly prepared cells whereas fixed preparations can be stored at -20 °C until required. We have used fixed infected monolayers on cover-slips because the morphology of the characteristic foci of infection produced by V-Z virus is preserved and specific fluorescence can be seen to occur only in these foci and not in the surrounding areas of uninfected cells.

MATERIALS AND METHODS

Patients with varicella-zoster infections

Ninety sera were tested from 14 students (11 males) aged 18–26 years with varicella, and 74 sera from 12 students (7 males) aged 19–29 years with herpes zoster. Serial specimens (5–9 per patient) were collected during observation periods ranging from 9 months to 4 years. All these students were seen by one of us (M.S.B.) and the diagnosis was confirmed in each case by observing a rise of at least fourfold in the titre of CF antibody.

In order to compare the antibody responses in patients of different ages we also examined sera (2–3 per patient) submitted for routine serological diagnosis. One hundred and ten sera were tested from 50 patients aged 10 weeks to 67 years with varicella, and 121 sera from 55 patients aged 12–89 years with zoster. The diagnosis was confirmed in 95 cases by observing a rise of at least fourfold in the titre of CF antibody, and in ten cases by observing a fall in titre. Rheumatoid factor, which may give false positive results in immunofluorescence tests for IgM antibody (Fraser, Shirodaria & Stanford, 1971) was not detected in any of these sera at a dilution of 1/8 by a slide latex agglutination test. Two sera from a patient with zoster which did contain rheumatoid factor have not been included.

Normal adults and contacts

Single specimens of serum from 100 normal students aged 18–25 years (80 males) were tested for V-Z-specific IgG, IgA and IgM antibodies in order to assess the prevalence of these antibodies in young adults and the titres in those who had had varicella in the past. Paired sera from 14 normal adults aged 19–57 years who had previously had varicella and were in close contact with cases of this disease were also tested for these antibodies; the first specimen was taken within a week of the onset of the rash in the index case and the second about 4 weeks later.

Patients with primary herpes simplex virus infections

Paired sera from 57 patients with HSV infections which were thought to be primary were tested for V-Z CF antibody and V-Z-specific immunoglobulins. The first serum was collected within a week of the start of the illness and the second between 9 days and 6 weeks after the onset. The diagnosis was confirmed by detecting a rise of fourfold or more in the titre of HSV CF antibody (all cases) and by virus isolation (40 cases). Twenty-five patients had no V-Z IgG antibody in the first serum and were assumed not to have had varicella in the past; 23 of these were children aged 6 months to 10 years with stomatitis, and 2 were adolescents aged 15 and 16 years with genital infections. Thirty-two patients had V-Z IgG antibody in the first serum and were assumed to have had varicella in the past; 11 of these were aged $3\frac{1}{2}$–31 years and had herpes infections of the mouth, throat, lips or face; 21 were patients aged 18–28 years with genital infections.

Cover-slip preparations for fluorescent staining

Cover-slip cultures were made with the MRC 5 line of human diploid cells (Jacobs, Jones & Baille 1970). These cells were grown in minimal essential medium (Wellcome Reagents Ltd) containing 10% fetal calf serum, 10% Difco tryptose phosphate broth and 2 mM glutamine. They were maintained in Eagles basal medium (Grand Island Biological Company) containing 2% fetal calf serum.

Pieces of cover-slip measuring 30×5 mm. were inserted into $4 \times \frac{1}{2}$ in. tissue culture tubes to which 1·4 ml volumes of growth medium containing about 300000 cells per ml were then added. These tubes were incubated in a sloped position at 36 °C for 24 h. The growth medium was then replaced by maintenance medium and the cultures were reincubated for a further 1–2 days. The monolayers were infected by replacing the medium with a suspension of MRC 5 cells infected with V-Z virus (see below). The tubes were then incubated in a sloped position for 24 h at 36 °C. and for a further 24 h at 30 °C. Finally the cover-slips were removed, fixed in acetone for 5 min, dried in air, divided into smaller fragments and stored at −20 °C. as previously described (Cradock-Watson *et al.* 1972).

Varicella-zoster virus

The Brownlee strain of V-Z virus which was used for making preparations for fluorescent staining was isolated from a patient with varicella by inoculating vesicle fluid into tube cultures of MRC 5 cells. When these cultures showed a

typical cytopathic effect (CPE) the cells were dispersed with a solution of trypsin (Difco 0·06%) and versene (0·04%), suspended in 10 ml of maintenance medium, and were then inoculated onto a fresh monolayer in a 100 ml flat bottle. When this monolayer showed a 25% CPE the cells were dispersed with trypsin/versene solution, suspended in 9 ml of growth medium and 1 ml of dimethyl sulphoxide (DMSO), and were then stored in liquid nitrogen in 1 ml volumes. When virus was required for infecting cover-slips (see above) 1 ml of infected cell suspension was thawed and inoculated onto MRC 5 cells in a 100 ml flat bottle. This culture was incubated at 36 °C and the maintenance medium was changed after 24 h. After 3 days the cells were dispersed with trypsin/versene and suspended in 10 ml of growth medium. This was further diluted (usually 1/50) in maintenance medium to produce sufficient working suspension for infecting monolayers on cover-slips; any remaining undiluted cells were frozen with 10% DMSO to replenish stocks as required. The virus was thus passaged and stored in the form of infected cells and no attempt was made to obtain cell-free preparations.

Immunofluorescent technique

The cover-slips were stained by methods similar to those used in studies of rubella antibody (Cradock-Watson *et al.* 1972). Briefly, the preparations were treated with dilutions of serum (from 1/8) or sucrose density gradient fractions and were then stained with fluorescein-labelled globulins prepared against human IgG, IgA or IgM (Wellcome Reagents Limited). The cover-slips were finally mounted in glycerol and examined by dark-ground illumination from a quartz-halogen lamp.

Sucrose density gradient centrifugation

Selected sera were fractionated by centrifugation on sucrose density gradients. This was carried out as previously described, but without prior absorption with chick red cells (Cradock-Watson, Ridehalgh & Chantler, 1976).

Complement fixation tests

Complement fixation tests were performed in microtitre trays by the method of Bradstreet & Taylor (1962). Serial specimens from students were tested with Behring antigen (Hoechst Pharmaceuticals); other sera, submitted for routine serological diagnosis, were tested with antigen from the Standards Laboratory of the Central Public Health Laboratory. All sera from any one patient were tested simultaneously.

RESULTS

Microscopic appearances of fluorescent staining

In preparations stained for IgG antibody fluorescence was confined to the typical oval foci of cytopathic effect which result from the cell to cell spread of V-Z virus. Fluorescence was not seen in the intervening areas, which were occupied by normal, presumably uninfected, cells. In the centre of each focus cytopathic damage was too great for the intracellular distribution of fluorescence to be

Table 1. *Varicella-zoster antibodies in sucrose density gradient fractions from the serum of a patient aged* 20 *years,* 9 *days after varicella*

Fraction no.	Immunoglobulin detected by gel diffusion			Immunofluorescent titre of V-Z-specific immunoglobulin		
	IgG	IgA	IgM	IgG	IgA	IgM
1	–	–	tr	–	–	2
2	–	–	+	–	–	16
3	–	–	+	–	–	64
4	–	tr	+	2	16	16
5	+	+	–	64	64	–
6	+ +	+ +	–	256	64	–
7	+ +	+ +	–	1024	32	–
8	+ +	+ +	–	512	16	–
9	+	+	–	256	8	–
10	+	tr	–	32	2	–
11	tr	–	–	2	–	–
12	tr	–	–	2	–	–
Whole serum	.	.	.	2048	256	128

discerned. At the edges of the lesions, where the cells were more recently infected and relatively undamaged, fluorescence consisted mainly of fine granules distributed throughout the cytoplasm. Nuclear staining was less bright and in many preparations the nucleus appeared as an almost unstained central area in the cell. An example of staining for IgG antibody is shown in Plate 1. When preparations were stained for IgA and IgM antibodies the appearances were usually similar, but occasionally nuclear staining predominated. The end-point of a titration was taken as the last dilution in which specific fluorescence could be clearly seen.

Sucrose density gradient centrifugation

The sedimentation pattern of V-Z-specific immunoglobulins from a representative case of varicella is shown in Table 1. Sera from cases of herpes zoster gave a similar pattern of results. IgM antibody was found in fractions 1–4 (rarely in fraction 5), with a peak in fraction 3. IgG antibody was found in fractions 4–12, with a peak in fraction 7 or 8. The IgG and IgM conjugates were evidently highly specific, since they did not stain the peak IgM and IgG fractions, respectively. IgA antibody was usually found in the IgG-containing fractions, but showed slightly more overlap with IgM and had a peak in fraction 5 or 6. The IgA conjugate was also evidently highly specific, since it did not stain fractions from cord sera which contained IgG but no IgA.

Antibody responses in adults aged 18–26 *years with varicella*

The titres of CF, IgG, IgA and IgM antibodies in serial specimens from 14 students with varicella are summarized in Table 2. CF antibody appeared in all subjects and reached maximum titres 6–40 days after the onset of the rash, but the peak values varied widely and ranged from 10 to 1280 in different individuals.

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Table 2. *Antibody titres in serial specimens from* 14 *patients aged* 18–26 *years with varicella*

Time after onset of rash	No. of sera with antibody/no. tested	Range of titres	Median titre
	Complement-fixing antibody		
0–1 day	0/7	< 5	< 5
2–3 days	1/4	< 5–30	< 5
4–5 days	3/5	< 5–120	20
6–7 days	3/4	< 5–160	60
8–21 days	10/11	< 5–1280	80
22–42 days	14/14	10–480	40
6½–13 weeks	10/11	< 5–480	40
14 weeks to 1 year	17/20	< 5–120	20
13 months to 2 years	7/7	10–40	30
25 months to 4 years	3/6	< 5–30	< 5
	IgG antibody		
0–1 day	2/7	< 8–32	< 8
2–3 days	4/4	16–256	91
4–5 days	4/5	< 8–4096	1024
6–7 days	4/4	512–4096	1448
8–21 days	11/11	512–8192	4096
22–42 days	14/14	512–4096	2048
6½–13 weeks	11/11	256–4096	1024
14 weeks to 1 year	21/21	64–2048	512
13 months to 2 years	7/7	64–512	512
25 months to 4 years	6/6	64–512	181
	IgA antibody		
0–1 day	0/7	< 8	< 8
2–3 days	2/2	< 8–32	< 8
4–5 days	3/5	< 8–256	128
6–7 days	4/4	64–512	91
8–21 days	11/11	32–256	128
22–42 days	12/14	< 8–64	16
6½–13 weeks	4/11	< 8–32	< 8
14 weeks to 1 year	9/21	< 8–32	< 8
13 months to 2 years	0/7	< 8	< 8
25 months to 4 years	0/6	< 8	< 8
	IgM antibody		
0–1 day	0/7	< 8	< 8
2–3 days	1/4	< 8–128	< 8
4–5 days	3/5	< 8–256	128
6–7 days	4/4	64–512	181
8–21 days	11/11	32–512	256
22–42 days	14/14	16–256	64
6½–13 weeks	6/11	< 8–64	16
14 weeks to 1 year	7/21	< 8–32	< 8
13 months to 2 years	0/7	< 8	< 8
25 months to 4 years	0/6	< 8	< 8

The median titre was greatest 8–21 days after the rash and then declined. CF antibody was detected in all six students who were tested 1–2 years after the illness, but in only three out of five who were tested after the second year.

IgG antibody appeared in all subjects and persisted throughout the period of study. It was detected in two out of seven sera taken within 24 h of the appearance of the rash and in all sera obtained after the second day, except in one subject who still had no antibody on the fourth day. IgG reached maximum titres of 2048–8192 after 6–40 days and then declined, but was still present in titres of 64–512 in five subjects who were tested 2–4 years after the illness. IgG titres were always greater than CF titres.

IgA antibody appeared in all subjects and followed a transient course. It was first detected 2 days after the onset of the rash and reached maximum titres of 32–512 after 5–11 days. Three weeks after the rash titres were declining and after 3 months IgA was present only in low titres in a minority of specimens.

IgM antibody also appeared in all subjects. It was first detected 3 days after the onset of the rash, reached maximum titres of 64–512 after 8–11 days and followed a transient course similar to that of IgA. There was considerable individual variation in the duration of the IgA and IgM responses and these two classes of antibody were not always found together in the same serum. Neither IgA nor IgM was detected in specimens taken from eight subjects more than a year after the illness.

Specific immunoglobulin titres in sera from patients aged 10 *weeks to* 67 *years with varicella*

Although these patients covered a wide range of ages the temporal pattern of response was similar to that which occurred in young adults (Table 3). All classes of antibody appeared 2–5 days after the onset of the rash. IgG and IgM antibodies were detected in all 50 patients and IgA antibody in 45 (90 %). Titres increased rapidly and the median values for all three classes were maximum after 8–21 days. IgG antibody continued to be present in all sera, but titres diminished steadily. IgA and IgM titres declined more rapidly and after 6 weeks these antibodies were detected only in low titres in a minority of specimens.

Specific immunoglobulin titres in normal young adults and in contacts of cases of varicella

V-Z IgG antibody was detected in 88 and CF antibody in 36 out of 100 normal students in titres of 8 or greater. IgG titres in 50 individuals who possessed this antibody ranged from 8 to 1024 with a geometric mean titre (GMT) of 79. IgA antibody was detected in 8 out of 100 students (titres 8–16) and IgM in only 2 (titres 8–16).

The titres of IgG antibody in 14 close contacts of cases of varicella ranged from 32 to 1024. IgA antibody was present in a titre of 8 in three contacts, but IgM was not detected. No change in titre of more than twofold was observed in any of these subjects 4 weeks after contact.

Table 3. *Specific immunoglobulin titres in* 110 *sera from* 50 *patients aged* 10 *weeks to* 67 *years with varicella*

Time after onset of rash	No. of sera with antibody/no. tested	Range of titres	Median titre
	IgG antibody		
0–1 day	0/8	< 8	< 8
2–3 days	12/22	< 8–1024	8
4–5 days	7/8	< 8–4096	128
6–7 days	7/7	64–4096	1024
8–21 days	44/44	256–16384	2048
22–42 days	8/8	512–4096	1024
6½–13 weeks	7/7	128–4096	1024
14 weeks to 1 year	6/6	128–512	256
	IgA antibody		
0–1 day	0/8	< 8	< 8
2–3 days	4/22	< 8–128	< 8
4–5 days	6/8	< 8–64	16
6–7 days	7/7	8–128	64
8–21 days	40/44	< 8–1024	64
22–42 days	4/8	< 8–16	8
6½–13 weeks	1/7	< 8–16	< 8
14 weeks to 1 year	1/6	< 8–8	< 8
	IgM antibody		
0–1 day	0/8	< 8	< 8
2–3 days	3/22	< 8–128	< 8
4–5 days	6/8	< 8–128	45
6–7 days	5/7	< 8–1024	128
8–21 days	44/44	32–2048	128
22–42 days	6/8	< 8–64	23
6½–13 weeks	2/7	< 8–64	< 8
14 weeks to 1 year	0/6	< 8	< 8

Antibody responses in adults aged 19–29 *years with herpes zoster*

The titres of CF, IgG, IgA and IgM antibodies in serial specimens from 12 students with zoster are summarized in Table 4. CF antibody reached titres ranging from 20 to 640, 9–20 days after the appearance of the rash, and then declined at about the same rate as in students with varicella. The geometric mean values of the highest observed CF titres were 108 for students with zoster and 66 for those with varicella, but because of the wide range the difference is not significant.

IgG antibody was present in all specimens; titres increased rapidly after the fifth day, reached maximum values of 2048–16384 after 9–23 days and then declined at about the same rate as in students with varicella.

IgA antibody appeared in all subjects and followed a transient course, as in varicella. It was not detected in specimens taken during the first 5 days, but then increased rapidly and reached maximum titres of 16–128 after 6–23 days. IgA then declined and was present in only a minority of specimens taken after 3 months. After a year it was detected in only one out of seven subjects.

Table 4. *Antibody titres in serial specimens from* 12 *patients aged* 19–29 *years with herpes zoster*

Time after onset of rash	No. of sera with antibody/no. tested	Range of titres	Median titre
	Complement-fixing antibody		
0–1 day	1/1	15	15
2–3 days	2/4	< 5–30	< 10
4–5 days	2/3	< 5–30	10
6–7 days	5/6	< 5–320	40
8–21 days	15/15	20–640	120
22–42 days	8/8	20–480	80
6½–13 weeks	12/12	20–320	60
14 weeks to 1 year	17/17	10–120	30
13 months to 2 years	3/3	15–40	30
25 months to 3 years	2/3	< 5–40	30
	IgG antibody		
0–1 day	1/1	128	128
2–3 days	4/4	32–128	64
4–5 days	3/3	32–128	64
6–7 days	6/6	64–2048	512
8–21 days	15/15	128–16384	4096
22–42 days	8/8	1024–4096	2048
6½–13 weeks	12/12	512–4096	2048
14 weeks to 1 year	17/17	256–2048	512
13 months to 2 years	4/4	128–512	256
25 months to 3 years	3/3	64–128	128
	IgA antbody		
0–1 day	0/1	< 8	< 8
2–3 days	0/4	< 8	< 8
4–5 days	0/3	< 8	< 8
6–7 days	3/6	< 8–64	11
8–21 days	14/15	< 8–128	64
22–42 days	6/8	< 8–32	23
6½–13 weeks	7/12	< 8–64	11
14 weeks to 1 year	6/17	< 8–32	< 8
13 months to 2 years	1/4	< 8–8	< 8
25 months to 3 years	0/3	< 8	< 8
	IgM antibody		
0–1 day	0/1	< 8	< 8
2–3 days	0/4	< 8	< 8
4–5 days	0/3	< 8	< 8
6–7 days	0/6	< 8	< 8
8–21 days	10/15	< 8–128	8
22–42 days	1/8	< 8–32	< 8
6½–13 weeks	2/12	< 8–16	< 8
14 weeks to 1 year	0/17	< 8	< 8
13 months to 2 years	0/4	< 8	< 8
25 months to 3 years	0/3	< 8	< 8

IgM antibody was found in only nine subjects when whole serum was examined. It was first detected 9 days after the rash and appeared only briefly. Titres were usually much lower than in students with varicella, and in seven subjects IgM was detected in titres of only 8–16 in sera taken 9–14 days after the onset. Titres of 32 and 128 were recorded in two students, who still had titres of 16 after 7 and 9 weeks respectively. In previous work on the detection of IgM antibody in acute and congenital rubella we found that IgM could often be demonstrated more successfully by staining sucrose density gradient fractions than by staining whole serum, possibly because IgG antibody in whole serum may block antigenic sites during staining (Cradock-Watson *et al.* 1972; 1976). In the hope of revealing IgM antibody in more specimens from this group of patients we fractionated the majority of sera taken 8–42 days after the rash and some additional sera taken at times up to 4 months after the onset. The staining of fractions confirmed the presence of IgM antibody in sera which had already given positive results, although titres were never greater in fractions than in whole serum. In addition, the staining of fractions revealed traces of IgM in seven sera which were obtained at times between 6 days and 4 months after the rash and which had previously given negative results. In this way we detected traces of specific IgM in two additional patients, but there was no evidence that IgM staining was being depressed by IgG antibody and it is clear that IgM responses in these young adults with zoster were generally weaker than in patients of the same age with varicella.

Specific immunoglobulin titres in sera from patients aged 12–89 *years with herpes zoster*

Although there was more individual variation, the temporal pattern of response was in general similar to that which occurred in young adults (Table 5). IgG antibody was present in all sera. IgA was detected in 54 out of 55 patients (98%) and IgM in 43 (78%). Titres increased rapidly and the median values for all three classes of antibody were maximum after 8–21 days. IgA was still present in the majority of sera 3 months to 1 year after the illness, but titres were low. IgM was present in only a minority of sera taken after three months.

Antibody in sera taken before the onset of herpes zoster

Sera taken before the appearance of the zoster rash were available from six patients. The results from these are given separately in Table 6. IgG antibody, not detectable by complement fixation, was found in all pre-zoster sera in titres from 4 to 128. In one patient (A.M.) there was no change one day after the onset of the rash, but in two patients (M.B. and S.P.) IgG titres showed significant increases 2 and 3 days after the rash, respectively.

Maximum titres of specific immunoglobulins in patients of different ages with varicella and herpes zoster

The median titres of all classes of antibody in patients with varicella and roster were always highest in sera taken 8–21 days after the appearance of the rash

Table 5. *Specific immunoglobulin titres in* 116 *sera from* 55 *patients aged* 12–89 *years with herpes zoster*

Time after onset of rash	No. of sera with antibody/no. tested	Range of titres	Median titre
	IgG antibody		
0–1 day	6/6	16–1024	256
2–3 days	9/9	16–512	128
4–5 days	14/14	64–8192	256
6–7 days	10/10	32–8192	724
8–21 days	46/46	256–16384	4096
22–42 days	16/16	64–16384	2048
6½–13 weeks	8/8	128–8192	512
14 weeks to 1 year	7/7	128–1024	512
	IgA antibody		
0–1 day	2/6	< 8–512	< 8
2–3 days	3/9	< 8–16	< 8
4–5 days	9/14	< 8–256	16
6–7 days	7/10	< 8–512	32
8–21 days	46/46	8–2048	128
22–42 days	16/16	8–1024	128
6½–13 weeks	8/8	8–256	32
14 weeks to 1 year	5/7	< 8–64	32
	IgM antibody		
0–1 day	1/6	< 8–512	< 8
2–3 days	0/9	< 8	< 8
4–5 days	4/14	< 8–256	< 8
6–7 days	5/10	< 8–512	< 8
8–21 days	34/46	< 8–512	32
22–42 days	13/16	< 8–256	23
6½–13 weeks	3/8	< 8–128	< 8
14 weeks to 1 year	2/7	< 8–32	< 8

(see Tables 2–5). We have therefore compared the heights of the antibody responses in patients of different ages, including students, by calculating for separate age groups the GMT of all sera collected during this time (Table 7). In varicella the IgA response was significantly weaker in young children than in older children and adults, and the difference was greatest when cases were divided into those under and over the age of 6 years ($P < 0{\cdot}001$ by the t test). The mean IgG titre was lower in children under the age of 1 year than in patients over the age of 6 years ($P < 0{\cdot}02$). The mean IgG titre in patients aged 18–29 years with zoster appeared to be slightly greater than in patients of the same age with varicella, and slightly less than in older patients with zoster, but the differences are not significant. The mean IgA titre in young adults with zoster was lower than in those with varicella ($P < 0{\cdot}01$) and much lower than in older patients with zoster ($P < 0{\cdot}001$). The mean IgM titre in young adults with zoster was much lower than in those with varicella ($P < 0{\cdot}001$) and also much lower than in older patients with zoster ($P < 0{\cdot}001$).

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Table 6. *Varicella-zoster antibody titres before and after the appearance of the rash in* 6 *patients with herpes zoster*

Patient	Age (years)	Time before or after onset of rash	V-Z antibody titre IgG	V-Z antibody titre CF
A.M.	16	4 years before	32	< 5
		1 day after	32	< 5
		15 days after	2048	160
M.B.	22	5 days before	8	< 5
		2 days after	32	< 5
		10 days after	2048	120
S.P.	18	7 weeks before	32	< 5
		3 days after	256	5
		17 days after	4096	40
R.L.	12	8 months before	32	< 5
		6 days after	1024	30
J.D.	19	15 months before	128	< 5
		5 months after	1024	60
S.M.	16	3 years before	4	< 5
		16 months before	4	< 5
		11 months after	128	10

Table 7. *Geometric mean titres of specific immunoglobulins in sera from patients of different ages* 8–21 *days after varicella or herpes zoster*

Age group	Type of illness	No. of patients	Geometric mean titre IgG	Geometric mean titre IgA	Geometric mean titre IgM
< 1 year	Varicella	7	927	20*	200
< 6 years	Varicella	14	1413	23*	160
⩾ 6 years	Varicella	41	2684	119*	165
18–29 years	Varicella	28	2689	126	181
18–29 years	Herpes zoster	33	3217	58	12*
⩾50 years	Herpes zoster	20	3327	219	53*

* Titres < 8 were regarded as = 4 for the purpose of calculating geometric means.

Antibodies to varicella-zoster virus in patients with primary herpes simplex virus infections

Twenty-five patients with primary HSV infections had no V-Z IgG or CF antibodies in the first serum and were assumed not to have had varicella in the past. No V-Z CF antibody appeared in any of these patients, but in eight cases (32%) V-Z IgG staining was observed in the convalescent serum in titres ranging from 8 to 64. No V-Z IgA or IgM staining was detected.

Thirty-two patients had V-Z IgG titres of 16–1024 in the first serum, presumably from previous varicella. V-Z IgG rises of 4- to 32-fold occurred in 22 patients (69%), and in 9 of these IgA antibody also appeared in the convalescent serum or showed a significant increase in titre compared with the previous specimen. V-Z IgM antibody was not detected. Twenty of the 22 patients with V-Z IgG rises also had increases of 4- to 16-fold in the titre of V-Z CF antibody.

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DISCUSSION

Our results show that during an attack of varicella all three classes of antibody appear 3–4 days after the onset of the rash, increase simultaneously and reach maximum titres during the second and third weeks. IgM antibody then declines rapidly and disappears after a variable number of weeks. IgA follows a similar transient course, but may not always disappear completely since low titres are present in a small proportion of normal young adults. IgG titres decline slowly after the third week, but lower values probably persist for life since IgG was found in 88% of normal young adults and studies now in progress show that its prevalence increases with age. CF antibody in our patients with varicella followed a course broadly similar to that described by Gold & Godek (1965), but complement fixation was less sensitive than the fluorescent IgG test, which detected antibody more often and in higher titres.

IgM responses in patients with varicella were good at all ages, but IgG and IgA responses were weaker in very young children than in older children and adults. These age-related differences may be attributable to the different rates of maturation of the three immunoglobulin systems: total IgM concentrations increase rapidly after birth and reach adult values at the age of 1–2 years, but IgG and IgA concentrations do not reach adult values until the age of six years or more (Allansmith *et al.* 1968; Buckley, Dees & O'Fallon, 1968).

Hope-Simpson suggested that the antibody titre during the period of latency might be increased by contact with a case of varicella. Marked rises in neutralizing antibody were reported by Caunt & Shaw (1969) in a mother whose three children developed chickenpox, and by Asano *et al.* (1977) in a child who had been immunized with attenuated vaccine and was in contact with another child with the disease. Brunell *et al.* (1975), using membrane immunofluorescence, reported fourfold rises in IgG and IgA titres in two contacts, but other workers, using either immunofluorescence or complement fixation, have found no significant changes (Gershon, Steinberg & Brunell, 1974; Gershon & Krugman, 1975; Gold & Godek, 1965). Our own failure to detect significant rises in IgG or IgA in 14 close contacts suggests that such responses are uncommon, and we doubt if they help significantly in maintaining antibody.

If zoster is due to reactivation of latent virus in a person whose immunity has waned, then antibody should be present, probably in low titre, before the onset of the disease. This is difficult to demonstrate because reactivation presumably starts several days before the rash appears; moreover a low titre may not be detectable by complement fixation. We were able to test pre-zoster sera from 6 patients aged 12–22 years: all had IgG antibody in titres of 4–128, but negative CF tests, confirming the reports by Brunell *et al.* (1975) and Gerna, Achilli & Chambers (1977), who detected antibody in pre-zoster sera from a total of 11 patients by the immunofluorescence and immunoperoxidase tests, respectively. In five of our patients the IgG titres were low ($\leqslant 32$), but we are reluctant to draw any general conclusions from this because the number of cases is small, all the patients were young, and we have not been able to measure pre-zoster

titres in older persons who are in fact more likely to get the disease. When zoster occurs, the antibody titre can evidently rise soon after the rash, since increases in IgG were detected after 2 and 3 days, respectively, in two patients who had been tested before the illness (M.B. and S.P., Table 6). Our results, however, provide no support for the commonly expressed view that antibodies develop more rapidly after zoster than after varicella.

Previous comparisons of the heights of the antibody responses in varicella and zoster have not all given similar results but have generally indicated stronger responses after zoster: Weller & Witton (1958) and Schmidt & Lennette (1975) found no difference between convalescent titres of CF antibody in the two diseases, but Taylor-Robinson & Downie (1959), Brunell & Casey (1964) and Miller & Brunell (1970) reported that CF titres were generally higher after zoster; Caunt & Shaw (1969) reported higher titres of neutralizing antibody after zoster than after varicella, and better responses have been demonstrated by means of precipitation (Taylor-Robinson & Rondle, 1959), platelet-aggregation (Palosuo, 1972) and the agglutination of tanned red cells (Furukawa & Plotkin, 1972). When we compared patients of similar ages with the two diseases we found that CF and IgG antibody titres were slightly higher after zoster, but the difference was not significant. There appeared to be a further slight increase in IgG titres in older patients with zoster, but the mean value was still not significantly greater than in young adults with either zoster or varicella. Consequently we cannot confirm that IgG production is more rapid or abundant than after varicella, but immunofluorescence gives no indication of the biological function of antibody and it is clear that further studies of the relationship between fluorescent and neutralizing titres are necessary for all three classes of immunoglobulin.

We detected transient IgM antibody in 25 out of 33 young adults with zoster (76%), but titres were generally much lower than in patients of the same age with varicella. Weak or absent IgM responses are thought to be characteristic of the secondary type of reaction, but the improved response in older patients was unexpected and is difficult to explain. It is unlikely to be due to age *per se*, but it might be related to the duration of latency, to a general waning of immunity and memory, or perhaps to the clinical severity of the illness which tends to be worse in elderly persons.

Immunofluorescence was more sensitive than complement fixation in detecting rises in V-Z antibody in patients with HSV infection. We detected weak V-Z IgG staining in convalescent sera from some patients who had not previously had varicella, and rises in V-Z IgG to much higher titres in patients who already possessed this antibody. These cross-reactions are probably due to common antigens which provoke a secondary type of response when HSV infects a person who has had varicella in the past. In our patients, all of whom were children or young adults, these secondary reactions consisted mainly of IgG, sometimes IgA, but never IgM. Whether older patients would give better IgM responses, as they do in zoster, is an interesting possibility which we have not been able to test. It seems unlikely that cross-reactions from antibodies elicited by past HSV infections can have affected the V-Z titres in our patients with chickenpox and

zoster, although they might cause difficulties of interpretation when using immunofluorescence to determine the immune status of high-risk individuals who have been in contact with varicella.

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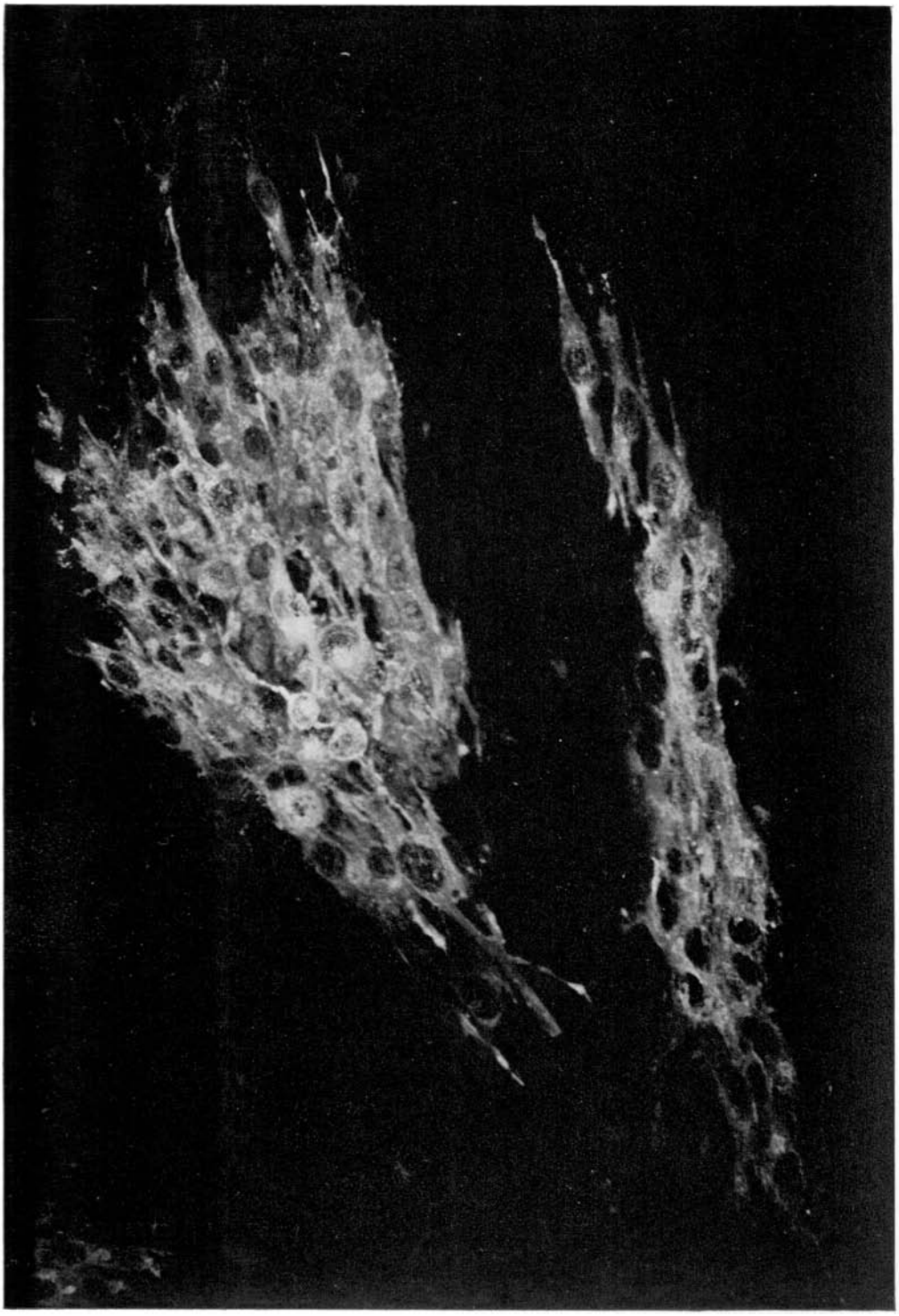
EXPLANATION OF PLATE

Immunofluorescent staining of IgG antibody to varicella-zoster virus. × 250.
Serum dilution 1/128.



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