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Distribution and titres of rotavirus antibodies in different age groups

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SUMMARY

Three hundred and fifty-seven sera selected at random from hospital patients of all ages were examined for rotavirus antibodies using indirect immunofluorescence (FA) and complement fixation tests (CFT). Three hundred and fourteen of these were also tested for neutralizing antibodies to human rotavirus. Sera from patients admitted with a diagnosis of acute gastroenteritis were excluded from this survey.

FA antibodies were found in newborn infants but fell to undetectable titres at 3 months. The highest titres were found in children between the ages of one and three years. In older age groups, the modal titre fell gradually with increasing age until, in sera from those above 70 years of age, FA antibodies were almost undetectable. The same pattern was observed with neutralizing antibodies. A high modal titre of CF antibodies was only found in sera from those aged one to three years.

INTRODUCTION

It has been shown that CF and FA tests can detect group specific antigen, probably situated within the inner capsid layer and common to all the known rotaviruses (Flewett *et al.* 1974; Woode *et al.* 1976; Thouless *et al.* 1977). Human serum antibody titres against rotavirus infection were studied by FA (Ørstavik, Figenschau, Haug & Ulstrup, 1976; Blacklow, Echeverria & Smith, 1976), and CFT (Kapikian *et al.* 1975; Blacklow *et al.* 1976). A new method (Banatvala, Totterdell, Chrystie & Woode, 1975; Bryden, Davies, Thouless & Flewett, 1977) for detecting human rotavirus in cell cultures has made it possible to titrate neutralizing antibodies against the human virus as well.

This survey was undertaken to investigate the epidemiology of rotavirus infections in man and, in particular, to see if the evidence would indicate whether repeated infections through life were common or not.

METHODS

Human sera

A total of 357 serum samples from patients referred to East Birmingham Hospital between 1974 and 1976 were examined. These patients were suffering from a wide variety of illnesses and could be considered to be representative of the population as a whole. Sera from those diagnosed as acute infectious gastro-

enteritis were excluded from the survey. Ages of the patients ranged from newborn to over 80 years and both sexes were equally represented. The sera were stored frozen at −20 °C until examined.

Cell cultures

Primary calf kidney (PCK) monolayers were seeded at $3{\cdot}0 \times 10^5$ cells/ml and prepared in rolling Winchester bottles and 4 oz medical flats. They were used mainly for the preparation of secondary cultures and for the production of tissue culture adapted calf virus.

Secondary calf kidney (SCK) monolayers were seeded $1{\cdot}5 \times 10^5$ cells/ml and prepared in Leighton tubes with coverslips.

Monolayer cultures of LLC-MK_2 cells, a continuous cell line originally derived from a rhesus monkey kidney (Flow Laboratories Ltd). For the microtitre plate technique, a volume of 0·2 ml cell suspension containing approximately 6×10^4 cells was put into each well, to be used for the neutralization tests (NT).

Media

Growth medium was Eagle's Minimum Essential Medium (MEM) with Hanks' salts supplemented with 10% (v/v) fetal calf serum (FCS), gentamicin 40 μg/ml, fungizone 10 μg/ml and for the LLC-MK_2 cells, 1% (v/v) non-essential amino acids (Flow Laboratories Ltd) was added. Maintenance medium was Eagle's MEM with Earle's salts buffered with 20 mmol of N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES) instead of sodium bicarbonate and supplemented with 2% (v/v) FCS.

Viruses

Calf rotavirus was propagated in rolling Winchester bottles containing confluent monolayer cultures of PCK. Before inoculation each monolayer was washed three times with phosphate-buffered saline (PBS). Each bottle was then inoculated with 0·6 ml of the virus suspension contained in 20 ml of maintenance medium without serum. The virus was allowed to adsorb for 2 h at 37 °C (Mebus, Kono, Underdahl & Twiehaus, 1971), after which the nutrient fluid was made up to a volume of 100 ml/bottle. Twenty-four hours later 2% (v/v) FCS was added. After 7 days' incubation, the cells were scraped off and the resulting suspension was sonicated for 3 min in an Electrosonic Model H60-2 waterbath, clarified by centrifugation at 3000 rev./min for 10 min and the virus concentrated by centrifuging the supernatant at 90,000 *g* for 1 h at 4 °C in the 8 × 35 AR60 rotor of an MSE superspeed 65 ultracentrifuge. Finally, the virus was harvested from the deposit, examined by electronmicroscopy, divided into 1 ml volumes and stored at −70 °C until used.

Human rotavirus suspensions were prepared from the faeces of infected children. Ten to 20% (v/v) faecal suspensions were made in PBS, pH 7·3 and clarified by centrifugation at 3000 rev./min for 15 min and then at 7000 *g* for 30 min in an MSE 8 × 35 angle head rotor. Supernatants were filtered through a series of membrane filters (Sartorius membrane filters, West Germany) ending with one of

0·45 μm a.p.d. The filtrate was concentrated by ultracentrifugation at 155,000 *g* for 30 min at 4 °C in a Spinco SW50 rotor. The pellets were resuspended in sterile PBS and checked by E.M. for the presence of virus particles.

Screening of human sera

Indirect immunofluorescence (FA)

Each serum was tested at a dilution of 1/10. Those giving positive fluorescence were then titrated using doubling dilutions in PBS from 1/20 to 1/320 to determine the immunofluorescent titre, which was the highest serum dilution giving positive fluorescence.

Leighton tubes with confluent coverslip cultures of SCK cells were used. The fluid was changed to 0·9 ml maintenance medium without serum and each tube inoculated with one drop of tissue culture adapted calf virus preparation. All tubes were incubated at 37 °C for 24 h after which coverslips were removed, cells fixed in cold acetone (at 4 °C) for 7 min and allowed to dry in the air.

The serum sample to be tested was allowed to cover each coverslip, and was incubated at 37 °C for 1 h in a humid atmosphere. Coverslips were then washed in PBS for 30 min using a magnetic stirrer and allowed to dry in the air.

Fluorescein labelled antihuman immunoglobulin (sheep) (Wellcome Laboratories Ltd) was used in a dilution of 1/20 in PBS to cover the coverslips, which were then incubated at 37 °C for 1 h, washed in PBS for 30 min, counterstained in Evans' blue (1/150,000) for 10 s, washed in water, left to dry, mounted in 90 % glycerol in PBS of pH 8·3, and examined.

Negative controls were done with each sample using virus free cultures of SCK and whenever possible a positive control treated with known positive 1/10 dilution of calf antiserum prepared from a gnotobiotic calf convalescent from an experimental infection and 1/10 dilution of fluorescein labelled anticalf serum.

Titrations were read on 'coded' specimens.

Complement fixation tests (CFT)

Tissue culture adapted calf rotavirus at its optimum dilution for human serum was used as CF antigen (Thouless *et al.* 1977). All reagents were diluted in veronal buffered saline (VBS) and complement was used at 3 HD50. A negative control antigen was prepared from uninfected cell cultures. Sera were screened at a dilution of 1/5 and those giving a positive reaction were titrated. The end point was the highest serum dilution giving 50 % lysis with the optimum dilution of the antigen.

Neutralization tests (NT)

Neutralization tests were carried out by the microtitre plate technique already described (Bryden *et al.* 1977; Thouless *et al.* 1977). The stock human virus was titrated using triplicate dilutions and the working dilution of the virus taken as that dilution giving, on average, 50–100 fluorescing foci per well. Neutralizing antibody titres were recorded as the highest serum dilution giving an average reduction of 50 per cent or more of the fluorescing foci compared with the control.

Table 1. *Modal titres of the sera tested, calculated from the results of FA, CFT and NT*

Group	Age (years)	Test	No. of sera examd	No. AC*	No. of sera with reciprocal titre of									Reciprocal modal titre†
					<5	5	10	20	40	80	160	320	640	
I	0–¼	FA	37	.	5	0	2	7	11	7	5	0	0	40
		CFT	37	7	16	3	5	2	3	1	0	0	0	–
		NT	26	.	0	2	1	4	8	3	5	3	0	40
II	¼–½	FA	22	.	14	0	3	1	2	2	0	0	0	–
		CFT	22	1	14	2	3	1	1	0	0	0	0	–
		NT	17	.	0	2	5	6	2	1	0	1	0	20
III	½–1	FA	25	.	11	0	1	1	5	2	5	0	0	–
		CFT	25	3	11	0	2	0	6	3	0	0	0	–
		NT	21	.	0	1	7	2	4	1	4	2	0	10
IV	1–3	FA	25	.	2	0	2	2	3	6	10	0	0	160
		CFT	25	1	8	0	2	2	3	9	0	0	0	80
		NT	24	.	0	1	1	1	3	3	1	9	5	320
V	3–6	FA	25	.	3	0	1	4	5	10	2	0	0	80
		CFT	25	2	12	1	1	8	1	0	0	0	0	–
		NT	21	.	0	0	1	1	4	4	9	2	0	160
VI	6–9	FA	26	.	4	0	2	5	3	9	3	0	0	80
		CFT	26	3	14	1	2	2	2	2	0	0	0	–
		NT	25	.	0	1	2	2	4	6	8	1	1	160
VII	10–19	FA	26	.	5	0	0	1	16	2	2	0	0	40
		CFT	26	1	18	0	3	1	3	0	0	0	0	–
		NT	26	.	0	0	1	1	7	9	5	3	0	80
VIII	20–29	FA	22	.	5	0	1	2	12	1	0	0	0	40
		CFT	22	.	21	0	1	0	0	0	0	0	0	–
		NT	22	.	0	0	2	4	3	8	5	0	0	80
IX	30–39	FA	20	.	5	0	0	12	3	0	0	0	0	20
		CFT	20	.	20	0	0	0	0	0	0	0	0	–
		NT	20	.	0	0	0	6	6	7	1	0	0	80

Table 1 (*cont.*)

Group	Age (years)	Test	No. of sera examd	No. AC*	No. of sera with reciprocal titre of									Reciprocal modal titre†
					<5	5	10	20	40	80	100	320	640	
X	40–49	FA	22	.	6	0	4	10	2	0	0	0	0	20
		CFT	22	.	22	0	0	0	0	0	0	0	0	–
		NT	22	.	0	0	2	2	6	10	2	0	0	80
XI	50–59	FA	27	.	9	0	12	4	1	1	0	0	0	10
		CFT	27	1	26	0	0	0	0	0	0	0	0	–
		NT	23	.	0	0	3	3	12	4	1	0	0	40
XII	60–69	FA	42	.	18	0	19	3	1	1	0	0	0	10
		CFT	42	1	41	0	0	0	0	0	0	0	0	–
		NT	30	.	0	3	2	9	13	2	1	0	0	40
XIII	70–79	FA	26	.	18	0	7	0	1	0	0	0	0	–
		CFT	26	.	25	0	1	0	0	0	0	0	0	–
		NT	25	.	0	2	3	7	11	2	0	0	0	40
XIV	80	FA	12	.	9	0	3	0	0	0	0	0	0	–
		CFT	12	.	12	0	0	0	0	0	0	0	0	–
		NT	12	.	0	2	2	4	2	2	0	0	0	20

*AC = anticomplementary. † - = 5.

Table 2. *Comparison between the results of FA and CFT in the different age groups*

		FA			CFT			
Group	Age in years	Number examined	Number positive	%	AC*	Net total	Number positive	%
I	0–¼	37	32	87	7	30	14	47
II	¼–½	22	8	36	1	21	7	33
III	½–1	25	14	56	3	22	11	50
IV	1–3	25	23	92	1	24	16	67
V	3–6	25	22	88	2	23	11	48
VI	6–9	26	22	85	3	23	9	39
VII	10–19	26	21	81	1	25	7	28
VIII	20–29	22	17	77	0	22	1	5
IX	30–39	20	15	75	0	20	0	0
X	40–49	22	16	73	0	22	0	0
XI	50–59	27	18	67	1	26	0	0
XII	60–69	42	24	57	1	41	0	0
XIII	70–79	26	8	31	0	26	1	4
XIV	⩾ 80	12	3	25	0	12	0	0

* AC, anticomplementary.

All specimens were coded and the NT titres were compared with those of FA and CFT only after the results had been recorded. Modal titres were calculated for each test in each group.

RESULTS

Serum samples were classified according to age in 14 groups. Tables 1 and 2 and Fig. 1 show a summary of results. It was noticed in group IV of highest age incidence (1–3 years) that all male sera examined showed positive fluorescence, while 85·7% of female sera within the same group gave positive results. *P* value was calculated and found to be 0·085 (8·5%). Therefore, no statistical difference in incidence between males and females was found in this study. As age advanced, the percentage of both male and female sera with detectable antibody started to drop. The percentage of male sera with positive FA in groups V to XIV was: 94·7, 90·9, 82·4, 66·7, 88·9, 75·0, 70·0, 62·1, 62·5 and 28·6 with (72·2 ± 19·4)% expressed as mean ± 1 S.D. The corresponding percentage of female sera with positive FA was: 66·7, 80·0, 77·8, 90·0, 63·6, 70·0, 57·1, 46·2, 16·7 and 20·0 respectively with (58·8 ± 24·6)%. All sera with negative FA were also negative by CFT and there was no obvious relation between FA and CF antibody titres, so that a serum with a high FA titre might give low, moderate or negative CF titre depending – possibly – on the date of onset and duration of the disease. Fluorescent antibodies persist while CF antibodies rapidly decline after infection. Table 2 shows that FA test could detect rotavirus antibodies in human sera in 64% of cases up to the age of one year, 88% between 1 and 9 years and 62% between 10 and > 80 years. By CFT, these figures dropped to 44%, 51% and 5% respectively.

In general, the modal NT titres were higher than those of FA in all age groups but the pattern of both was the same (Fig. 1).

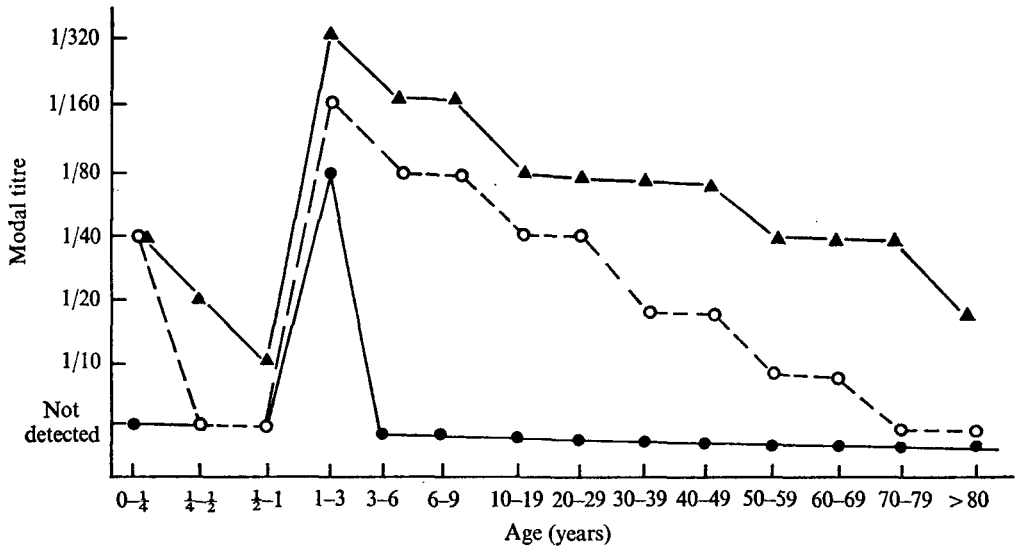

Fig. 1. Relationship between age and modal serum titres as measured by neutralization tests (▲—▲), FA (○—○) and CFT (●—●).

DISCUSSION

In this survey, the highest FA, CF and NT titres were obtained in group IV (Table 1 and Fig. 1). As these serum samples were taken from children not suffering from gastroenteritis on the day of sampling the blood, it appeared that the maximum incidence for rotavirus gastroenteritis was in children at about one year of age and this is in broad agreement with the findings reported by Middleton *et al.* (1974); Kapikian *et al.* (1976*b*) and Blacklow *et al.* (1976). Shepherd *et al.* (1975) reported that males were more commonly affected by rotaviruses than females. They studied hospital infants suffering from gastrointestinal symptoms due to infection with human rotavirus identified by electron microscopy of faecal extracts. Since no statistical difference in incidence between males and females was found in this study, males might be considered more severely but perhaps not more commonly infected with rotaviruses than females.

The percentage of children's sera having rotavirus antibodies detectable by CFT (Table 2) were in broad agreement with the findings reported by Blacklow *et al.* (1976). On the other hand, Kapikian *et al.* (1975) reported higher figures of 86% at age 3–4 years and 75% at age 4–5 years. These differences might be related to the different types of CF antigens used as well as to the composition of the various study groups.

It appeared that FA tests were more sensitive than CFT in detecting rotavirus antibodies in human sera. This is in agreement with Wyatt *et al.* (1974), Kapikian *et al.* (1975) and Kapikian *et al.* (1976*a*). However, NT was by far the most sensitive serological test for the detection of rotavirus antibodies and therefore the test of choice for epidemiological surveys. Modal titres were indicated in each test and for each group since they represented the titre most commonly repeated

in sera of the general population examined. A modal titre of a group of sera might be 'negative' although some sera in the group gave positive results.

Where virus infection is persistent in the tissues for life (as in herpes) or repeated (as in influenza), antibody titres do not normally decrease significantly with age. On the other hand, the falling modal titre in rotavirus infection in the older age groups suggests that infection is not persistent and reinfection does not frequently occur; or if rotavirus infection is repeated, it does not provide much antigenic stimulus.

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