J. Hyg., Camb. (1972), **70**, 49
Printed in Great Britain

An unusual plaque variant of rubella virus

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(*Received* 9 *July* 1971)

SUMMARY

In a comparative study of 24 different rubella virus strains, all but three formed small plaques in RK 13 cell cultures; of these one was the vaccine strain HPV-77, one was isolated from a congenitally infected infant, and the third was recovered from an adult who contracted severe rubella while handling this congenital strain. Whereas the plaque size of the vaccine strain was stable after further passage in cell culture, the plaque size of the other two rapidly diminished when the virus was passed in monkey kidney cells, and one of them was also reduced by passage in RK 13 cells. Cell culture passage of the typical small plaque strains did not result in altered plaque size.

INTRODUCTION

Lawrence & Gould (1969) showed that different rubella virus strains could be distinguished on the basis of plaque size and presented preliminary evidence that rapid changes in plaque size were associated with cell culture history. Evidence for this was also presented in a paper by Parkman, Meyer, Kirchstein & Hopps (1966), describing the vaccine strain HPV-77, the virus having changed from a small to a large plaque type during prolonged passage in monkey kidney cell culture; but it was not suggested that this change represented a profound alteration in the virus. It is generally accepted that rubella virus exists as one serotype (Best & Banatvala, 1970; Kono, 1969) although Fogel & Plotkin (1969) presented evidence which does not seem to support this view. The latter group used a plaque reduction technique for serotyping rather than the haemagglutination inhibition or tube neutralization methods employed by others. Whether or not only one serotype exists, it is clear that plaque morphology is not uniform, and this paper presents a comparative study of the plaque type of a wide selection of rubella virus strains and records in detail certain changes observed in two unusual cases.

MATERIALS AND METHODS

The twenty-four strains employed in this investigation are listed in Table 1 which shows the categories into which they fall and their cultural history. Virus was isolated from vaccinees or infected animals, from throat swabs, nasal swabs or washings, and from clinical material from the human fetus by macerating the sample in growth medium and subjecting it to three cycles of freezing and thawing

after which the fluid was clarified. The strains were subjected to passage in cell cultures using the simple procedure of harvesting inoculated cultures when extensive cytopathic effects developed or at 7–10 days after infection. All virus preparations were stored at −70° C. or in the freeze-dried state at −20° C.

The cell culture systems employed were RK 13 cells, WI-38 cells, Vero cells and primary cells from *Erythrocebus patas* monkey kidney, rabbit kidney or chick embryo. All cultures were incubated at 36·5° C.

The plaque test was carried out in 60 or 35 mm. Falcon plastic Petri dishes seeded with sufficient cells to form a confluent monolayer in 72 hr.; this was normally in the order of 200,000 cells/ml. The plates were incubated at 32·5° C. in an atmosphere of 5 % CO_2 in air, and were washed once with buffered Eagle's minimum essential medium (MEM) before use. The volume of inoculum was 0·2 ml. for 60 mm. plates, 0·1 ml. for 35 mm., and the period of virus adsorption was 15–30 min. at room temperature. The overlay medium consisted of Eagle's MEM, 0·09 % sodium bicarbonate, 2 % fetal or agamma calf serum and 1 % Difco Noble agar (10 ml. was required for 60 mm. plates, 4 ml. for 35 mm. plates). The plates

Table 1. *History and characteristics of rubella virus strains*

Strain	History	Plaque size (mm.)
Category I. Virus isolated from post-natal infection of children and adults. Throat and nasal swabs		
Day	RK 13 (3)*	0·75–1·0
Sheppard	RK 13 (6)	
Judith	YRK† (3)	
Lesley	P.D.‡	
Janine	P.D.	2·25–2·5
Category II. Virus isolated from congenital infection		
(*a*) Foetal liver and kidney		
He	RK 13 (1)	1·0–1·25
Wright	RK 13 (2)	
(*b*) Placental		
Savva	P.D.	1·0
(*c*) Post-natal throat swab, urine, lens		
Lambert	RK 13 (1)	0·75–1·0
Pullen (9 months)§	RK 13 (2)	
Andrews (6 days), Thomas E. (4 weeks)	RK 13 (3)	
Dunning (6 months)	RK 13 (4)	2·25–2·5
Thomas, T. (< 4 weeks)	RK 13 (7)	0·75–1·0
Simoni, Hitchcock	MK (2)	
Carnwright, Lefebvre (1), Gabriel, Goldthorpe	MK (3)	
Lefebvre (2)	MK (6)	
Category III. Vaccine strains		
HPV-77	MK (78)	2·0–2·25
Cendehill	YRK (51)	1·25–1·5
RA27/3	WI-38 (30)	1·0–1·25

* Number of passages.
† Primary rabbit kidney.
‡ Material plaqued direct from clinical sample.
§ Age of child when virus isolated.

https://doi.org/10.1017/S0022172400022087 Published online by Cambridge University Press

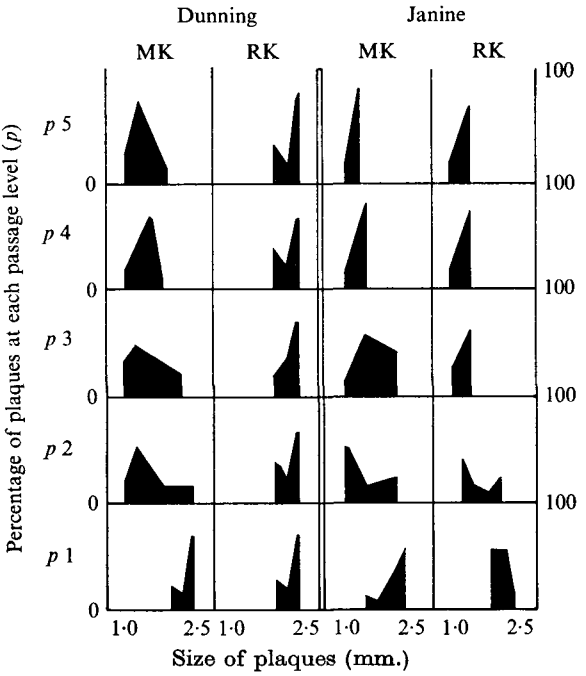

Fig. 1 Range of plaque size with passage.

were left at room temperature for 15–20 min. to allow the overlay to solidify before being returned to the cabinet. The cultures were incubated for 7 days, at which time the overlay was stripped off and the cell sheets stained with formalized crystal violet in phosphate buffered saline. Optimum results were obtained at 32·5° C. with RA27/3 virus and this temperature did not prejudice production of plaques by other strains. Deviation from this method resulted in either loss or variation in the size and quality of the plaques. Batches of agar were occasionally found to be toxic and changes in the concentration of agar or sodium bicarbonate adversely affected the test. Goat, rabbit, adult bovine, agamma and fetal calf sera were used experimentally for both growth and maintenance of the cells. Optimum results were obtained using adult bovine serum in the growth medium, and fetal or agamma calf serum in the maintenance medium.

RESULTS

Plaque sizes varied between 0·75 and 2·5 mm., the most common size being nearer the lower limit. Thus of the five strains examined in Category I, four formed plaques in the range 0·75–1·0 mm., and only one strain produced large plaques of 2·25–2·5 mm. Similarly, in category II most of the strains whether derived from fetal, placental or post-natal congenital material formed small plaques and only one strain produced a large plaque type. In the third category the vaccine viruses produced plaques in three size ranges, HPV-77 were large, RA27/3 were small and Cendehill formed plaques intermediate in size.

https://doi.org/10.1017/S0022172400022087 Published online by Cambridge University Press

Selected virus strains were then passaged in continuous cell and in primary tissue cultures. On passage in RK13 and monkey kidney cells, only Dunning and Janine showed significant change in plaque size. Strain Janine in both cell culture systems reduced from large to small within six passages. Dunning only altered in this way when passaged in monkey kidney cells, six passages in RK13 leaving it unchanged (Fig. 1). Detailed studies of these changes indicated that a high percentage of the plaques were large in the original material and a high percentage were small after six passages. During the intermediate passage levels a range of plaque sizes were seen. We noted that the pattern of these changes was strikingly similar in all cases where they occurred. No significant changes in plaque size were observed in other virus strains subjected to passage in RK13 or monkey kidney, those tested being Sheppard and Lesley (Category I), Savva, Thomas and Dunning (Category II), HPV-77 and RA27/3 (Category III). Preliminary results indicate that Dunning is also reduced in size after passage in Vero cells but not in WI-38. Janine has not yet been tested in this manner.

HPV-77 passed in chick embryo or primary rabbit kidney showed no evidence of changes and similarly studies of RA27/3, HPV, Cendehill and Sheppard after passage through man or monkey indicated the stability of the plaque type.

DISCUSSION

Our studies show that limited passage of rubella virus in cell culture or animals does not normally result in the selection of different plaque types. Furthermore, it would appear that the usual plaque type is small whether obtained from pre- or post-natal infection, and even amongst the vaccine strains only the high passage virus HPV-77 produced large plaques. The large-type plaque, therefore, is unusual and the two low passage examples we describe may have some interest, although the rapid loss of this character discourages speculation about its genetic significance beyond the possibility that heterogeneity may exist. We note that one of the large plaque strains, Dunning, was obtained from a 6-month-old infant who had experienced congenital infection and the second, Janine, was from an adult case contracted whilst handling the Dunning strain. This infection was of some interest being relatively severe with widespread rash, enlarged glands, swollen joints and arthralgia.

It is tempting to suggest that the large plaque strain may have been selected during prolonged postnatal replication; however, the Pullen strain isolated from a 9-month-old child produced normal 1·0 mm. plaques as did the Andrews and Thomas strains from 4-week and 6-day-old infants respectively. Unfortunately we were not always able to obtain the information concerning the age of the infant when the virus was isolated – thus it is not possible to speculate further on the importance of the length of term of viral replication. Nevertheless, it is possible that the congenitally infected infant may be a potential hazard to the population due to the provision of an unusual ecological niche for the emergence of rubella virus variants.

We acknowledge the technical assistance of Terry Bromley and John Rowe.

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