SHORT ARTICLE

TRANSMISSION OF HUMAN ROTAVIRUSES TO GNOTOBIOTIC PIGLETS

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PLATE L

RECENTLY there have been numerous reports of finding reovirus-like particles or rotaviruses in the faeces of young children with acute gastro-enteritis (Bishop *et al.*, 1973, 1974; Flewett, Bryden and Davies, 1973; White *et al.*, 1974; Cruickshank, Axton and Webster, 1974; Flewett *et al.*, 1974b; Holmes *et al.*, 1974; Kapikian *et al.*, 1974; Middleton *et al.*, 1974; Ørstavik, Figenschau and Ulstrup, 1974; Sexton *et al.*, 1974; Tan *et al.*, 1974; Davidson *et al.*, 1975). Similar viruses have been described in calves (Mebus *et al.*, 1969; Woode *et al.*, 1974), monkeys (Els and Lecatsas, 1972), piglets (Woode and Bridger, 1975) and infant mice (Much and Zajac, 1972; Kapikian *et al.*, 1974; Flewett, Bryden and Davies, unpublished).

Although the calf and human viruses are closely related morphologically and serologically (Flewett *et al.*, 1974a; Kapikian *et al.*, 1974), attempts to infect calves with the human virus have been unsuccessful (Flewett *et al.*, 1974a). The calf rotavirus, however, multiplies readily in piglets, inducing diarrhoea (Woode and Bridger, 1975). The present paper describes attempts to infect piglets and suckling mice with the human virus.

MATERIALS AND METHODS

Animal experiments. (i) *Gnotobiotic piglets.* Four samples of faeces (A, B, C and D), from four young children with acute enteritis, that had been found by electron microscopy to contain rotaviruses, were prepared as 30% (w/v) phosphate-buffered saline extracts, pH 7·3, and freed of bacteria by membrane filtration. The filtrates, in 1–2-ml amounts, were inoculated intranasally into 2–28-day-old gnotobiotic piglets (Tavernor *et al.*, 1971), as follows. Pooled filtrates A and B were given to two piglets, filtrate C to three piglets, and pooled filtrates C and D to three piglets. Control piglets were given 1–2 ml of tissue-culture medium only. Subsequently, faecal filtrates were prepared from some of these animals for further serial passages in gnotobiotic piglets. Piglets were bled 3 weeks after virus inoculation for antibody tests. (ii) *Suckling mice.* These were obtained both from our normal laboratory strain of mice (Rayleigh strain) and from a “nude”, thymus-deficient strain of mice (BALB C cross C3H). Both colonies are known to be free of mouse rotavirus. Faecal suspensions, which had been clarified by centrifugation (3300 *g* for 20 min.) but not filtered, were inoculated by placing drops of suspension over the nostrils of the mice.

Electron microscopy. Faeces or gut contents from the piglets were prepared by differential centrifugation and mixed with potassium phosphotungstate, pH 6·0, before examination in the electron microscope (Flewett *et al.*, 1974a); some specimens were further purified by centrifugation in a sucrose density gradient.

Serology. Sera were examined for antibodies by the indirect immunofluorescence (FA) technique, and by the neutralisation test, against a cell-culture-adapted calf rotavirus

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(Bridger and Woode, 1975). Virus-antibody reactions were also observed directly by immunoelectron microscopy (IEM) (Flewett *et al.*, 1974*a* and *b*).

Virus isolation in cell culture. Bacteria-free filtrates were prepared, as described above, from human and pig faeces containing rotavirus particles and were inoculated into primary pig-kidney (PK), calf-kidney (CK) and human-embryo-kidney (HEK) cell cultures. The preparation and maintenance of these cultures have previously been described (Woode *et al.*, 1974).

RESULTS

Clinical signs of infection. None of the piglets given intranasal inoculations of human rotavirus, whether prepared from human faeces or piglet faeces after serial passage in piglets, developed any obvious clinical signs of infection during the observation period of 3-4 weeks. Some of them passed faeces of looser consistency and altered colour, but this was also seen in some of the control piglets and was of dubious significance. In contrast, piglets infected with pig rotavirus became anorexic and produced profuse, watery, often floccular faeces 18-72 h after challenge.

Similarly, none of the mice given intranasal inoculations of human faecal filtrates were clinically affected, although the same mice when challenged with mouse rotavirus developed diarrhoea.

Virus excretion. Four of seven piglets given human faecal filtrates subsequently showed the presence of typical rotavirus particles in their faeces or gut contents, and virus was also found in the pooled faeces of piglets that had been inoculated with first or second piglet-

TABLE

Presence of virus particles in faeces and antibody in serum of piglets after intranasal inoculation of human rotavirus

Primary or serial passage	Inoculum	Piglet no.	Presence or absence of virus in faeces	Serum-antibody titres of	
				fluorescent antibody	neutralising antibody
Primary passage	A*+B	1	+ (4)†	160	≥ 160
		2	...	160	≥ 160
	C	3	-(3)	...	...
		4	+(4)	...	...
		5	+(5)	...	...
	C+D	6	+(6-13)	≥ 80	< 10
		7	-(2-13)	≥ 80	< 10
		8	-(5)	...	...
First serial passage	Pooled faecal filtrates from piglets 6, 7 and 8	9 } 10 } 11 }	+(4-8)	{ 160 320 ≥ 40	{ < 20 < 20 < 20
		12 } 13 }		{ 1280 1280	{ < 40 < 40
		14 } 15 }	+(2-8)	{ 640 640	{ < 40 < 40

* A-D = different batches of faecal filtrate prepared from children with rotavirus-positive gastro-enteritis.

† + = Present; - = absent; number in parenthesis = day on which faeces or gut contents were examined.

... = Not done.

passage material (table); particles with and without the outer capsid layer were found (figure). Two of the three virus-negative piglets were killed on the 3rd and 5th day respectively after challenge, and this short time-interval could account for the apparent absence of virus. The third negative piglet was one of a pair (piglets 6 and 7) challenged with the same inoculum and virus was not detected in ten samples of its faeces collected over a period of 13 days, although the litter-mate excreted virus from day 6 until day 13. However, both piglets developed antibody, suggesting that virus multiplication had occurred also in the virus-negative one. Rotaviruses were not found in faecal samples taken from piglets before challenge, or in any of the control piglets.

Virus particles were not detected in the faeces or intestinal tract of any of the mice challenged with human rotavirus. After re-infection with mouse rotavirus, however, virus particles were easily found.

Virus isolation in cell culture. Although the human virus was readily found in piglet faeces by electron microscopy, it could not be subcultured to PK, CK or HEK cell cultures, as demonstrable by cytopathic effect or staining by the FA technique. Similarly, filtrates of human faeces containing rotavirus particles could not be subcultured in these cells. In contrast, calf and pig rotaviruses, after passage through pigs, readily infected both CK and PK cells in tissue culture, and the infected cells stained specifically in FA tests with animal antisera prepared against human, calf, pig and mouse rotaviruses; fluorescence was also obtained with a high proportion of sera randomly collected from human subjects, pigs and calves. All the various sera gave the same pattern of fluorescence in the infected cells.

Serology. All 11 piglets examined developed fluorescent antibody against calf rotavirus but only two also had neutralising antibody (table); these two piglets had been infected with the same batch of human virus. None of the piglets had demonstrable antibody before inoculation of virus.

Immunoelectron microscopy. Cross-agglutination tests were carried out by electron microscopy with "smooth" virus particles (possessing their outer capsid layer) and "rough" virus particles (having lost their outer capsid layer) against convalescent sera from children with rotavirus gastro-enteritis, a serum from a gnotobiotic piglet infected with human rotavirus, convalescent sera from piglets infected with pig rotavirus and convalescent sera from calves infected with calf rotavirus. The human convalescent sera and the pig anti-human-rotavirus antiserum (piglet number 1) agglutinated both "smooth" and "rough" rotavirus particles of man, pig and calf. The pig and calf convalescent sera agglutinated "smooth" and "rough" particles of both pig and calf rotaviruses but only "rough" human rotavirus particles.

DISCUSSION

The excretion of human rotavirus in the faeces for several days, even after three serial passages, followed by the development of antibody, indicates that this virus replicates in experimentally infected piglets. The poor neutralising antibody response (two piglets only), compared with the more regular production of fluorescent antibody (11 piglets), could have been due to either the use of different batches of human virus or inherent variability in the responses of the animals used. We have found similar differences in naturally infected human subjects and pigs when their sera were tested against calf rotavirus (unpublished). Thus, among 23 randomly selected human sera, 15 had fluorescent antibody but only five had neutralising antibody; among 56 randomly selected pig sera, 54 had fluorescent antibody but only 36 neutralising antibody. So far, all sera with neutralising antibody have been positive for fluorescent antibody.

An alternative explanation for the development of neutralising antibody in only two of our piglets is laboratory contamination of the inoculum with calf virus; rotaviruses are very stable and occur in faeces in great numbers. However, great care was taken to avoid laboratory contamination and all piglets were kept under gnotobiotic conditions. Moreover, the absence of diarrhoea in the two piglets and the failure to isolate virus from their faeces in tissue cultures make the presence of either pig- or calf-rotavirus contaminants in the inoculum most unlikely; these animal rotaviruses normally cause profuse diarrhoea in piglets and grow readily in PK and CK tissue cultures after passage through piglets. In addition, the

piglet anti-human-rotavirus antiserum resembled human convalescent sera, rather than pig or calf convalescent sera, in the IEM tests. Animal variation or strain differences among human rotaviruses are therefore the most likely explanations for the differences in serological response of the experimentally infected piglets.

Naturally occurring cross-infection between animal species might account, at least partly, for the presence of cross-reacting antibodies in randomly collected human and pig sera. However, only piglets are readily infected with human, calf and pig rotaviruses while calves appear to be susceptible only to the calf virus and fail to produce antibodies after ingestion of either human (Flewett *et al.*, 1974a) or pig rotavirus (Woode and Bridger, unpublished). The extensive cross-reactions that are found between human, pig and calf sera are therefore more likely to be due to the close antigenic relationships of the three viruses. Unfortunately, complete cross-antigenic studies are not yet feasible because only the calf virus can be grown in tissue culture. However, the recent report that the human virus multiples in organ cultures (Wyatt *et al.*, 1974) may provide a useful technique for further serological comparisons between the members of the rotavirus group. Replication of the human virus in gnotobiotic piglets will facilitate the production of large quantities of both animal-derived virus and valuable monospecific serum.

SUMMARY

Faecal filtrates containing rotavirus particles, from children with acute infectious diarrhoea, were inoculated intranasally into gnotobiotic piglets. The piglets developed no symptoms, but virus was readily found by electron microscopy in their faeces during three serial passages. Among 11 piglets tested 3 weeks after inoculation of virus, all had developed fluorescent antibodies against tissue-culture-adapted calf rotavirus but only two had neutralising antibody. Growth of human rotavirus did not occur in either normal or "nude", thymus-deficient suckling mice.

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FIGURE.—Rotavirus particles in faeces of a gnotobiotic piglet after intranasal inoculation of human rotavirus. (a) Particles with outer capsid layer ("smooth" particles); (b) particles without outer capsid layer ("rough" particles). Electron micrograph. $\times 160,000$.

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