Effect of pH and temperature on the infectivity of human coronavirus 229E

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The stability of human coronavirus 229E infectivity was maximum at pH 6.0 when incubated at either 4 or 33°C. However, the influence of pH was more pronounced at 33°C. Viral infectivity was completely lost after a 14-day incubation period at 22, 33, or 37°C but remained relatively constant at 4°C for the same length of time. Finally, the infectious titer did not show any significant reduction when subjected to 25 cycles of thawing and freezing. These studies will contribute to optimize virus growth and storage conditions, which will facilitate the molecular characterization of this important pathogen.

Key words: coronavirus, pH, temperature, infectivity, human coronavirus.

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La stabilité de l'infectivité du coronavirus humain 229E était maximale à pH 6,0 aux températures de 4 ou 33°C. Cependant, l'influence du pH était plus marquée à 33°C. L'infectivité virale a été complètement perdue après une période d'incubation de 14 jours à 22, 33 ou 37°C, alors qu'elle était relativement constante à 4°C pour cette même période de temps. Finalement, le virus ne montra pas de baisse significative de titre lorsque soumis à 25 cycles de congélation–décongélation. Cette étude contribuera à optimiser les conditions de culture et d'entreposage du virus, ce qui facilitera la caractérisation moléculaire de cet important pathogène.

Mots clés : coronavirus, pH, température, infectivité, coronavirus humain.

The human coronavirus 229E (HCV-229E) (Hamre and Procknow 1966) is an important member of the *Coronaviridae*, a family of enveloped viruses which contain a single-stranded RNA of positive polarity (McIntosh 1974; Siddell *et al.* 1983*a*; Tyrrell *et al.* 1978) and which are responsible for a number of human and animal respiratory, neurological, and gastrointestinal infections (Macnaughton and Davies 1981; Siddell *et al.* 1983*b*). Virions are pleomorphic particles, 60 to 180 nm in diameter, which bear about 20 nm long club-shaped surface projections (Bradburne and Tyrrell 1971; Siddell *et al.* 1983*a*). Some strains of coronaviruses cause encephalitis and demyelination in mice and rats and it has been suggested that human coronaviruses could play a role in neurological disorders such as multiple sclerosis (Burks *et al.* 1980; Chaloner-Larsson and Johnson-Lussenburg 1981; Hovanec and Flanagan 1983; Johnson-Lussenburg and Zheng 1987; Madden *et al.* 1981; Salmi *et al.* 1982; Sorensen *et al.* 1986; Tanaka *et al.* 1976) or Parkinson's disease (Fishman *et al.* 1985). This emphasizes the importance of studies on the molecular biology of human coronaviruses (Talbot *et al.* 1988). As a preliminary step, it was critical to optimize *in vitro* virus growth conditions. We report here on the importance of pH and temperature on the stability of HCV-229E infectivity.

Previous studies on several members of the *Coronaviridae* indicated that infectivity of different strains of coronaviruses was differently influenced by changes of pH and temperature. For instance, Hess and Bachmann (1976) observed considerable differences in pH stability of eight strains of transmissible gastroenteritis virus. Furthermore, Bucknall *et al.* (1972) showed differences between thermal inactivation of two strains of human coronavirus. On the other hand, some properties appear to be common to a large number of coronaviruses. For example, most coronaviruses seem to show some decrease of infectivity when exposed to acid pHs at 37°C (Cowen *et al.* 1971; Hierholzer 1976) but appear to be relatively stable at 4°C (Cheever *et al.* 1949; Daniel and Talbot 1987).

The 229E strain of human coronavirus and the L132 human fetal lung cells (Chaloner-Larsson and Johnson-Lussenburg 1981) were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown at 37°C in growth medium consisting of one part of Earle's minimum essential medium, one part of Hank's M199, 5% (v/v) fetal calf serum, 0.13% (w/v) sodium bicarbonate, and 0.05 mg/mL gentamycin. A virus stock was obtained after two cycles of plaque purification on L132 cells and used directly for the incubations described below. Viral infectious titers were determined by plaque assay on L132 cells, using a method described by Daniel and Talbot (1987), except that plaques were revealed after a 7-day incubation period at 33°C.

The effect of pH on virus stability was evaluated by measuring the infectious titer remaining after an incubation period of 6 h at 4 and 33°C in buffers of various pHs (Daniel and Talbot 1987; legend to Fig. 1). As shown in Fig. 1, an optimal stability of viral infectivity was observed at pH 6.0 at both 4 and 33°C. However, the infectious titer was more stable at extreme pHs when virus was incubated at 4°C. Indeed, viral

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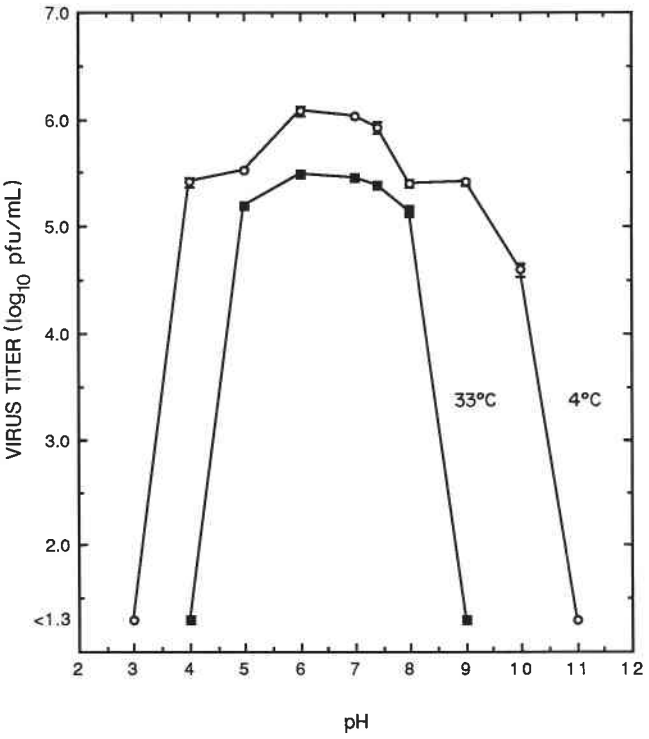

FIG. 1. Effect of pH on HCV-229E infectivity. A virus stock was diluted 10-fold in buffers at various pHs: pH 3.0 to 8.0 in 0.1 M citric acid and 0.2 M sodium phosphate dibasic; pH 9.0 to 12.0 in 0.1 M glycine in 0.1 M sodium chloride and 0.1 N sodium hydroxide. All buffers contained 150 mM sodium chloride and 5% (v/v) fetal calf serum. After incubation for 6 h at 4 or 33°C, the remaining infectious titers were measured by plaque assay on L132 cells. pfu, plaque-forming units.

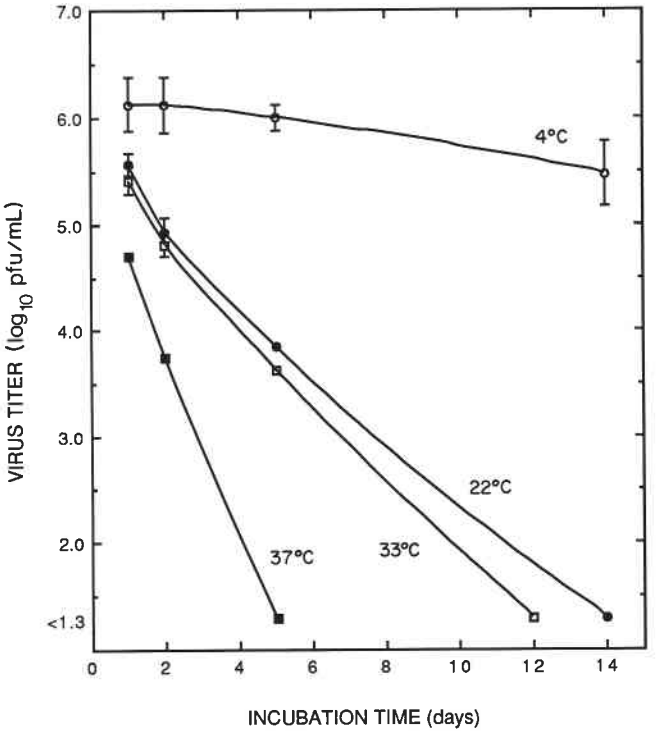

FIG. 2. Thermal inactivation of HCV-229E. A virus stock was diluted 10-fold in buffer at pH 6.0, as described in the legend to Fig. 1, and incubated in the dark at 4, 22, 33, or 37°C for various times, after which the remaining infectious titers were measured by plaque assay on L132 cells.

infectivity was undetectable after exposure to pH 4.0 or 9.0 at 33°C, whereas 93% (5.4/6.1 log_{10}) of viral infectivity remained after exposure to these pHs at 4°C. Moreover, 84% (4.6/6.1 log_{10}) of viral infectivity also remained after incubation at 4°C in medium buffered at pH 10. Similar results have been reported by Pocock and Garwes (1975) in their work on transmissible gastroenteritis virus. They found that virus infectivity was least affected by exposure to a pH of 6.5 when incubated at 37°C but when kept at 4°C for the same length of time, virus infectivity remained constant between pH 5.0 and 8.0. On the other hand, Alexander and Collins (1975) showed that stability of avian infectious bronchitis virus was directly related to pH between 6.0 and 8.0, being more stable at the acid pH values. Hierholzer (1976) reported that human coronavirus 229E was acid labile but did not mention the effect of alkaline pH on viral stability.

Thermal inactivation of viral infectivity was studied at 4, 22, 33, and 37°C, at the optimal pH of 6.0. As shown in Fig. 2, virus was relatively stable for at least 14 days at 4°C but showed a rapid decrease in infectious titer at 22, 33, and especially 37°C, being undetectable after 5 days of incubation at the latter temperature. Similarly, Daniel and Talbot (1987) found that murine hepatitis virus (MHV-A59) was stable for 3 months at 4°C, whereas infectivity was undetectable after 14 days at 22 and 37°C. Cheever *et al.* (1949) observed that MHV-JHM conserved its infectivity after 21 days at 4°C. Furthermore, Hirano *et al.* (1978) showed that MHV-2 was rapidly inactivated at 37°C, showing a decrease of infectious titer of 2.6 log_{10} after an incubation of 24 h.

Finally, the effect of freezing and thawing on virus infectivity was studied for practical storage purposes. One-millilitre samples of viral suspension were frozen for at least 2 h at −70°C and then thawed in a 37°C water bath. This operation was repeated 25 times and no significant reduction of viral titer was observed (data not shown). This is in accordance with Daniel and Talbot (1987), who reported that MHV-A59 was stable for at least 15 cycles of freezing and thawing.

Even though other viral growth conditions may contribute to an increase in infectious titers, pH and temperature constitute two important and conveniently controlled factors that will facilitate further molecular studies on human coronaviruses.

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