

## ISOELECTRIC FOCUSING OF HOG CHOLERA VIRUS \*

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### Résumé

ELECTROFOCALISATION DU VIRUS DE LA PESTE PORCINE CLASSIQUE. — Le point isoélectrique du virus de la peste porcine classique (souche Alfort) a été déterminé ( $pH_i = 4,8$ ). A ce pH, il y a une nette stimulation de son infectiosité. Le profil isoélectrophorétique de souches isolées sur le terrain ne montre pas de différences significatives.

### Introduction

Electrophoretic properties have often been used to compare different strains of the same virus (Polson and Decks, 1962; Thorne *et al.*, 1965; Breeze and Thorne, 1966; Magdoff-Fairchild, 1960; Douglas and Williams, 1969; Pringle, 1969). On the other hand, a comparative study of Hog Cholera virus (HCV) Alfort, Thiverval and 331 strains to pH sensitivity demonstrated an equivalent and great stability between pH 4 and 10 (Aynaud, 1972).

Thus, using an isoelectrofocusing micro-technique, it was of interest i) to determine the isoelectric point of HCV, an still unknown parameter of this virus. ii) to judge the eventual value of this criterion as a genetic marker for in vitro identification of field strains of HCV.

### Materials and methods

#### 1. — *Viruses and cells*

The origins of the strains used has been previously described (Aynaud *et al.*, 1972; Corthier *et al.*, 1974). The Alfort clone, was isolated from a wild-type strain highly pathogenic for pigs. The Thiverval clone, an attenuated mutant, was isolated from the Alfort clone. Strains 331 and Loud, isolated in the field by Mengeling and Paker (1969) in the U.S.A. and by Aynaud *et al.* (1974) in France, respectively, are responsible for the chronic forms of the disease.

Virus stocks were prepared in PK<sub>15</sub> cells as described previously (Aynaud *et al.*, 1972; Corthier *et al.*, 1974). Infected cells were grown in Eagle's medium lacking calcium and supplemented with 5% foetal calf serum.

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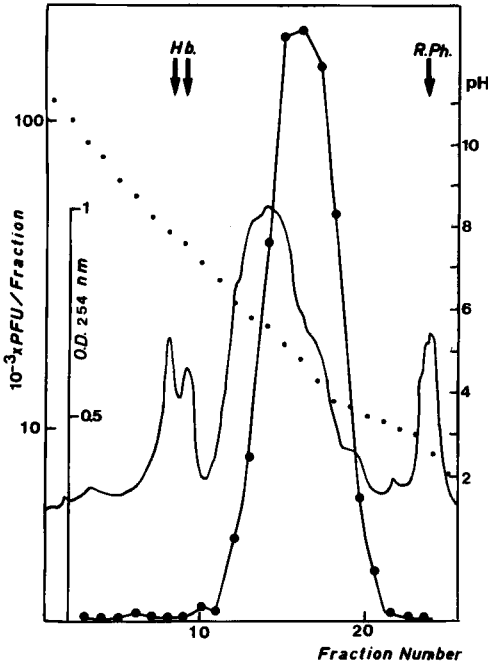


Fig. 1. Isoelectric focusing of the Alfort strain in a gradient from 3.5 to 10. Markers are sheep hemoglobin (Hb), phenol red (Ph. R.). ●—● : Infectivity; — : OD at 254 nm.

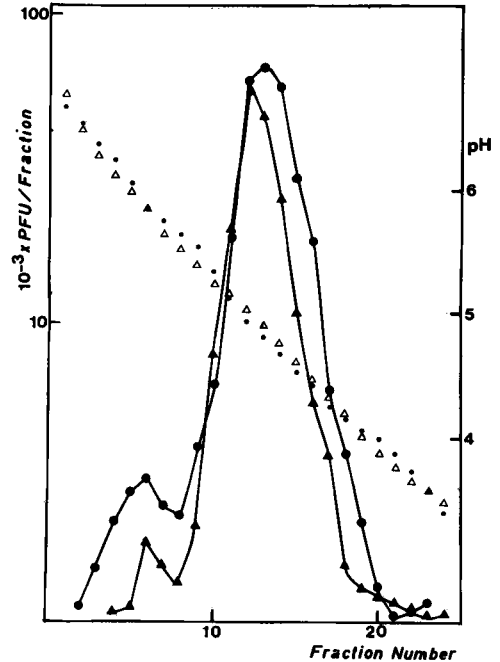


Fig. 2. Isoelectric focusing of Alfort (●—●) and Thiverval (▲—▲) strains in a gradient from 4 to 6. (○ and △).

After incubation, the cultures were frozen at  $-20^{\circ}\text{C}$  in the presence of 5% dimethylsulfoxide. The solutions were clarified by centrifugation at 7,000 g for 20 min just prior to use.

#### 2. — Virus titration

The infectivity was measured by a method of direct immunofluorescence on a monolayer PK<sub>15</sub> layer (Carbrey *et al.*, 1965), previously described by Aynaud (1968).

#### 3. — Isoelectric focusing

Isoelectric focusing (Svensson, 1961) of the virus was done in a liquid stream in small volume columns (6 ml), according to the method of Korant and Lonberg-Holm (1974) which we adapted to a Gelphor (Gilson) electrophoresis apparatus. The 10-40% (w/w) sucrose density gradient contained 1% Ampholines (LKB). Two pH ranges were used, 3.5-10 and 4-6. The sample was introduced as 0.3 ml during the formation of the sucrose gradient in a zone near its final migration point. A constant power generator was used (LKB PS-2103); maximum current was 2 mA per column and the voltage

reached 1000 V after 6 hours of migration. Afterwards, fractions of the gradient are collected on an ISCO fractionator. Thirty 0.2 ml samples were collected and absorbance was automatically monitored at 254 nm. The pH at  $20^{\circ}\text{C}$  was quickly measured in the fractions.

#### 4. — Buffer solutions

— McIlvaine's buffer (1921), pH 4.8 : 0.1 M citric acid, 0.2 M disodium phosphate.

— Ten buffer, pH 7.2 :  $10^{-2}$  M Tris-chloride, 1 mM EDTA, 0.1 M NaCl.

### Results

#### *Isoelectrophoretic properties of the HCV strains*

After focusing of the virus, the distribution of infectivity in a pH 3.5 to 10 gradient is represented as a broad peak (Fig. 1). After analysis in a restricted range of pH 4 to 6, the maximum of virus infectivity is found to be at  $\text{pH } 4.8 \pm 0.2$ . (Fig. 2). Absorbance measurements at 254 nm reveal that virus peak is highly contaminated by components having a cellular origin.

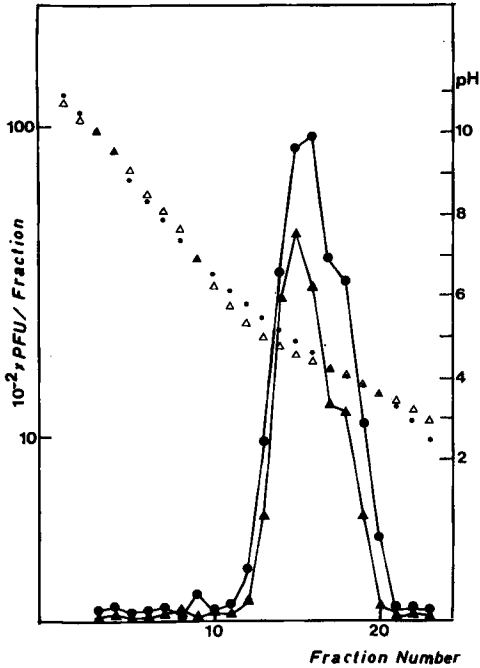


Fig. 3. Isoelectric focusing of 331 (●—●) and Loud (▲—▲) strains in a gradient from pH 3.5 to 10. (○ and △).

The isoelectrophoretic properties of the different strains studied seem not to be significantly different (Figs. 1-3).

**Influence of pH on virus infectivity**

Despite of the stability of HCV between pH 3 and 10 (Aynaud, 1972), in this electrofocusing experiments virus recovery is very low (~ 10 %) probably because of unfavorable ionic environment.

Otherwise, when buffered at pH 4.8, the infectivity of a virus suspension (Alfort clone) is tripled.

**Discussion**

— **Isoelectric point (pHi)**

In our experiments only infectivity was recorded to know where virus particles were migrating. Therefore, it must thus be asked if the infectious peak observed at pH 4.8 really corresponds to the pHi of the virus or merely to a pH where it exhibits a maximum specific infectivity. The analysis of the electrofocusing profile (Fig. 2) shows a difference of two log units between apex and base of the peak; the stimulation of infectivity (x3 at pH 4.8) mentioned above can thus not be

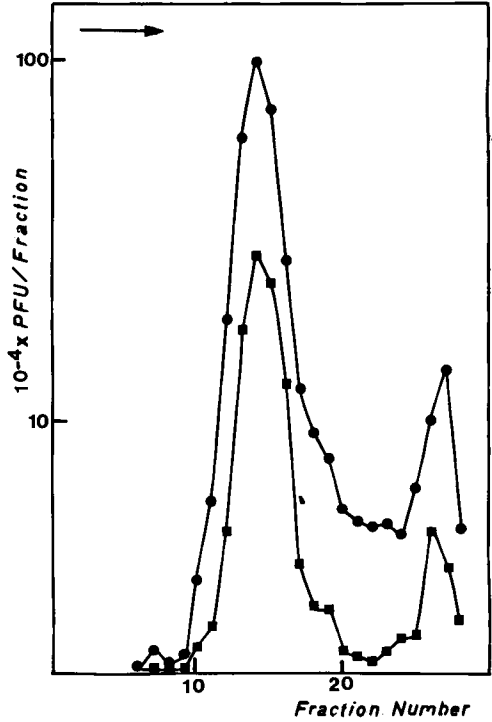


Fig. 4. Rate zonal centrifugation of an HCV sample in a 5-25 % (w/w) sucrose gradient in TEN pH 7.6. Rotor SW 40, 2 hr at 39,000 rev/mn. Migration from right to left. ●—● virus preincubated 10 min at pH 4.8; ■—■ : control virus at PH 7.6.

responsible for such difference in infectivity, and pH 4.8 must be considered as major pHi of HCV. This pHi is significantly different from that of the Semliki Forest virus (pHi ~ 6 : Kennedy, 1974), another virus of the Toga family.

On the other hand, a phenomenon of infectivity stimulation at a given pH could explain the presence of minor peaks of infectivity (as at pH 6). The existence of minor pHi values, however, implying an equilibrium among several conformational states of the particles, has already been reported in certain viruses (Mandel, 1971; Korant *et al.*, 1975). In our experiments, we have not verified if this bimodal distribution persists after an additional analysis of fractions isolated after focusing.

— **Influence of pH on virus infectivity**

When a HCV suspension is buffered at pH 4.8, a clear stimulation of its infectivity

is observed which persists even after a return to neutral pH. The hydrodynamic properties of viral particles pre-incubated for 10 min at pH 4.8 seem very similar to those of particles remaining at pH 7.6 (Fig. 4); this fact would favor the hypothesis of a modification of their specific infectivity. It should be noted also that the coincidence of the stimulation maximum with the isoelectric point has not been established precisely.

Be that as it may, this is not the first time that an increase in the infectivity of HCV after acidic treatment is reported. Work in the U.S.A. had showed that conservation of virulence had an optimum near pH 5 and that it persisted three times longer than at neutral pH (anonymous, 1938). Subsequently, Collins (1960) observed that the infectivity was ten times higher at pH 5.5 than at pH 8.5. Lastly, Aynaud (1972) noted that the stability

of strain 331 had a maximum at pH 5.6. Since the isolation of this virus is sometimes difficult, it might be more judicious to effect the homogenization of suspected organs at pH 5 rather than at neutral pH.

#### — Comparison of strains

Under our experimental conditions (unpurified virus) no significant differences were shown in the electrophoretic properties of the different strains examined. This probably reflects the fact that Alfort, 331 and Loud strains have only minor antigenic differences (Corthier *et al.*, 1974).

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#### Summary

The isoelectric point of the Hog Cholera virus (Alfort strain) has been determined (pHi = 4.8). A clear stimulation of its infectivity can be recorded at this pH. The isoelectrophoretic profiles of strains isolated in the field show no significant differences.

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