Fractionation of HVJ* by Zone Electrophoresis on Starch

Recently some viruses have been shown by zone electrophoresis and by chromatography, to consist of physico-chemically different infective fractions.[1,2,3,4]

During the studies on the structure of HVJ, the authors have found that the infective particles of HVJ are also heterogeneous zone-electrophoretically.

HVJ was collected from infected allantoic fluid of 10 day old embryonated eggs after 72 hours incubation and purified by differential centrifugation at 3,000 rpm for 20 minutes and 22,000 rpm for 30 minutes using a Spinco L ultracentrifuge fitted with a No. 30 rotor.

The purified and concentrated HVJ (200,000 HA units/ml) was suspended in borate-phosphate buffer (μ=0.05, pH=8.35) and was applied on starch for zone electrophoresis in the cold.

After electrophoresis for 14 hours, the starch column was divided into 1 cm segments and each segment was eluted with 5 ml of isotonic saline. The protein contents[5], infectivity, hemagglutinating, hemolytic and fusing activities[6] of each fraction was determined. Results, except the fusing activity, are shown in Fig. 1.

The HVJ preparation separates into two main fractions (II and III) and another small fraction (I) which remained at the origin. In repeat experiments, the fast fraction (III) showed a tendency to separate further into two fractions. As seen in Fig. 1, the protein content per HA unit was lowest in fraction II, and highest in fraction 1. The distribution of infectivity, the hemagglutinating activity, and the protein content showed good parallelism, and the hemolytic activity per 400 HA units (for fractions No. 9–16) and the fusing activity per 1,000–2,000 HA units (for fractions No. 8–20) were not significantly different in the different fractions, although the hemolytic activity of fraction No. 8 was a little higher than that of the other fractions and from fraction No. 21 it decreased progressively in titer.

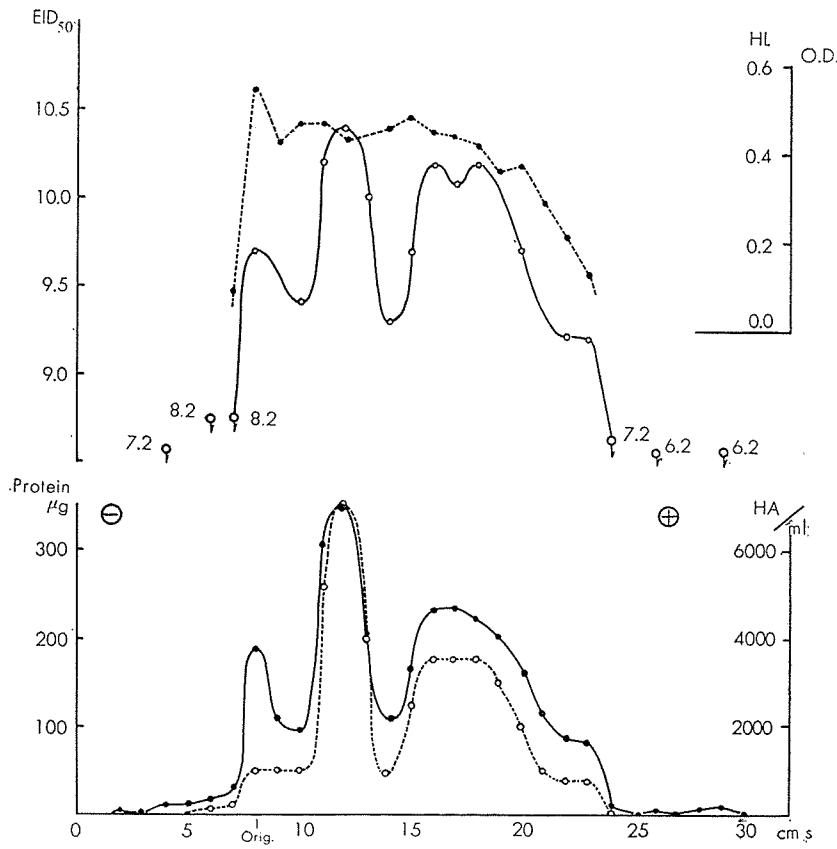
On re-zone-electrophoresis of HVJ of each fractions, it migrated to the similar position, which indicates that these electrophoretic mobilites are characteristics specific for the fractionated virus.

Next the subunits of HVJ of the three fractions were separately obtained by Emasol-ether disintegration treatment[7], concentrated by centrifugation at 40,000 rpm for 60 minutes and diffused against anti-HVJ rabbit serum in agar gel. Like the ether disintegrated preparation of unfractionated HVJ, all preparations showed R and H precipitation lines against anti-HVJ serum[8] in gel diffusion.

Then morphology of virus of each fraction was studied. A virus preparation was obtained by inoculation of a 10^{-6} dilution of infected chorioallantoic fluid. In this case no morphological difference was detected by electron microscopy between the fractions but in undiluted inoculation, bag-like particles were observed

* Abbreviation of Hemagglutinating Virus of Japan, so called Sendai virus.

Fig. 1. Fractionation of HVJ purified preparation by zone electrophoresis on starch



Distance along the electrophoretic column

Conditions: Borate-phosphate buffer, pH=8.35, μ=0.05
Current, 7mA at 750 volts for 14.5 hours at 10°C;
column size, 30 cm×2 cm×1 cm

The hemagglutinating activity was expressed as reciprocal of viral dilution at agglutinating titration end point against 0.5 ml of 0.5% fowl red cells. Hemolytic activity was expressed as the optial density at 540 mμ of the supernatant of a 2 ml suspension of 2% fowl red cells hemolysed by 1 ml of virus sample at 37°C for 60 minutes.

Upper figure —○—○— infectivity/ml
- - -●- -●- - - hemolytic Lctivity/ml
Lower figure ——●——● protein content/ml
– – –○– –○– – – hemagglutinating activity/0.5 ml

in fraction II and filamentous and doughnuts shaped particles were dominant in fractions I and III.

Although virus preparation of various inoculum sizes and at various growth

stages were used it was not possible to obtain electrophoretically homogeneous fractions. The progeny produced by successive inoculation of limiting dilutions of fractions (II and III) were similarly separated electrophoretically, showing that the difference in electrophoretic behavior between the fractions was not a genetical one.

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YASUSHI HOSOKAWA
YASUHIRO HOSAKA
KONOSUKE FUKAI

Department of Preventive Medicine
Research Institute for Microbial Diseases
Osaka University, Osaka

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