Bull. Inst. Zool., Academia Sinica **26**(3): 201-214 (1987)

CHARACTERISTICS OF A INFECTIOUS PANCREATIC NECROSIS LIKE VIRUS ISOLATED FROM JAPANESE EEL (*Anguilla japonica*)

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(Received March 11, 1987)
(Revision received April 3, 1987)
(Accepted April 8, 1987)

Jen-Leih Wu, Chi-Yao Chang and Ya-Li Hsu (1987) Characteristics of a infectious pancreatic necrosis like virus isolated from Japanese eel (*Anguilla japonica*), *Bull. Inst. Zool., Academia Sinica* **26**(3): 201-214. The infectivity of E1S, which was isolated from the spleen of diseased Japanese eel, was tested in various cell lines and CHSE-214 cells showed the best one for viral multiplication. Under EM observation, the unenveloped icosahedral particles of E1S virus were presented in the cytoplasm near endoplasmic reticulum of CHSE-214 cells. With a single layer capsid, these particles showed 59.8 nm in diameter. From the metabolic inhibitor tests, the E1S was identified to be an RNA virus. The buoyant density of the purified E1S in CsCl was 1.33 g/ml. The results of neutralization test indicated that E1S had cross reaction with EVE (Eel Virus, European). The biochemical, serological and electron microscopic studies revealed that E1S is an IPNV-like virus and belongs to Birnaviridae. The virus multiplication was inhibited by UV irradiation, but at low fluences, survival curve of E1S displayed a minor shoulder. The virus infectivity was reduced 10^7 $TCID_{50}$/ml under 15,000 $J \cdot m^{-2}$ tensity of UV irradiation, and $10^{4.5}$ $TCID_{50}$/ml when 1 μg/ml of virazole was added.

Infectious pancreatic necrosis virus (IPNV) is an economically important pathogen of salmonid fishes (Hedrick *et al.*, 1983b; Wolf *et al.*, 1960; Wu *et al.*, 1983). These viruses were also isolated from aquacultured non-salmonid fishes including Japanese eel (Hedrick *et al.*, 1983b; Ueno *et al.*, 1984), tilapia (Hedrick *et al.*, 1983b), loach (Chen *et al.*, 1984) and clam in Taiwan (Chen *et al.* 1985). It became the prototype of a new group of viruses and was referred as birnaviruses (Dobos *et al.*, 1979). These new virus group all have unenveloped, icosahedral capsid (Dobos, P., 1976; Macdonald and Yamamoto, 1977), and two segments of double stranded RNA. The buoyant density of IPNV was found to be 1.33 g/ml in CsCl and size was ranged from 57 to 74 nm in diameter (Dobos and Roberts, 1983).

The annual epizootics among pond cultured Japanese eels (*Anguilla japonica*) in Shizuoka prefecture Japan have been reported since 1969 (Sano, T., 1976). This virus was also isolated from those imported European eels. Because the virus showed an antigenic relationship to a European strain of IPNV, it was designated as eel virus European (EVE). The polypeptide and RNA composition of the purified virions of EVE were compared to those of three strains (VR-299, Sp, Ab) of IPNV isolated from trout. All three IPNV strains could be distinguished by the relative mobilities of either the virion

1. Paper No. 292 of the Journal Series of the Institute of Zoology, Academia Sinica.
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polypeptides or the dsRNA genome segments. The eel virus had a similar polypeptide profile to strain Ab IPNV but differences in the migration of genome segments between the two viruses indicated each was unique (Hedrick *et al.*, 1983a). In this paper, the virus which was isolated from the spleen of diseased Japanese eel no. 1 (designated as E1S) was investigated. The biochemical and electron microscopic studies indicated that the viral agent possessed RNA genome and unenveloped icosahedral capsid with 59.8 nm in diameter. The neutralization test revealed that E1S is related to EVE. Therefore, E1S is an IPNV-like virus and belongs to birnaviridae .The viral activity is sensitive to UV irradiation and virazole treatments. The possible prevention and control measure of this virus was discussed.

MATERIALS AND METHODS

Cells and viruses

The cell lines of chinook salmon embryo (CHSE-214) (Nims *et al.*, 1970), rainbow trout gonad (RTG-2) (Wolf and Quimby, 1962), Atlantic salmon (AS) (Nicholson and Byrne, 1973), blue gill fry (BF-2) (Wolf and Quimby, 1966), fathead minnow (FHM) (Gravell and Malsberger, 1965), and epithelioma popillosum cyprini (EPC) (Tomasec and Fijan, 1971), and the primary cells of tilapia kidney (TK), carp fin (CF), loach fin (LF), perch kidney (PK), perch heart (PH), and perch liver (PL) were used for virus isolation and cultivation. The cells were grown either in minimum essential medium MEM) in Earles' salts (Flow Laboratories) or Leibovitz's L-15 medium (L-15) (Flow Laboratories) supplemented with fetal calf serum to 10% (MEM-10), penicillin to 100 IU/ml, streptomycin to 100 μg/ml, gentamicin to 25 μg/ml, and fungizone to 0.25 μg/ml. Infectious pancreatic necrosis virus (VR-299 strain) and eel virus European (EVE) were obtained from Dr. B. L. Nicholson and Dr. T. Sano.

Virus isolation

Tissues of liver, kidney and spleen from diseased eels were homogenized with a polytron in 10 volumes of MEM containing double strength antibiotics (penicillin, streptomycin, gentamicin sulfate and fungizone). The homogenate was centrifuged at 1500-2000×g for 5 min, and the supernatant was filtrated through 0.45 μm membrane. Two ml of this filtrate was independently placed on monolayer cultures of RTG-2, FHM and EPC cells. Then they were incubated at 18°C. When the cytopathic effect (CPE) was observed, the culture medium was filtrated and infected the fresh cells again as described before.

Cell susceptibilities to virus (E1S)

Twelve fish cell lines and primary cells were chosen to determine the virus infectivity in different host cells. Monolayers of different cells grown in 25 cm^2 tissus culture flasks were infected with 10^4 $TCID_{50}$/ml of E1S. After 7 days incubation at 18°C, the culture media were collected and examined for virus infectivity in CHSE-214 cells by the procedure of Reed and Muench (1938).

Virus purification

For large quantity of virus cultivation, the E1S was propagated in CHSE-214 cell monolayers at a multiplicity of infection (MOI) of 0.01 to 0.1. Infected cultures were incubated at 18°C until extensive CPE was observed. The culture medium was collected and the infectious titer was determined by $TCID_{50}$ assay. The virus was stored at −70°C.

The procedure of viral purification was modified from Chang *et al.* (1978), Dobos and Rowe (1977) and Hedrick *et al.* (1983a). Briefly, the culture fluid was centrifuged at 6,800×g for 15 min. The virus suspension was adjusted to contain 2.2% (w/v) NaCl and 5% (w/v) polyethylene glycol (PEG, MW, 15,000-20,000, Sigma Co.). The mixture was stirred at 4°C for 9-10 h, and the precipitated material was pelleted by centrifugation at 10,000×g for 75 min at 4°C. The pellet was resuspended in 2 ml of TNE buffer (0.1 M Tris-0.1 M NaCl-1 mM EDTA, pH 7.3). The cell-associated virus (cell debris part) was

repeatedly extracted with Freon 113. The aqueous sample was collected, then the PEG-concentrated and Freon-extracted virus suspension were combined and reextracted with Freon 113. A few drops of 40% (w/v) CsCl was added to the aqueous sample, mixed well and centrifuged at low speed (500-1000×g) for 3 min. The supernatant was treated with ribonuclease (RNase) at a final concentration of 100 μg/ml at 37°C for 1 h. The samples were layered over a three-step caesium chloride gradient (3 ml 40%, 3 ml 30%, 1 ml 20%) and centrifuged at 190,000×g for 16 h at 4°C. The virus band was withdrawn and the density of this fraction was adjusted to 1.33 g/ml in 5 ml of TNE buffer, and then centrifuged at 160,000 ×g for 16 h at 4°C. The virus band was collected again and diluted to 5 ml TNE buffer, and was centrifuged at 160,000×g for 1 h at 4°C. The virus pellet was resuspended in 1 ml of TNE buffer, and layered over a continuous sucrose gradient (15% to 50%, w/v) and centrifuged at 130,000×g for 2 h at 4°C. The virus band was collected again and diluted to 5 ml TNE buffer, and centrifuged at 160,000×g for 1 h at 4°C for pellet the virus.

Determination of virus density

Caesium chloride was added into the purified virus suspension until the final density reached 1.33 g/ml. The virus was then centrifuged at 160,000×g for 16 h at 4°C (SW 55 rotor). Eight drop fractions of this centrifuged virus suspension were collected from the bottom using polystaltic pump (Buchler instruments), and the refractive index of each fraction was determined by using an Abbe-3L refractometer. Each fraction was assayed for virus titer in CHSE-214 cells.

Electron microscopy

CHSE-214 cells infected with virus were harvested one day after inoculation by centrifugation at 1,000×g for 3 min. The samples were fixed with 2.5% glutaraldehyde in phosphate buffer (pH 7.4) overnight. Tissues were postfixed in 1% osmic tetraoxide for 2 h at 4°C, washed in distilled water, stained with uranyl acetate for 2 h, dehydrated in serial alcohol, and then embedded in epon. Thin sections stained with uranyl acetate and lead citrate were observed using JEM 100S electron microscope at 40 kv. For negative stain, purified virus pellet was fixed with 3% glutaraldehyde in phosphate buffer (pH 7.4), placed on formvar film coated 300-mesh grids, and then stained with uranyl acetate. The virus preparations were observed under a JEOL 100 cx electron microscope.

Metabolic inhibitors test

The monolayer cultures of CHSE-214 cells were treated with 100 μM 5-fluoro-2′-deoxyuridine (FURD) and 0.01-2 μg/ml actinomycin D in MEM as described by Rovozzo and Burke (1973).

Neutralization test

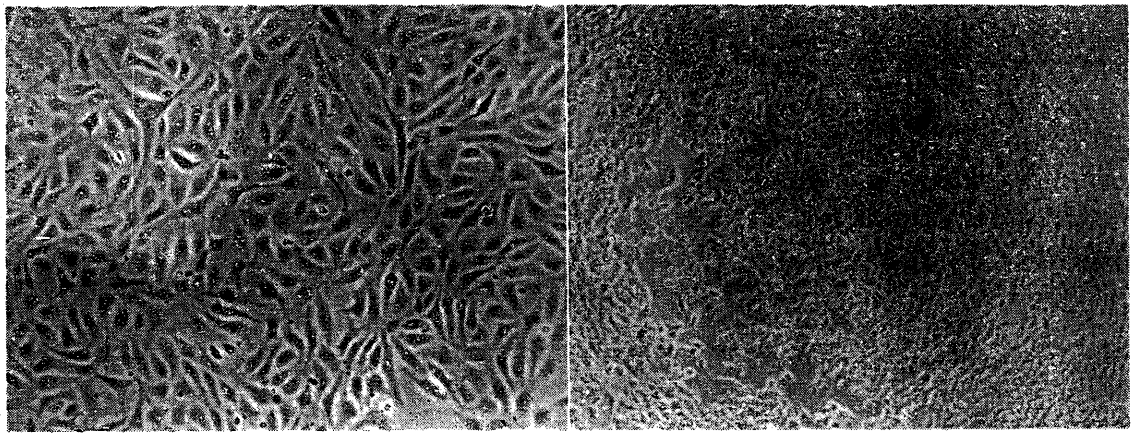
The rabbit anti-EVE serum was obtained from Dr. R. P. Hedrick. Serum neutralization test was performed as described by Rovozzo and Burke (1973).

UV irradiation

Two ml virus suspensions (containing phenol red) were placed to a 60 mm diameter petri dishes. The dishes were exposed below a UV lamp with a wave length of 254 nm. The intensity of the light striking the surface of the suspensions was measured and fixed at 4.4 $J \cdot m^{-2} \cdot sec^{-1}$ using a model J225 Blak-Ray Ultraviolet Intensity meter. Samples were removed at various time and held at 0°C until titration (MacKelvie and Desantels, 1975).

Virazole treatment

Preliminary test indicated that CHSE-214 cells showed no abnormalities below 20 μg/ml of virazole. Therefore, virazole was diluted in different concentrations from 0.001 μg/ml to 20 μg/ml with MEM medium. For each drug concentration, one 96-well microtiter plate of CHSE-214 cells was used, and MEM



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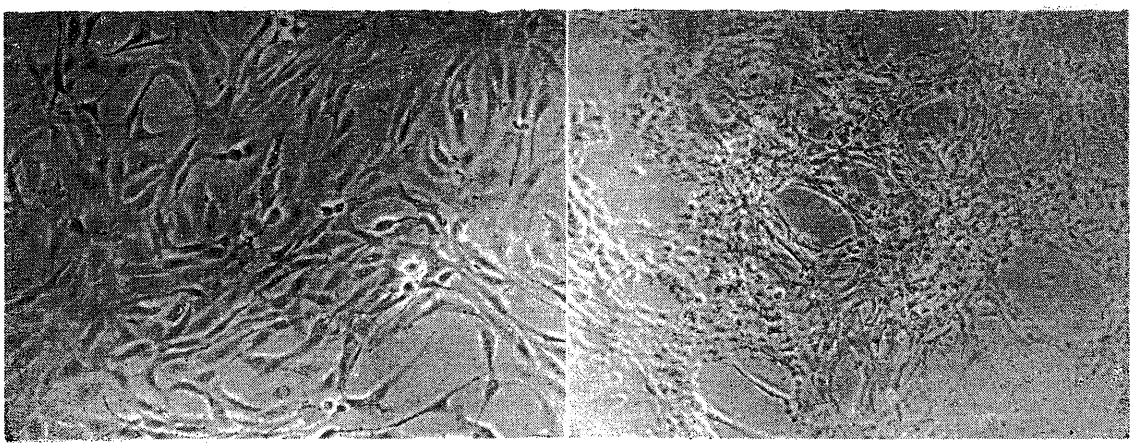

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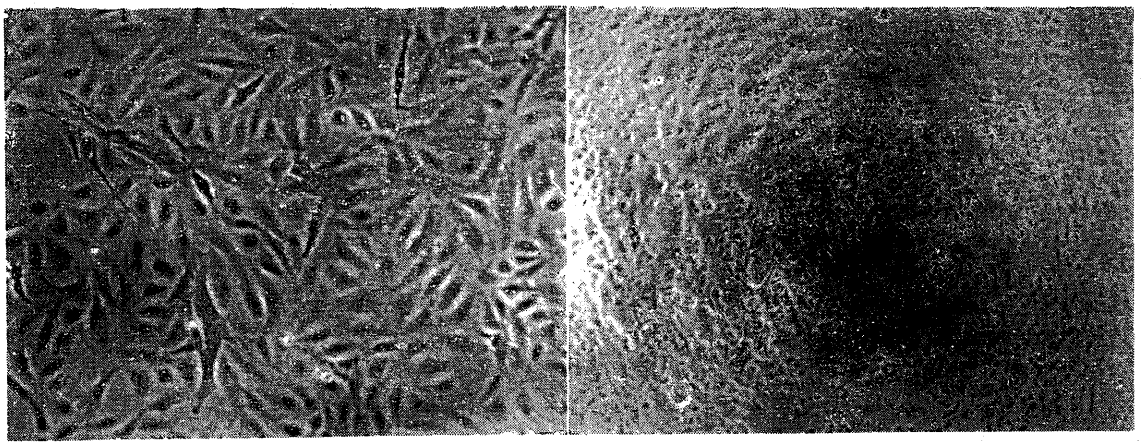

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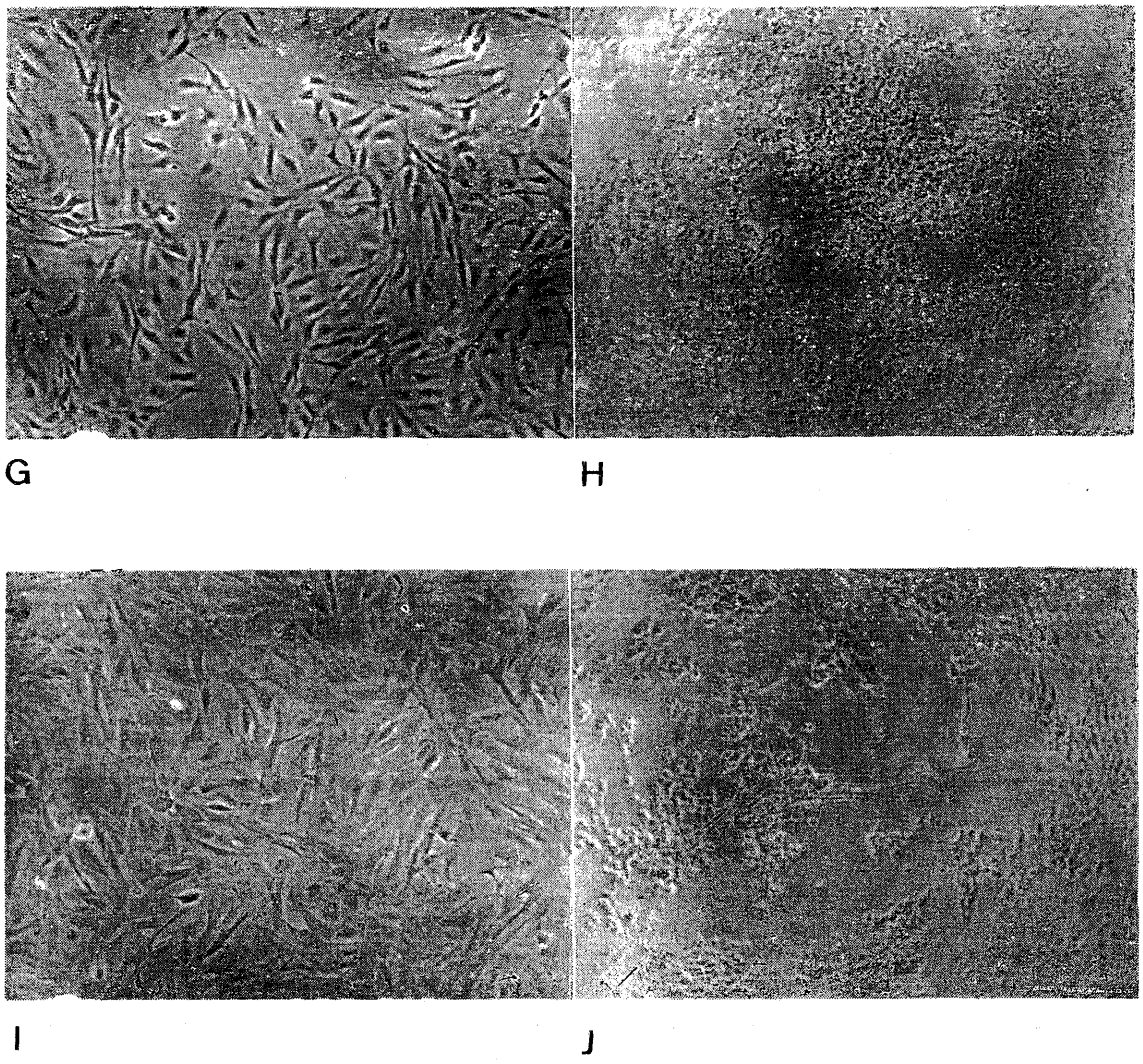

Fig. 1. The morphology of E1S infected and uninfected fish cell lines and primary cells. (240×)
(A) uninfected and (B) infected CHSE-214 cells;
(C) uninfected and (D) infected RTG-2 cells;
(E) uninfected and (F) infected AS cells;
(G) uninfected and (H) infected BF-2 cells;
(I) uninfected and (J) infected PH cells.

medium only was served as control (Migus and Dobos, 1980).

RESULTS

Virus isolation

Specimens of diseased Japanese eels were collected from culture farms located in Lu Kang, Taiwan. These eels showed some pathological symptoms such as ulcer lesion over the body, congestion of fin, hypertrophy and necrosis of the liver, atrophic muscle and deformed trunk. Cell lines of RTG-2, EPC and FHM were used to isolate eel virus, each showed different susceptibilities to the same isolate. The virus isolated from the spleen of eel no. 1 (E1S) showed the highest infectivity was chosen for further studies.

Cell susceptibilities to E1S

Twelve fish cell lines and primary cells were chosen to determine the virus infectivities in different fish cells. The results as shown in Table 1, E1S infected CHSE-214 cells showed the highest titer (1×10^9 $TCID_{50}$/ml) after 7 days incubation at 18°C. Subsequently the E1S infectivity orders were RTG-2, AS, BF-2, PK, PH, PL and BB cells. Substantially, only slight infectivity was shown on EPC and FHM cells and no CPE on CF and LF cells. This indicated the E1S virus could propagate well in salmonid fish cell lines, but less well or not replicate in cyprinoid fish cell lines. The CPE of E1S virus on CHSE-214 cells showed broken wrinkling of cell aggregations, large circular transparent cells and sloughted cell sheet (Fig. 1). Additionally, a few spindle shape cells remained on the surface. The CPE of E1S showed the similar phenomenon as that of EVE and IPNV (Sano *et al.*, 1981).

Purification and buoyant density of virus

The virus suspension was concentrated 100 fold by using 2.2% (w/v) NaCl and 5% (w/v) PEG, and almost got 100% recovery of the infectivity of E1S virus from CHSE-214 cells. After the Freon 113 extraction, the virus aqueous phase was separated from cellular debris. After CsCl buoyant density centrifugation and sucrose gradient centrifugation, the purified E1S virus solution was subjected to buoyant density determination by CsCl equilibrium centrifugation. As shown in Fig. 2, the peak fraction 9 containing a virus titer of 4.6×10^{10} $TCID_{50}$/ml which equivalent to a buoyant density of 1.33 g/ml. Electron microscopic examination of this fraction revealed that it contained a majority of complete particles and was free of cellular debris. The density of E1S is the typical value of IPNV (Dobos, P., 1977; Macdonald and Yamanoto, 1977).

Intracellular location and structure of E1S virus

After virus infection on CHSE-214 cells for 24 h, electron dense viral particles were found to be near the endoplasmic reticulum

TABLE 1
The multiplication of the isolated virus (E1S) in different fish cell lines and primary cells

Cell line or primary cell	Abbreviation	Titer $TCID_{50}$/ml	Cell line or primary cell	Abbreviation	Titer $TCID_{50}$/ml
Chinook salmon	CHSE-214	1.0×10^9	Perch liver*	PL	1.0×10^8
Rainbow trout	RTG-2	4.6×10^8	Brown bullhead	BB	1.0×10^8
Atlantic salmon	AS	3.2×10^8	Carp	EPC	3.2×10^3
Bluegill	BF-2	3.2×10^8	Fathead minnow	FHM	3.2×10^2
Perch kidney*	PK	2.1×10^8	Carp fin	CF	1
Perch heart*	PH	1.0×10^8	Loach fin	LF	1

* Primary cell

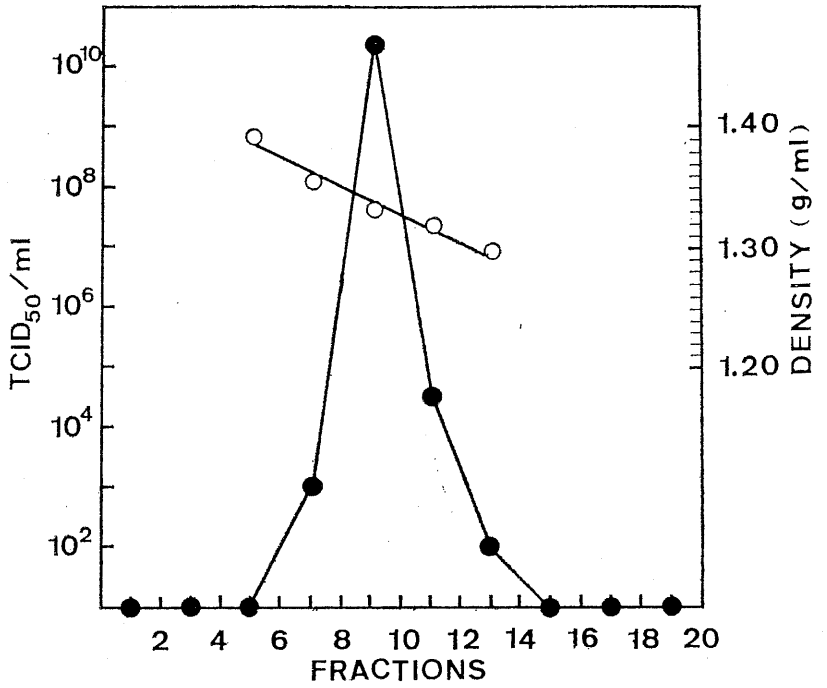

Fig. 2. Isopycnic sedimentation of E1S in CsCl gradient.
● The virus titer of each fraction was determined by $TCID_{50}$/ml.
○ The density was determined by measuring the refractive index.

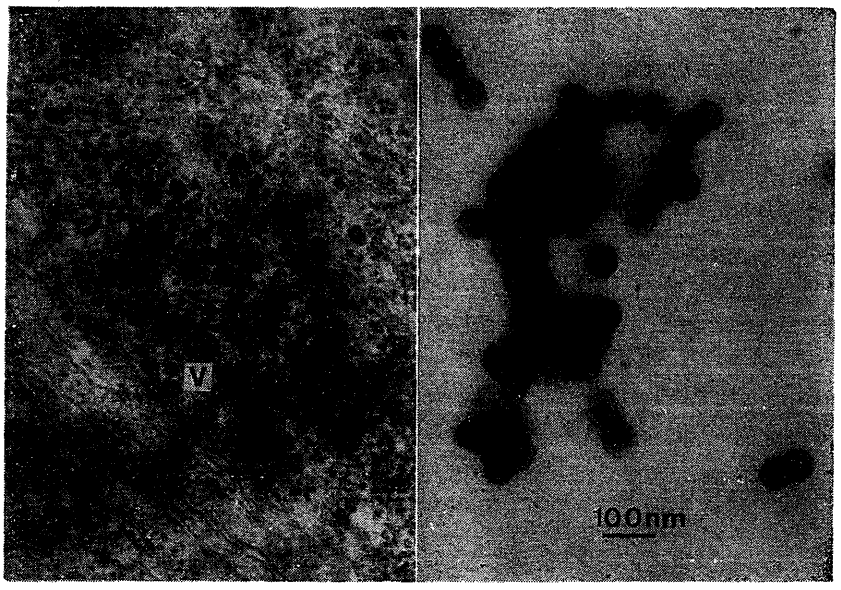

Fig. 3. Electron micrograph of 24 h postinfection of E1S infected CHSE-214 cells. 3A, thin section of infected cells. (36,720×) Viral particles (V) are closed to endoplasmic reticulum (ER) in cytoplasm. 3B, negative stain of purified virus. (50,100×) Dark internal particles probably lack the core or RNA.

in cytoplasm (Fig. 3A). The unenveloped hexagonal particles with 59.8 nm diameter were presented in negative stained picture (Fig. 3B). The four structural components per facet edge of the viral particle was observed, suggesting that E1S virus capsid was made up of 92 capsomers. The complete hexagonal particles with electron dense in the center were also shown in Fig. 3B. Those may be the empty particles (Dobos *et al.*, 1977) without RNA or core component. These results resembled that reported in EVE (Nishimura *et al.*, 1981) and IPNV (Cohen *et al.*, 1973; Dobos *et al.*, 1977; Kelly and Loh, 1972) and might be due to artificial disruption during purification or capsomers self-assembling before virion maturation.

No inhibition by actinomycin D and FUDR

Actinomycin D is a polypeptide antibiotic which binds tightly to double helical DNA and inhibits all DNA dependent RNA synthesis (Reich *et al.*, 1961). FUDR irreversible inhibits thymidylate synthetase and thereby inhibits the replication of DNA (Salzman *et al.*, 1964). The results as shown in Table 2 indicated actinomycin D and FUDR did not inhibit the virus replication even at the concentration of 2 μg/ml and 100 μM, respectively, therefore, E1S was proved to be RNA type virus.

Neutralization by EVE antiserum

E1S, E1K (Eel no. 1, Kidney) and E4L (Eel no. 4, Liver) viruses were neutralized with 10^{-5} diluted anti-EVE rabbit serum, and no CPE was shown on the infected RTG-2 cells. Obvious CPE was appeared on the EVE and E1S control groups (Table 3). This result revealed that the viruses isolated from spleen, kidney and liver of illed eels were closely related to EVE which was isolated from gill and kidney of illed eels in Japan.

Inactivation by UV irradiation

By using UV irradiation below 1,000 J$\cdot$m^{-2},

TABLE 2
The effect of metabolic inhibitors on the isolated virus in CHSE-214 cells

Virus	Treatment	Concentration	Titer $TCID_{50}$/ml
E1S	Actinomycin D	0.00 μg/ml	4.6×10^{8}
		0.01 μg/ml	3.2×10^{8}
		0.50 μg/ml	1.0×10^{8}
		2.00 μg/ml	3.2×10^{8}
E1S	FUDR	0 μM	3.2×10^{9}
		100 μM	3.2×10^{9}
IPN (VR-299 strain)	FUDR	0 μM	2.1×10^{9}
		100 μM	3.2×10^{9}

TABLE 3
The neutralization test of the isolated virus and EVE against anti-EVE serum in RTG-2 cells

Virus	Treatment	Cytopathic effect	Virus	Treatment	Cytopathic effect
E1S	Anti-EVE	−	E4L	Anti-EVE	−
	Control	+		Control	+
E1K	Anti-EVE	−	EVE	Anti-EVE	−
	Control	+		Control	+

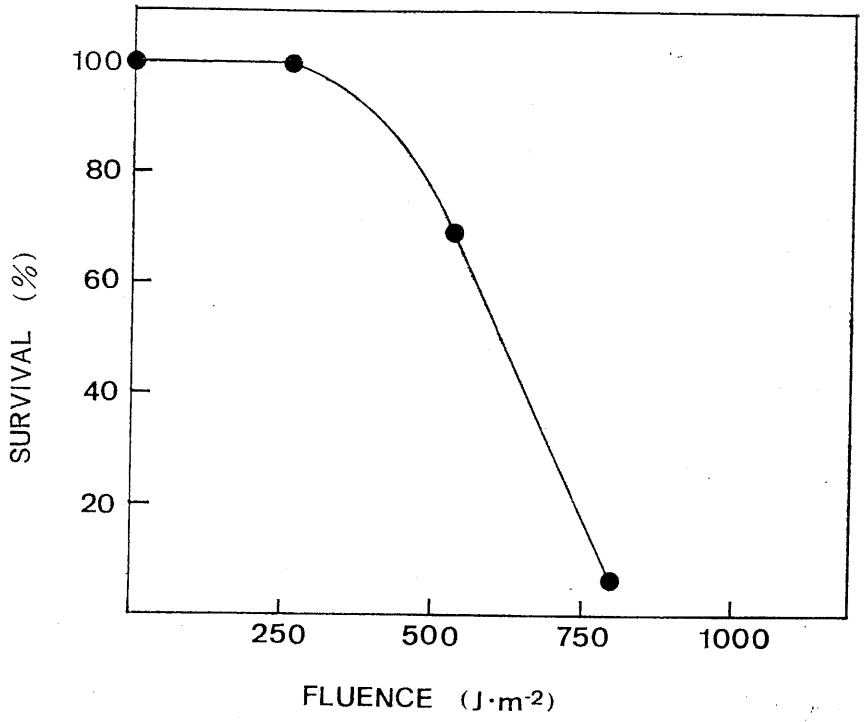

Fig. 4. The survival curve of E1S virus as a function of 254 nm UV fluence.

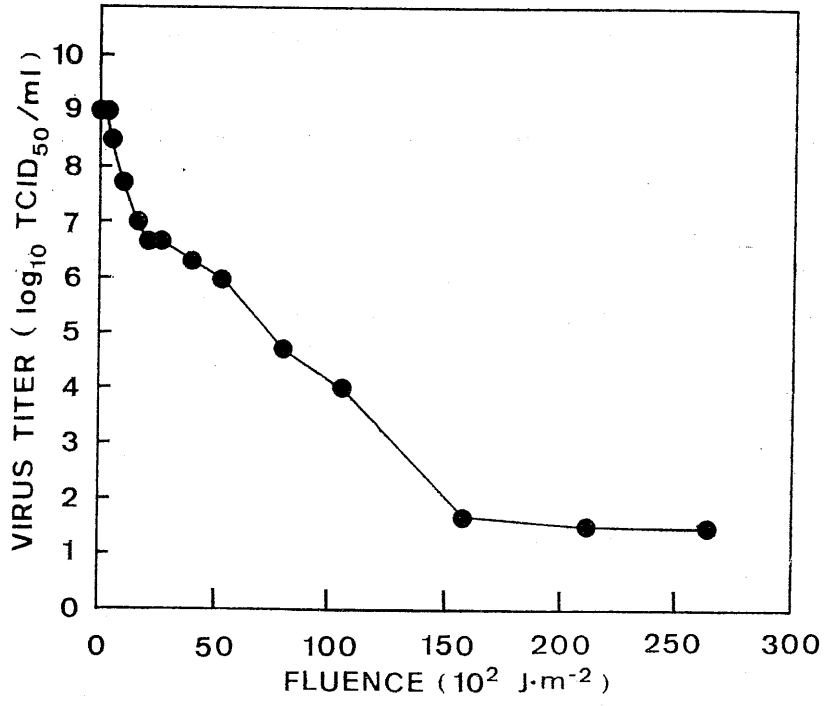

Fig. 5. The inactivation of E1S replication in CHSE-214 cells under UV irradiation.

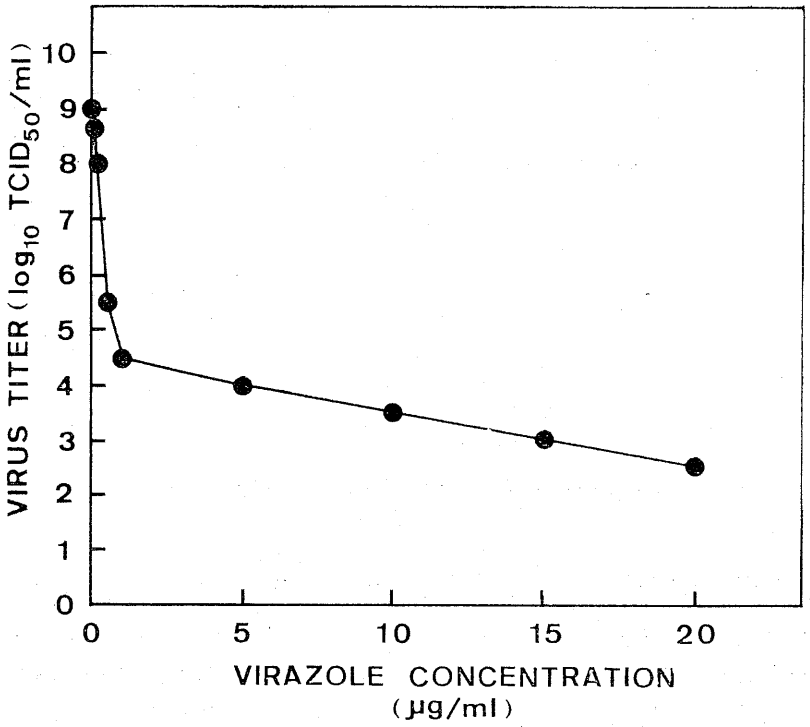

Fig. 6. The inactivation of E1S replication in CHSE-214 cells by virazole treatment.

a shoulder curve can be seen in the survival of E1S virus as a function of UV fluence (Fig. 4). When E1S was exposed to 2,000 J•m^{-2}, the infectivity of the virus abruptly decreased two log scale of $TCID_{50}$/ml (Fig. 5). As the UV fluence was risen to 15,000 J•m^{-2}, the virus titer declined to $10^{1.7}$ $TCID_{50}$/ml with less steep (Fig. 5). However, the UV fluence was risen from 15,000 to 26,000 J•m^{-2}, the virus titer remained at $10^{1.5}$ $TCID_{50}$/ml (Fig. 5).

Inactivation by virazole

The CHSE-214 cells showed slight cytotoxicity by virazole treatment at the concentration of 20 μg/ml. No any cyto-effect when the virazole concentration lower than 20 μg/ml. However 7 days incubation at 40 μg/ml of virazole treatment, the cells were disrupted. Therefore, less than 20 μg/ml virazole concentrations were used in the inhibition of virazole to the E1S replication in CHSE-214 cells. By using 0.1 μg/ml virazole, the virus infectivity decreased one log scale of $TCID_{50}$/ml. Under 1.0 μg/ml virazole treatment, the virus titer declined rapidly of about 4.5 log scale of $TCID_{50}$/ml (Fig. 6. While higher concentrations were treated, a slight decline of virus infectivity was observed (Fig. 6).

DISCUSSION

We found that the isolated E1S virus can multiply very well in salmonid fish cell lines such as CHSE-214 and RTG-2, less well in EPC and FHM cells with very low titer and has no multiplication in CF and LF cells (Table 1). But from the primary isolation of E1S virus, no CPE was observed in FHM cells. IPNV Ab strain also failed to form plaques on FHM cells (Macdonald and Gower, 1981), especially at the virus attachment level (Darragh and Macdonald, 1982).

E1S has the icosahedral in structure and the size ranged from 49 nm to 67 nm with an average diameter of 59.8 nm (Fig. 3B).

Thus, the shape and size of E1S are very

similar to IPNV. Both the E1S and IPNV virions have a buoyant density of 1.33 g/ml (Fig. 2). As shown in the metabolic inhibitor tests, actinomycin D and FUDR can not inhibit the virus replication (Table 2), which indicated that E1S is an RNA virus. In the meantime, E1S confines its replication and morphogenesis process in cytoplasm (Fig. 3A). To draw a preliminary conclusion from the above data, E1S is similar to IPNV or IPN like virus.

Since E1S was isolated from eel, a neutralization test was conducted with anti-EVE serum. The results as shown in Table 3, the anti-EVE rabbit serum could inactivate E1S infectivity which indicated that E1S was serologically related to EVE, which was isolated from gill and kidney of illed eels in Japan. However, there are some differences between EVE and E1S, especially in their particle size and temperature stability. Under electron microscope observation, the average diameter of E1S and EVE were 59.8 nm and 65.5 nm, respectively. E1S decreased two log $TCID_{50}$/ml after one week at −20°C and −70°C, and four log $TCID_{50}$/ml infectivity after 5 months at −70°C. However, no infectivity decreased in EVE after 30 and 32 month at −20°C and −80°C, respectively (Sano *et al.*, 1981). Much smaller in size and unstable at low temperature of E1S, both revealed the differences between E1S and EVE. Cross neutralization studies by Okamoto *et al.* (1983) indicated that European eel virus (EVE) was antigenically related to the Ab strain of IPNV ($1/r=1.8$). Whether E1S is more close to Ab strain or EVE. The relationship between these 3 viruses needed further biochemical studies. Occasionally, disrupted particles and empty particles were discovered in the purified E1S virus. Nishimura *et al.* (1981) reported that more disrupted and empty particles were discovered by the same purified procedures of EVE rather than IPNV VR-299 strain, and suggested a major difference in virus stability between EVE and IPNV.

Nicholson and Dun (1974) reported that IPNV interfered by homologous viral interference and decreased its infectivity after successive passages of partially purified virus at high input multiplicities. E1S also showed this decreased titer from 10^8–10^9 $TCID_{50}$/ml to 10^5–10^6 $TCID_{50}$/ml after several passages at high viral input multiplicities. This phenomenon can be recovered by the serially diluted infection.

After the exposure of short wave UV light below 300 nm, organism produced cyclobutyl dipyrimidies, pyrimidine adducts, spore photoproducts, pyrimidine hydrates and nucleic acid-protein cross-reaction photoproducts, these factors caused the organism unable to replicate (Harm, W., 1980). We tried to apply UV light for virus sterilization. According to the target theory, the survival curve of E1S virus under UV irradiation below 1,000 $J \cdot m^{-2}$, displayed minor shoulder (Fig. 4), this case meant one more hit needed to inhibit organism survive. This also conjectured that E1S virus probably possessed double stranded genome (Harm, W. 1980), and perhaps possessed repair system. Under 15,000 $J \cdot m^{-2}$ UV irradiation, E1S virus titer decreased from $10^{8.7}$ $TCID_{50}$/ml to $10^{1.7}$ $TCID_{50}$/ml, higher intensity of UV light treatment such as 26,000 $J \cdot m^{-2}$, the virus titer of $10^{1.5}$ $TCID_{50}$/ml still remained. Thereby, it is dangerous only to treat with UV irradiation for E1S virus sterilization.

Virazole (1-β-D ribofuranosyl-1, 2, 4-triazole-3-carboxamide) is a synthetic nucleoside which inhibits cellular nucleic acid synthesis, and also to a lesser degree of cellular protein synthesis. It blocked the guanosine monophosphate synthesis pathway at a step which involved the conversion of inosine monophosphate to xanthosine monophosphate (Streeter *et al.*, 1973). So far, at least 16 DNA viruses were inhibited by virazole, including herpes simplex virus, vaccinia virus, vesicular stomatities virus and influenza virus (Katz *et al.*, 1976; Sidwell *et al.*, 1972; Streeter *et al.*, 1973). At a concentration of 10 μg/ml, virazole decreased $10^{3.5}$ $TCID_{50}$/ml infectivity of IPNV

on CHSE-214 cells and RTG-2 cells (Migus and Dobos, 1980). In this paper, we found that virazole was more efficient to control E1S virus replication on CHSE-214 cells. Only at 1 μg/ml concentration, virazole could cause the decrease of E1S virus infectivity from 10^9 $TCID_{50}$/ml to $10^{4.5}$ $TCID_{50}$/ml. We suggested that virazole could be used as a powerful remedial drug to control E1S virus epizootics in culture fish.

Acknowledgements: This work was supported by the National Science Council, Republic of China under a cooperative program between NSC and NSF of U. S.

We wish to thank Dr. B. L. Nicholson for providing cells and viruses and technical assistance; Dr. R. P. Hedrick for providing anti-EVE serum; Dr. S. N. Chen for providing cells; Dr. Chu-Fang Lo and Dr. Kuo-Chum Liu for performing the electron microscopy.

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日本鰻所分離出感染性胰臟潰瘍類病毒之特性

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從患病之日本鰻（*Anguilla japonica*）脾臟所分離出之病毒命名爲 E1S。在數種細胞株中試驗此病毒感染力，以在 CHSE-214 細胞中表現最好之增殖能力。在電子顯微鏡觀察下，此病毒呈不具外套膜之二十面體，位於 CHSE-214 細胞質中鄰近內質網處，具有單層蛋白質外鞘之病毒顆粒，直徑爲59.8 nm。經由代謝抑制試驗，E1S 鑑定爲核醣核酸型病毒。而其在氯化銫中之浮力密度爲 1.33 g/ml，中和試驗結果顯示 E1S 病毒和 EVE 病毒密切相關。由生化、血清和電子顯微鏡的研究顯示 EIS 是感染性胰臟潰瘍類病毒，屬於兩段核醣核酸病毒。

病毒增殖可被紫外線和 virazole 抑制，在低能量紫外線照射下，E1S 病毒的殘存曲線呈肩形，而在 15,000 $J \cdot m^{-2}$ 强度紫外線照射下，病毒感染力下降 10^7 $TCID_{50}$/ml；當處以 1 μg/ml virazole 時，降低 $10^{4.5}$ $TCID_{50}$/ml 病毒感染力。