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Effect of Temperature on Survival and Growth of Infectious Pancreatic Necrosis Virus[1]

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Infectious pancreatic necrosis virus was stable for 10 days at 4 C in stream and well water, after which the virus had a half-life of 7.5 days. At 15 C, the virus was stable for 5 days, and then had a half-life between 5 and 6 days. Viral antigen in infected cells developed much more slowly at 4 C than at 20 C. Infected cells released infectious viral particles at temperatures as low as 4 C. Nutrition had a greater effect on the production of infectious virus at 4 C than at 20 C.

Achieving control of viral diseases of fish requires a knowledge of the stability and growth of viruses at various temperatures. A recently described immunofluorescent cell (IFC) assay procedure (4) was used to study the survival of infectious pancreatic necrosis (IPN) virus in fresh water and to determine the effects of temperature and nutrition on the production of viral antigen and infectious IPN virus. The results obtained are reported in this communication.

(This report is based in part on the dissertation submitted by Kuen-Ching Tu in partial fulfillment of the Ph.D. degree at Utah State University.)

Viruses, cells, and media. The ATCC-VR 299 and the Kamas Brook strains of IPN virus were grown in rainbow trout gonad (RTG-2) cells in autoclavable, Eagle minimal essential medium containing 10% fetal calf serum (FCS) as described previously (4).

Immunofluorescent cell assay. Confluent RTG-2 cell monolayers grown on circular cover slips 15 mm in diameter were infected with 0.02 ml of inocula of IPN virus (4). After 1 to 1.5 h of adsorption at 20 C, medium containing Tricine buffer was added and the cultures were incubated an additional 8 to 10 h. The cells were then stained with antiviral fluorescent antibody and the fluorescing cells on each cover slip were counted. Maximum variation in the counts in this procedure is ±23%. The methods used in antibody labeling, staining, and viral assay by IFC counting have been described (4).

Survival rate of IPN viruses in fresh water. Two different sources of water were used in the study. Stream water was obtained from the first impoundment of the Logan River adjacent to the Water Research Laboratory, Utah State University. Well water was supplied by the Fisheries Experiment Station, Logan, Utah. Aliquots of the water samples were incubated at 15 or 4 C for 2 h before inoculation with ATCC-VR 299 or Kamas Brook IPN virus. At various postinoculation time intervals, water samples were assayed for infectious IPN virus by the IFC counting technique.

Effects of nutrition and temperature on viral growth. Cover-slip cultures of RTG-2 cells were grown in Eagle medium, supplemented with 2, 5, 10, or 20% FCS. Each culture, after being infected with ATCC-VR 299 IPN virus, received medium containing the concentration of serum in which they were initially grown. After 12 h of incubation at 20 C or 10 days at 4 C, the cultures were stained with antiviral fluorescent antibody and the infected cells were counted. Other sets of cells in this experiment were cultured on cover slips in Eagle medium containing 10% FCS. After being infected, the cell sheets were washed three times with 5 ml of medium without serum. Five duplicate sets of petri dishes, each containing three cover slips, received 5 ml of medium containing 2, 5, 10, 15, or 20% FCS, respectively. One set of cultures was incubated at 4 C for 10 days, whereas the other set was incubated at 20 C for 12 h. The number of cells stainable by fluorescent antibody was determined.

In another experiment, 4-day-old RTG-2 cover-slip cultures grown in Eagle medium supplemented with 10% FCS were infected with

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ATCC-VR 299 IPN virus. After 1 h of adsorption at 20 C, the cell sheets were washed five times with cold medium without serum. Then the infected cultures were divided into five groups; each group contained 10 petri dishes with three cover-slip cultures per dish. Five milliliters of cold medium containing 2, 5, 10, 15, or 20% FCS was added to each of the five groups of cover-slip cultures, respectively. The infected cultures were then incubated at 4 C. At daily intervals beginning 3 days after infection, cultures were examined for cytopathic effects (CPE). After microscopic examination, the medium was removed from the cultures and assayed by the IFC procedure for virus released from the cells. The cells were subsequently stained by antiviral fluorescent antibody, and the number of infected cells on each cover slip was counted.

Survival of IPN virus in water. The results of survival of Kamas Brook virus in stream water are presented in Fig. 1. The data obtained with well water were almost identical to those shown in Fig. 1 for stream water. The Kamas Brook IPN virus was stable in stream and well water for about 10 days at 4 C. From 10 to 45 days after inoculation, the loss of infectivity was exponential; the half-life of the virus was about 7.5 days. No significant change occurred in the infectivity of the virus in stream and in well water during the first 5 days at 15 C, after the half-life of the virus was between 5 and 6 days. The response of the ATCC-VR 299 strain of IPN virus was similar to that of the Kamas Brook isolate.

Effect of cell nutrition and temperature on the growth of virus. The concentration of serum present in infected cultures incubated at 20 C had little effect on the number of infected cells observed after 12 h of infection (Table 1). Cultures incubated 12 h at 20 C produced from 3- to 10-fold more stainable cells than did cultures incubated for 10 days at 4 C in the presence of the same serum concentration.

The concentration of serum did affect the production of antigen and virus in cells incubated at 4 C (Table 2). Fewer cells contained

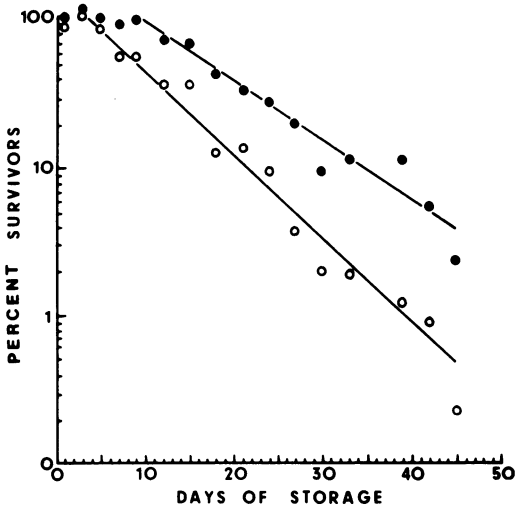

FIG. 1. *Survival of IPN (Kamas Brook) virus in stream water. Symbols: Water stored at 4 C (●); water stored at 15 C (○).*

TABLE 1. *Effect of cell nutrition and incubation temperature on the development of IPN (ATCC-VR 299) viral antigen*

Cell nutrition	Infectivity (ICC U/ml)[a]			
	20 C[b]		4 C[b]	
	A[c]	B[d]	A	B
Eagle medium	6.2×10^7	ND	6.9×10^6	ND
Eagle medium + 2% FCS	6.8×10^7	5.4×10^7	1.3×10^7	6.1×10^6
Eagle medium + 5% FCS	6.3×10^7	6.2×10^7	2.0×10^7	1.3×10^7
Eagle medium + 10% FCS	7.9×10^7	7.6×10^7	2.5×10^7	2.2×10^7
Eagle medium + 15% FCS	7.5×10^7	7.8×10^7	2.6×10^7	2.1×10^7
Eagle medium + 20% FCS	7.8×10^7	7.9×10^7	2.4×10^7	2.0×10^7

[a] ICC units: immunofluorescent cell counting units. ND, Not done (cell sheets came off of cover slips). FCS, Fetal calf serum.

[b] The number of cells producing IPN viral antigen in cultures incubated at 4 and 20 C was determined at 10 days and 12 h postinfection, respectively.

[c] A, Cells grown in Eagle medium + 10% FCS before infection; after 1 h of adsorption, the inoculated virus was removed by washing and the cultures were maintained in a medium containing FCS as indicated.

[d] B, Cells grown in the same type of medium before and after infection.

TABLE 2. *Effect of serum concentration on the development of IPN (ATCC-VR 299) viral antigen, cytopathic effects, and infectious virus in RTG-2 cells incubated at 4 C*[a]

Incubation time (day)	2% Serum			5% Serum			10% Serum			15% Serum			20% Serum		
	FC	CPE	IV[b]	FC	CPE	IV	FC	CPE	IV	FC	CPE	IV	FC	CPE	IV
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	6	—	—	4	—	—	19	—	—	12	—	—	4	—	—
5	30	—	—	46	—	—	60	—	—	48	—	—	22	—	—
6	50	—	—	195	—	—	277	—	—	227	—	—	156	—	—
7	67	—	—	218	—	—	387	—	—	356	—	—	189	—	—
8	78	—	—	260	—	—	418	—	—	401	—	—	285	—	—
9	88	—	—	269	—	—	420	—	—	289	—	—	351	—	—
10	122	—	—	248	—	—	438	—	D	413	—	D	381	—	D
11	130	—	—	250	—	D	AD	—	D	AD	—	D	399	—	D
12	102	—	D	AD	±	D	AD	±	D	AD	±	D	AD	±	D

[a] 2% serum: Eagle medium + 2% serum; FC: fluorescent cells; CPE, cytopathic effect; D, detectable; AD, viral antigen diffused over the cell sheet; —, negative result.

[b] IV, Infectious virus. The IV was released into the medium from the infected cells and was detected by inoculating the medium onto other RTG-2 cover-slip cultures.

viral antigen, and it required more time for infectious virus to be detectable when the cells were incubated after infection in a medium containing 2% serum.

Infectious pancreatic necrosis virus is the etiological agent of an acute, contagious disease that causes high mortality in salmonid fingerlings (8). Consequently, it is important to define its stability under various environmental conditions. Previous reports have shown that IPN virus is stable in sea water for at least 11 days but gradually loses infectivity in media of lower salinity (3). It remains infective in 50% glycerol for as long as 5 years at 4 C (5) and can survive in tissue culture media containing 2.5% FCS for 6 weeks at 4 C without significant loss of infectivity (1). Survival at 4 C is better if skim milk, lactalbumin hydrolysate, or lactose is added to infectious culture fluids (7).

The data in this report show that IPN virus, suspended in fresh stream or well water, retained almost all of its infectivity for about 10 days at 4 C and 5 days at 15 C. The long survival time for IPN virus in fresh water is not surprising. In the evolution of fish viruses, mutants capable of long survival in water would be most apt to survive.

The stability of IPN virus in fresh water has practical significance for hatchery managers. Good water flow must obviously be maintained to minimize the accumulation of virus. Further, its stability means that attempts to eliminate the IPN virus from a hatchery by removing the fish may fail unless the removal is followed by disinfection.

Precautions should also be taken in natural waters. Carrier fish should not be planted in streams or lakes without careful consideration of the possible spread of the disease to other fish. This is especially important in waters where fish spawn.

Malsberger and Cerini (2) reported that no IPN virus multiplication was detected at 4 C during 16-day incubation of infected RTG-2 cells in Eagle medium containing 5% FCS. They concluded that this was due to inhibition of a step in the replicative cycle of the virus subsequent to adsorption and penetration. This conslusion was based on the fact that the infected cells incubated at 4 C showed no CPE until they were transferred to a higher temperature. Wolf (6) suggested that the failure of virus to multiply at 4 C was due to the cells' nutritional deficiency. The results presented in Table 1 show that although the rate was slower at 4 C, IPN virus did adsorb, penetrate into RTG-2 cells, and initiate antigen production. The time of antigen appearance in cells maintained at 4 C did not vary among media containing different serum concentrations. However, the serum did have some effect on the amount of antigen developed in infected cultures, and on the time of release of infectious virus. Malsberger and Cerini may not have observed IPN virus multiplication at 4 C because of variations among IPN isolates. Another possibility is that the virus did multiply inside the cells in their experiments, but did not destroy enough cells to give a definite CPE.

The ability of IPN virus to multiply at 4 C provides a very useful tool for studying steps of macromolecule synthesis at low temperatures.

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