THE ROLE OF SEDIMENT IN TRANSMISSION OF CHANNEL CATFISH VIRUS DISEASE

Yolanda Juanita Brady

and

R. D. Ellender

University of Southern Mississippi
Hattiesburg, Mississippi

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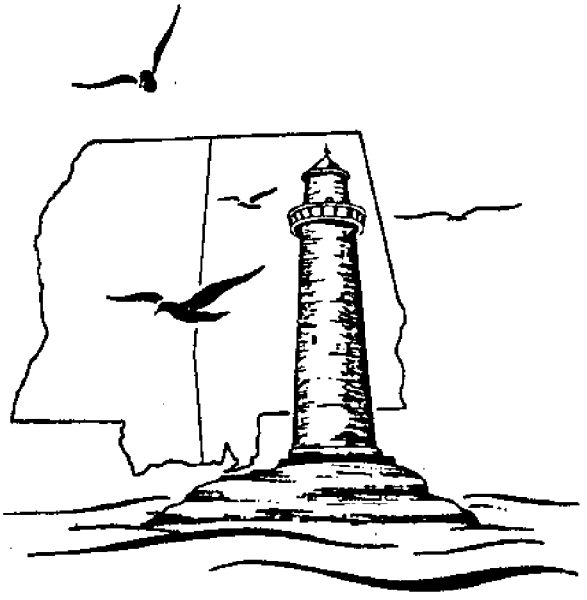

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CHAPTER I

INTRODUCTION

The channel catfish is an economically important species of cultured fish in the southern United States. Commercial production of channel catfish now exceeds the total production of all other cultured fish species in the United States. The channel catfish, Ictalurus punctatus (Rafinesque), has been experimentally cultured for more than 50 years, but commercial culture dates back to 1963 (1). With the increase in production there has also been increased losses due to mismanagement and disease. Channel catfish virus (CCV), a herpesvirus infecting young ictalurid fish, is responsible for approximately 95% of the observed mortality. The disease is prevalent in the southern states primarily during the summer months. This virus is readily transmitted to healthy fry and fingerlings when hatcheries are crowded. When channel catfish virus attacks this species, high fingerling mortality occurs and often leads to severe economic loss. Viral transmission usually occurs by contaminated tissue or water but in this study, the importance of sediment-associated virus was considered in the spread of disease. Virological investigations of

sediments are of current interest but the main thrust of that research has analyzed enteroviruses in sewage-polluted estuarine sediments. Little is currently known of the fate of fish viruses in freshwater or estuarine sediments.

The purpose of this investigation was to determine the possible role of sediment in the transmission of CCV disease. Studies included the effect of various physical and biological factors on virus survival as well as methods to elute sediment-bound particles.

The principal objective of the methods to be described was to assess the potential for transmission of CCV by pond sediments. To accomplish this objective, it was necessary to examine certain physical and chemical factors which are thought to influence the association between virus and sediment particles and to find suitable eluents that effectively demonstrate the presence of sediment-bound virus. This investigation is significant because it represents an initial study of fish virus transmission by sediments.

CHAPTER II

REVIEW OF LITERATURE

A. Introduction

Transmission of fish viruses can occur by either horizontal or vertical transmission. The best understood horizontal routes include the skin, gill tissues, and intestinal tract; minor routes are the eye and urogenital tract. Vertical transmission occurs when virus is genetically transferred from one generation to the next.

Transmission depends on the physical properties of the virus, the ability of the virus to survive environmental factors and the resistance mechanisms of the host which influence viral disease. For example, infectious pancreatic necrosis virus (IPN) is probably transmitted orally since the virus is highly resistant to digestive acids (24). Some viruses such as lymphocystis may be transmitted through abrasion of the skin (24), whereas channel catfish virus (CCV) can be experimentally transmitted via infected water, through gill swabs, or through injection (24). It has been stated that CCV may be transmitted by feeding (34); however that mode seems unlikely since CCV is sensitive to acidic conditions (27). The exact portal of entry for CCV as well as other fish viruses is not totally understood. Vertical transmission is suspected in the case of CCV but has yet to

be proven (30).

Since transmission of fish viruses is not well understood, it is important to control the various factors which could contribute to virus infection. The best control method is to avoid and minimize stressful conditions such as low oxygen tension, crowding, or excessive handling. Rearing fish in conditions which exclude the presence of virus is virtually impossible since the source of most growing waters include surface run-off or rivers, all of which contain unknown disease potential. Little is known about viral distribution in feral fish populations.

A good practice is to insure that incoming eggs and adult fish are virus-free. Viruses that are transmitted vertically may adhere to the surface of eggs as in the case of viral haemorrhagic septicaemia (VHS). This virus is lost after several days incubation in running water (24), but temperature is more important in the control of CCV (40) and infectious hematopoietic necrosis (IHN) (24). Plumb reported that 19°C was effective in reducing fingerling mortality (40). In a similar case, elevating water temperature above 15°C hindered the spread of IHN, but when the temperature was lowered, disease remained (24).

Possible control mechanisms include vaccination by injection, incorporation of the product in food or holding waters, or by the addition of agents that inhibit viral replication, and genetic selection of resistant strains

of fish. Vaccines may not be economically appropriate with channel catfish in which repetitive booster injections must be administered (15, 29). Genetic manipulation is being considered as in the case of a hybrid strain of channel catfish which is refractive to CCV under natural conditions (24).

Little can be done after fish have been infected with virus. Before restocking, the pond must be drained and treated with chlorine (56) to destroy any remaining virus in the sediment, or the pond bottom may simply be dried. Another common practice is to drain the pond, allow it to become anaerobic, then mix lime into the pond sediments. The fate of CCV in these types of environments is not known; however, if the pond is improperly treated, the disease potential remains.

B. Channel Catfish Virus Disease (CCVD)

Channel catfish virus is the only known virus that is pathogenic to ictalurid fish (50, 53, 56). An epizootic may result in losses as high as 95% among fry and fingerlings (28, 35, 49, 56, 64). Environmental stress, such as low dissolved oxygen levels, influence the rate of mortality and may trigger epizootics (42). Age, size of fish, temperature, crowding, and improper handling also contribute to epizootics (40). The virus has not been isolated from adult fish (38, 39, 40).

Fijan (10) first observed extensive mortality of

channel catfish (Ictalurus punctatus) which occurred about one week after fish were stressed by low oxygen concentration. The cause of mortality was determined to be of virus origin since bacteria-free filtrates injected into experimental fish produced the same symptoms as those observed in infected fish. Flexibacter (syn=Chondrococcus) columnaris was also isolated but was characterized as a secondary infection. The disease was first described in 1969 by Wellborn, Fijan, and Naftel (58). In two fish kills, the cause of death was again thought to be of bacterial origin; however treatment with antibiotics did not reduce mortality. Symptoms of the disease (11, 30, 31, 58, 61) were as follows: a) fish swam abnormally, rotating about the long axis; b) swimming pattern may be convulsive, after which the fish drop to the bottom; c) before death the affected fish hang vertically at the water surface; d) hemorrhagic areas appear on the fins and abdomen; e) the abdomen becomes distended due to fluid accumulation; f) the gills become pale or hemorrhagic; g) hemorrhagic areas appear in the musculature, liver, kidneys, and spleen; and h) the stomach becomes distended with yellowish mucoid secretion. Positive identification can only be made through cell culture (34).

The virus will traverse a filter having a 0.2μ , but not a 0.1μ porosity, and is heat labile (60°C, 1 hour) (58). The virus multiplies and produces cytopathic effect (CPE) in cultures of brown bullhead (BB) (ATCC CCL-59), channel

catfish ovary (5, 6, 7), and primary catfish cells (63, 66, 67). The virus induces giant cell formation typical of herpesvirus infections and will not hemagglutinate red blood cells (62). Under experimental conditions it is possible to infect healthy fingerlings within 48 hours by placing them in water with diseased fish, by swabbing the gills of susceptable fish with virus, by intraperitoneal or intramuscular injection, or by feeding infected food (35). It is suspected that the virus is vertically transmitted from broodstock to offspring (35, 36). It has also been observed that healthy fish seem to be more susceptible to CCV than fish in poor condition (39).

Replication of CCV in the BB cell line occurs from 10° to 33°C, with an optimum temperature of 25 to 33°C. Cell nuclei became basophilic during the first hour of infection and by the second hour, margination of chromatin is observed and the beginning of syncytium formation. Intranuclear inclusions are evident in some cells by the third hour and later in most nuclei. These inclusions differ somewhat from the Cowdry type A inclusions in that they are granular and irregular in shape. Syncytia formation continues and by the sixth hour the disintegration of nuclei and basophilic condensation begins. At the eighth hour nuclear disintegration is common. By the tenth hour cytoplasmic portions of syncytia undergo fragmentation followed by continuation of nuclear disintegration (62, 66, 67).

Three types of nuclear particles have been observed by electron microscopy including single-membrane particles, double-membrane particles, and single-membrane particles with electron dense cores. Virus particles outside the cell are usually enveloped and contain cores. The enveloped particle is approximately 175 to 200 nm in diameter and is composed of 162 capsomeres (24, 41, 46, 62, 66). Envelopment occurs at the inner lamellar of the nuclear envelope and by budding into nuclear vacuoles.

Based on the evidence of Wolf and Darlington (65), the virus is classified as a herpesvirus and is inactivated by treatment with 20% ether for 24 h at 4°C or treatment with 5% chloroform for 5 min at room temperature. In other studies, the virus was found to be glycerol labile (40) and inactivated by sodium hypochlorite and acidic conditions (27).

The density of CCV DNA as determined by a cesium chloride gradient is 1.715 gm/ml (27, 62) which corresponds to a base composition of 56.1% G + C, molecular weight = 8.5×10^7 daltons (27, 50). Additional studies involving the effect of temperature, pH, salinity, and ultraviolet irridiation on CCV have been performed (52).

Plumb (35, 37) determined that the extent of mortality depended on the condition, size, and age of the fish as well as environmental factors and stress. Tissue distribution of virus has been determined and the kidney

appeared to be the primary target organ; the gastrointestinal tract, liver, and skeletal muscle also contained virus. No virus was detected in the brain until 96 h post-inoculation which correlated with the pattern of erratic movement observed four days post-infection (35).

Histopathologic studies have been performed on experimentally infected fish (26, 46, 47, 66). Infection resulted in systemic edema and necrosis of kidney, liver, digestive tract (66), and spleenic tissues (46). Intranuclear inclusion bodies (crystalline arrays) and lamellar structures were associated with virus replication and are thought to produce hemorrhage, necrosis, and tissue edema.

The biological aspects of channel catfish virus disease have been discussed by Plumb (37). McGlamery and Gratzek (30) reported that channel catfish which survived exposure to CCV did not grow to normal size. There was no alteration of tissue and all attempts to isolate the virus failed. It is suspected that survivors shed virus under stress but a study of this type has never been performed, since survivors of CCV epizootics are usually destroyed to prevent the possibility of further transmission (30).

A study of the immune response of channel catfish was conducted by McGlamery, Dawe, and Gratzek (29). They determined that significant antibody response occurred in those catfish immunized with either bovine serum

albumin or vesicular stomatitis virus in Freund's complete adjuvant (29, 62). Heartwell (15) found that specific channel catfish immunoglobulins were macromolecules which had characteristics similar to those of human IgM and most fish immunoglobulins. Antisera to CCV has been prepared in channel catfish, but host response declines unless repetitive booster innoculations are given (15).

Biochemical studies have been conducted which involve purification of CCV (50) and the overall arrangement of nucleotide sequence in CCV DNA (8, 51).

Several investigators recently attempted to improve methods of study of channel catfish virus by the use of a new cell line (5, 6, 7, 43). Channel catfish ovary appears to be more sensitive to CCV replication than BB cells and was preferred since BB cells supported the replication of CCV, but the fish itself (Ictalurus nebulosus [LeSueur]) did not (7).

Studies involving different strains of channel catfish indicate significant differences in mortality ranging from 10% to 71% with hybrid strains exhibiting the lowest mortality (47). One study has determined that the blue catfish (I. furcatus [LeSueur]) and a hybrid strain of channel catfish and blue catfish could only be infected by injection and that other means of infection similar to those in natural conditions did not transmit disease (44).

C. Sediment Virology

The viral protein coat has the potential to be electrostatically charged which allows individual viral particles to adhere to natural surfaces. Such interactions have a strong influence on the viability of viruses in the environment (3, 19, 54, 55). Animal viruses are incapable of multiplying in such environments as water or soil and are easily destroyed by natural phenomenon. Nevertheless, certain factors such as surface adsorption tend to retard virus inactivation. Sediment is known to bind virus, to protect it from destruction, and to act as a reservoir for disease transmission (3).

The majority of research in the area of sediment virology has been conducted with enteroviruses since they represent a human health hazard (57). Enteroviruses can be transmitted by direct or indirect contact and are thought to cause common infections in children and adults.

Survival of human virus depends on chemical composition, pH, and temperature of the surrounding environment. It has been determined that enteroviruses survive longer in freshwater than in seawater unless sediment is present and it is recognized that waterborne enteroviruses present a definite health risk (19, 55).

In earlier studies on bacterial interaction in sediment, it was found that Escherichia coli survived longer in natural seawater when sediments were present

(9, 12, 33). In polluted waters it has been demonstrated that viral particles also bind to particulates and that wave motions and bottom currents in shallow areas can aid the release of virus from sediment under appropriate natural conditions (9, 19). Sediment protection could result from physical entrapment in surface openings or by stabilization of the virion by electrostatic forces (55). Sediment also provides protection from thermo-inactivation (23). The virus-sediment complex (9, 13) which exists in nature can dissociate and indicates that reversible binding does occur between virus and sediment particles (21, 60).

Several elution techniques have been formulated to meet the need of various environmental situations and sediment types (2, 3, 13, 14, 18, 20, 33, 59, 60). For example, Gerba et al. (18) found glycine and EDTA efficient for elution of enteroviruses from estuarine sediments. Beef extract was an effective eluent (22) for virus bound to wastewater sludge. Other studies demonstrated that deionized water caused the desorption of virus from soil (20) and nutrient broth released virus from membrane filter material (16). Landry et al. (22) demonstrated that organic eluents desorb virus more efficiently than inorganic eluents.

CHAPTER III

MATERIALS AND METHODS

A. Laboratory Procedures and Equipment

Aseptic techniques were used throughout this study. Media and reagents were prepared with double distilled water and sterilized either by an autoclave (121°C, 15 psi, 15 min) or filter sterilization (0.45μ filter, Millipore Co.). Aseptic conditions were maintained for cell cultivation, media preparation, and virus titrations with the aid of a verticle flow, laminar air hood (Bio-Gard Hood, Baker Co., Inc.).

All glassware exposed to virus was placed in a concentrated Lysol solution before sterilization. Glassware, including pipets, was then soaked overnight in a solution of 7 X liquid detergent (Linbro Division, Flow Laboratories), washed in tap and distilled water, and air dried. Glassware was wrapped in aluminum foil before sterilization in a hot air oven (Blue M Electric Co.) for 4 to 5 h at 165°C. Pipets were plugged with cotton and sterilized in metal canisters.

B. Cell Cultures

The brown bullhead (BB) cell line (ATCC No. CCL-59) was used in this investigation; it is a continuous line of

mixed epithelial fibroblast-like cells derived from caudal trunk tissues of the brown bullhead, Ictalurus nebulosus. The medium for growth was Leibovitz L-15 (Appendix A) (67) prefiltered and aseptically sterilized using a 0.45μ (Millipore) membrane. Stock cultures were maintained in 150-cm^2 disposable flasks (Corning) incubated at 28°C (Forma Scientific Model 20 Incubator). For subculture, cells were removed from the surface of confluent flasks by three washings of single strength (1X) ATV (Appendix A) (25). Flasks were held at room temperature until the cells detatched; fresh growth media were added (subcultivation) ratio of 1:5) and the suspension was used to prepare other stock and working cultures. Cells between passages 142-176 were used in all experiments.

C. Virus

Pools of channel catfish virus (CCV) (ATCC No VR-665) were prepared by inoculating 0.1 ml of stock CCV into confluent BB cultures. The virus was allowed to adsorb to the cells for 15 min at room temperature, followed by the addition of fresh growth media to the inoculated monolayer. The flask was incubated at 28°C until cytopathic effect (CPE) was evident and the entire cell sheet had detatched from the flask. The growth fluid containing virus was then centrifuged at 1000 x g to remove excess cell debris and 1 ml of the supernatant was aseptically dispensed into sterile 2-ml ampules (Vangard Inter.).

The ampules were stored at -70°C (Forma Bio-Freezer, Forma Scientific) and individual ampules rapidly thawed in a 37°C water bath prior to use. Viral dilutions were made in a buffer of glycine-tris-sodium acetate (GTSA) at pH 7.0 (Appendix A).

D. Titration

Virus titrations were performed in Linbro plates (Flow Laboratories) using four replicates per dilution. Each well received 102,000 cells along with 0.1 ml of appropriate virus dilution. The plates were observed daily until there was no change in titer. The titer was calculated using the Reed-Muench equation for determination of 50% end points (48).

E. Sediment

Sediment for this study was obtained from a private catfish pond in Hattiesburg, Mississippi. Sediment was collected from the same 2-ft^2 area during the months of Jan 1980, Nov 1980, and July 1981. In experiments using dry sediment, the sediment was washed twice in distilled water, and all debris removed prior to drying overnight in a hot air oven (165°C) before sterilization. In experiments with wet sediment all debris was removed and the sample autoclaved.

F. Physical Factors

1. Effect of pH on virus infectivity. The

objective of this experiment was to determine the optimum pH values at which CCV is stable. This experiment consisted of three divisions: a) using pH values of 3.0, 5.0, 7.0, 9.0, and 11.0; b) using pH values of 3.0 3.5, 4.0, 4.5, 5.0 and 7.0 as the control; and c) using pH values of 9.0, 9.5, 10.0, 10.5, 11.0, and 7.0 as the control. All virus dilutions were made in sterile GTSA buffer (Appendix A) standardized to the desired pH with 1N NaOH or 1N HCl. The virus was added to the buffer solution at the appropriate pH and incubated for 1 h at room temperature before titration.

2. Effect of temperature on virus infectivity. In this portion of the study, the effect of temperature on virus survival was determined. A 0.1-ml aliquot of virus stock was added to tubes of sterile GTSA buffer at pH 7.0 and held at 4°C, room temperature (approximately 25°C), 37°C, 45°C, and 60°C for one hour. After the incubation period, the virus was titered as described in Section D (page 15).

3. Effect of NaCl concentration on virus infectivity. Brown bullhead cells were introduced into salt concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 3.0% in sterile GTSA buffer at pH 7.0 to determine their salt tolerence. The virus (0.1-ml stock) was also incubated for 1 h in each salt concentration and titered along with

a salt-free control. Titrations were also performed using 1M, 2M, 3M, and 4M salt concentrations.

4. Effect of agitation on virus. A 10^{-1} dilution of virus was made in sterile GTSA buffer and placed on a mechanical shaker for 1 h at 250 rpm to determine if agitation affected virus titer. The sample was filtered to maintain sterility and titered.

5. Virus adsorption to pond sediment. This study was performed to examine the relationships of pH to the adsorption of virus to sediment and to analyze variable sediment-constant virus experiments designed to determine the characteristics of virus-sediment mixtures. A 0.1-ml aliquot of virus was added to 9.9 ml of buffer containing varying amounts of sediment. The virus-sediment combinations were placed on a mechanical shaker (New Brunswick Scientific Co., Inc.) for 30 min. followed by centrifugation at 6000 x g (Sorvall Model RC2-3) for 20 min. The supernate was filtered through a 0.45μ filter (Gelman) and titered and the amount of remaining virus in the supernate compared to the original titer. This procedure is illustrated in Figure 1.

6. Virus elution from pond sediment. Several eluents were used (Table 1) in attempts to elute virus from sediment. The stability of virus in all eluent mixtures was tested and is described in Figure 2. Ten

FIGURE 1

Virus Adsorption Procedure

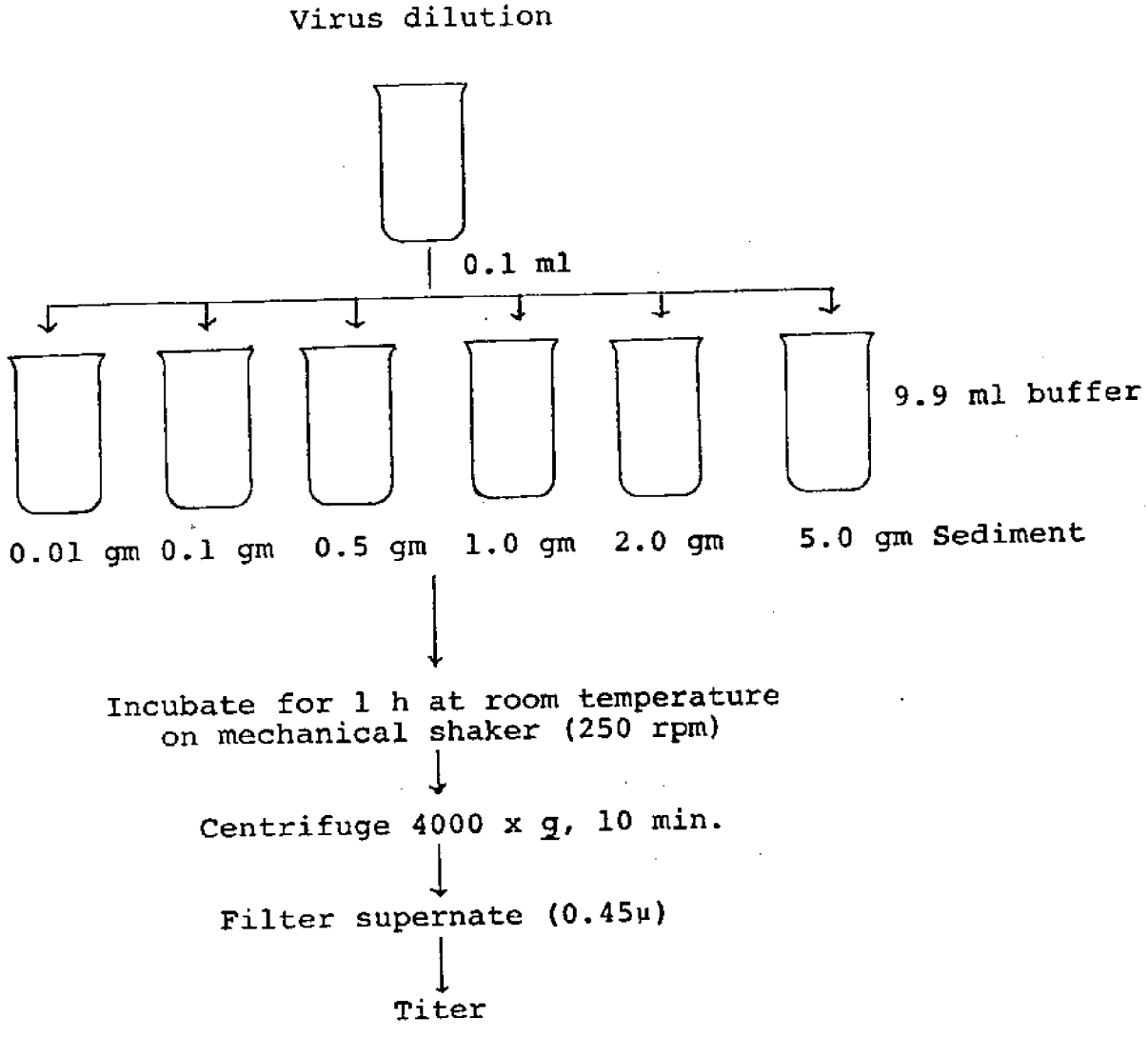


TABLE 1

List of Eluents

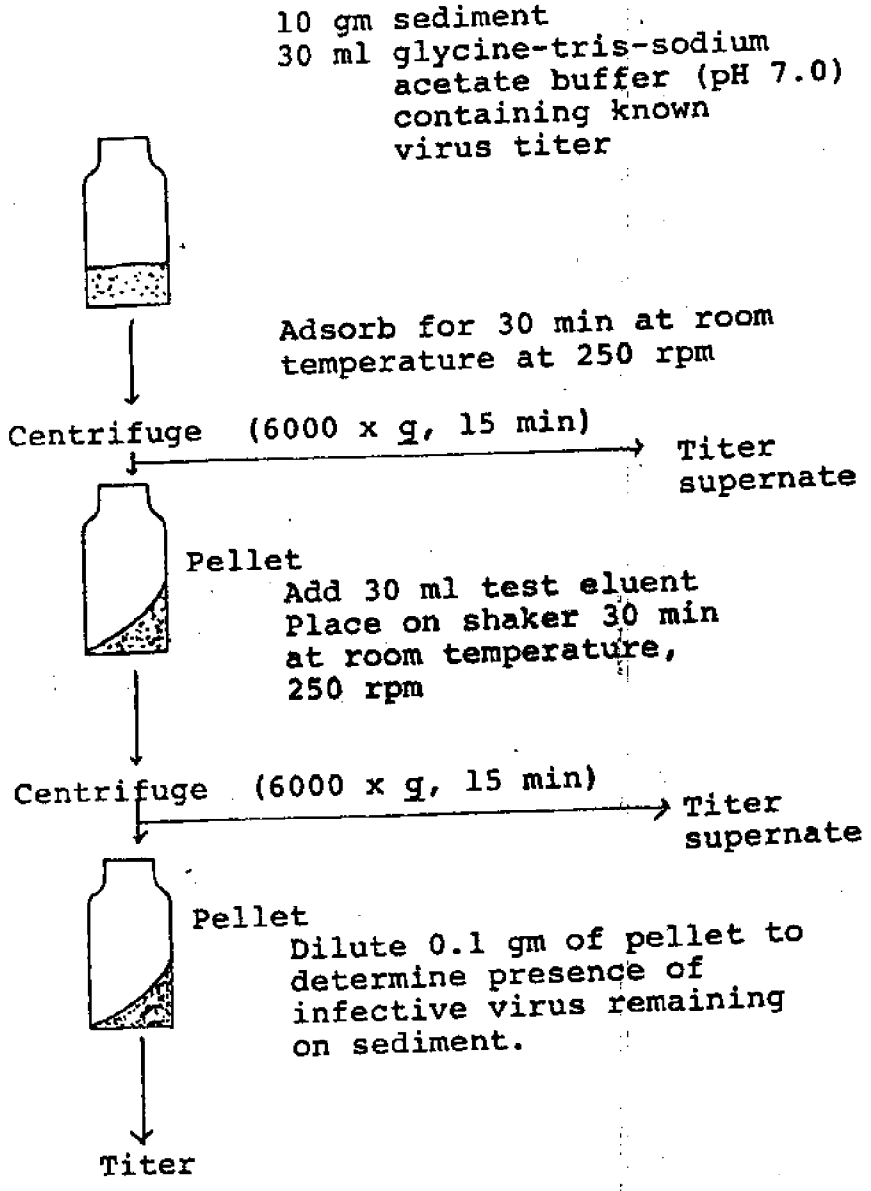
ELUENT	BUFFER	pH
4% Nutrient broth	Tris (0.2M)	7.5
4% Nutrient broth	Tris (0.2M)	9.0
4% Nutrient broth	Tris (0.2M)	10.5
4% Nutrient broth	PBS	7.5
4% Nutrient broth	PBS	9.0
3% Beef extract	Tris (0.2M)	7.5
3% Beef extract	Tris (0.2M)	9.0
3% Beef extract	PBS	7.5
3% Beef extract	PBS	9.0
15% Beef extract	Tris (0.2M)	7.5
15% Beef extract	Tris (0.2M)	9.0
15% Beef extract	PBS	7.5
15% Beef extract	PBS	9.0
4% Nutrient broth	PO_4-1M NaCl	7.5
4% Nutrient broth	PO_4-1M NaCl	9.0
3% Beef extract	PO_4-1M NaCl	7.5
3% Beef extract	PO_4-1M NaCl	9.0
15% Beef extract	PO_4-1M NaCl	7.5
15% Beef extract	PO_4-1M NaCl	9.0
4% Nutrient broth	GTSA-2M NaCl	7.5

Table 1 continued

ELUENT	BUFFER	pH
4% Nutrient broth	GTSA-2M NaCl	9.0
3% Beef extract	GTSA-2M NaCl	7.5
3% Beef extract	GTSA-2M NaCl	9.0
15% Beef extract	GTSA-2M NaCl	7.5
15% Beef extract	GTSA-2M NaCl	9.0
Heparin (100 g/ml)		8.0
Isoelectric casein (0.5%)		8.0
10% Newborn calf serum	Tris (0.2M) 1M NaCl	7.5

FIGURE 2

Elution Procedure



grams of wet sediment were mixed with 30 ml of buffer containing 1 ml of virus suspension in 400 ml centrifuge bottles. Virus-sediment combinations were mixed on a mechanical shaker and centrifuged. The supernate was titered and the sediment pellet resuspended in approximately 30 ml of test eluent. The eluent-sediment mixture was again shaken and centrifuged, followed by titration of the eluent supernate. A 0.1-g sample of the final sediment pellet from eluents composed of 4% nutrient broth (Difco), pH 7.5, 4% nutrient broth (Difco) pH 9.0, 3% beef extract (Inolex) pH 7.5, 3% beef extract (Inolex) pH 9.0, 15% beef extract (Inolex) pH 7.5, and 15% beef extract (Inolex) pH 9.0 in phosphate buffer with 1M NaCl was placed into 9.9 ml of GTSA buffer and diluted until no turbidity could be detected. One milliliter of each dilution was added to four replicates of confluent BB cells in 60-mm petri plates (Corning) along with 5 ml of complete growth media. Initial supernate, final eluent-supernate titrations, and petri plates were observed daily for CPE.

7. Virus survival in sediment. Thirty milliliters of buffer containing a known virus titer were mixed with 10 g of sediment on a mechanical shaker for 30 min followed by centrifugation. The supernate was diluted and titered to determine the amount of unadsorbed virus. A 0.1-g sample of sediment was immediately diluted in sterile buffer and titered in petri plates containing confluent BB cells.

The rate of virus survival was determined initially by sampling sediment after 4 days; however the sampling period was lowered to 24 h with samples taken at 6-h intervals.

CHAPTER IV

RESULTS AND DISCUSSION

A. Introduction

Experiments were performed to determine the effect of physiochemical conditions on virus stability and to select a suitable eluent which would not decrease the titer significantly nor be detrimental to viral infectivity. Because of the lack of research involving elution of fish viruses from sediment, several initial experiments were conducted to determine virus stability with regard to pH, temperature, and salt concentration. Many of the elution experiments were based on variations of the data of other investigations which analyzed the release of enteroviruses from estaurine sediment. Sediment composition was taken into consideration in this elution study; a summary occurs in Table 2.

B. Physical Factors

Effect of pH on CCV. Experiments pertaining to the effect of pH on the virus titer were performed (Table 3). In part A of this experiment, no survival of virus was noted at pH 3.0, the virus titer was similar at pH 5.0, 7.0, and 9.0, but the titer declined at pH 11.0. In part B, CCV did not survive at pH 3.0, nor was cytopathic effect

TABLE 2

Composition of Pond Sediment

Sample	Sediment type	% Composition
Pond sediment[1]	Sand (Very fine)	27.8
	Silt	43.8
	Clay	28.4

1. Sediment was always collected in the same 2-ft^2 area of the pond. Sample date, July 1981.

TABLE 3

Virus Survival (TCID50) at Various Levels of pH

	pH	Mean of 3 trials
A.	3.0	0
	5.0	3.02×10^7
	7.0	4.02×10^7
	9.0	4.20×10^6
	11.0	2.00×10^5
B.	3.0	0
	3.5	0
	4.0	0
	4.5	2.00×10^5
	5.0	1.40×10^7
	7.0 (control)	2.03×10^8
C.	9.0	2.67×10^7
	9.5	2.10×10^7
	10.0	3.00×10^7
	10.5	1.00×10^7
	11.0	2.00×10^5
	7.0 (control)	1.53×10^8

(CPE) evident at pH values of 3.5 or 4.0. Only after pH 4.5 was reached was CPE evident in the BB cells; however the titer was much lower than the pH 7.0 control. In part C, CPE was observed at all pH values but at pH 11.0 the titer was significantly lower than the control.

An experiment of this type was necessary to determine which pH would destroy virus infectivity so that a suitable eluent could be developed. The effects of chemicals such as glycerol, chloroform, and ether are commonly determined on all viruses and have been reported for CCV (27, 40, 58); however, data on virus in the free state are lacking. Robin and Rodrique (52) reported alkaline conditions favored the maintenance of virus activity over a 24-h period. McAllister (27) reported inactivation of CCV at pH 3.0, but detailed information on optimum pH for virus survival has not been described.

There was no survival from pH 3.0 to 4.0 in any trial. Only after pH 4.5 or higher was any CPE observed in the cells. The optimum range was between pH 5.0 and 9.0 with a decline in titer as either pH extreme was reached. Results of this study agree with those which concern virus survival at the basic pH range (52); however, a narrow pH range was studied using values of pH 6.0 to 8.0. Also the incubation period in this study was shorter (1 h) as compared to the 24-h incubation period in the Robin and Rodrique study (52).

Effect of temperature on virus titer. Table 4 summarizes results of the effect of temperature on virus survival. There was no significant difference in titer at any temperature until 60°C was reached. Previous studies (38, 39) indicated virus inactivation at 60°C. In this study the titer exhibited a two-log decrease. Virus elution is usually performed at room temperature and in the case of CCV there should be no loss of titer which could be attributed to this parameter.

Effect of salt concentration on virus titer. Salt concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 3.0% in sterile GTSA buffer (pH 7.0) were first introduced into a virus-free system to determine if certain concentrations were toxic to the cell line. In three trials there was no change in the cell line after 6 days (Table 5). The virus was also inoculated into various salt concentrations and incubated for 1 h at room temperature. There was no significant change in virus titer between virus and salt titrations and the salt-free control (Table 5). In later experiments, increased salt concentrations of 1M, 2M, 3M, and 4M were added to the cell line to determine toxic levels and again titrations with salt concentrations. As seen in Tables 6 and 7, the BB cell line tolerated an increased salt level until 3M was reached. All cells were dead at 3M and 4M within 6 days. Titrations were possible at increased salt concentrations. Cytopathic effects

TABLE 4

Effect of Temperature on Virus Infectivity[1]

Temperature	Trial 1	Trial 2	Trial 3	Mean
4°C	1×10^7	5×10^6	3×10^6	6.00×10^6
25°C (control)	3×10^6	3×10^6	5×10^6	3.33×10^6
25°C (1-h control)	1×10^7	6×10^6	6×10^6	7.33×10^6
37°C	5×10^6	6×10^6	2×10^7	1.03×10^7
45°C	2×10^6	1×10^6	3×10^6	2.00×10^6
60°C	3×10^4	2×10^4	3×10^4	2.67×10^4

1. Virus incubated at various temperatures in sterile GTSA buffer (pH 7.5).

TABLE 5

Effect of Salt Concentration on Virus Titer ($TCID_{50}$) and Cell Line

% NaCl	Final Concentration	Mean of 3 trials[1]	Mean of 3 trials[2]
0.5% (9.03M)	.005% (0.0003M)	NC[3]	5.67×10^7
1.0% (0.05M)	.010% (0.0005M)	NC	6.67×10^7
1.5% (0.08M)	.015% (0.0008M)	NC	5.00×10^7
2.0% (0.11M)	.020% (0.0011M)	NC	8.00×10^7
3.0% (0.16M)	.030% (0.0016M)	NC	4.50×10^7
Control[4]		NC	6.67×10^7

1. Effect of salt concentration on cells.
2. Effect of salt concentration on virus.
3. NC = no change in cell line after 6 days.
4. Buffer as diluent.

TABLE 6

The Effect of NaCl on Cell Survival

NaCl Concentration	Final Concentration	Cell Survival[1] Trial 1	Trial 2	Trial 3
Control[2]		++++	++++	++++
1 M[3]	.01M[4]	++++	++++	++++
2 M	.02M	+++	+++	+++
3 M	.03M	0	0	0
4 M	.04M	0	0	0

1. Survival code: No change in cells (++++)
   75% survival (+++)
   No survival (0)
2. Buffer (GTSA) without salt added to cells.
3. NaCl concentrations added to confluent BB cells; cells were checked for 6 days after addition of salt to determine cell survival.
4. Final concentration after 0.1 ml buffer was added to cells.

TABLE 7

Titration of Virus ($TCID_{50}$)[1] at Various NaCl Concentrations

NaCl Concentration	$TCID_{50}$/ml			
	Trial 1	Trial 2	Trial 3	Mean
Control[2] (0 time)	1×10^7	1×10^8	2×10^7	4.33×10^7
Control[3] (1 h)	3×10^7	3×10^7	2×10^7	2.67×10^7
1 M[4]	2×10^7	2×10^7	5×10^6	1.50×10^7
2 M	3×10^7	2×10^6	5×10^5	1.08×10^7
3 M	2×10^6	1×10^6	3×10^5	1.10×10^6
4 M	2×10^6	1×10^6	3×10^5	1.10×10^6

1. $TCID_{50}$ = Tissue culture infective dose, 50%.
2. GTSA buffer (pH 7.0) without NaCl
3. Titration in GTSA buffer after 1 h to determine loss of virus under control conditions.
4. Virus incubated in all salt concentrations for 1 h.

were noted in the cell line in each of three trials within 2 days after inoculation of virus into buffer containing each salt concentration. The virus was only incubated in the salt concentrations for 1 h and when dilutions were made they were made in sterile GTSA buffer at pH 7.0 (without NaCl) thereby diluting the NaCl concentration to a level suitable for cell survival. This was necessary because results from studies on the effect of NaCl indicated that the cells could not tolerate concentrations in excess of 2M. Robin and Rodrique (52) reported the inactivation of CCV in artificial seawater after 12 days. In this study, virus was exposed to NaCl for only 1 h with no decline in titer; however the NaCl was prepared in a buffer which may aid in virus survival. Also it was not necessary to expose virus to salt for longer than 1 h since elution procedures did not require longer periods of time. The results indicated that the virus could withstand a high salt concentration for at least an hour of incubation; however, the BB cell line could not tolerate salt concentrations over 2M.

Effect of agitation on virus. The virus was subjected to agitation in sterile buffer at 250 rpm on a mechanical shaker. This was done to determine the effect of the procedure which would be used later in adsorption and elution studies. Table 8 gives the findings of this study. A slight decline in titer was observed in all cases,

TABLE 8

Effect of Agitation on Virus Titer

Trial	Input[1] Control	Recovery[2] Control	Sample[3] 1	2	3	Mean
1	2×10^6	2×10^6	5×10^4	3×10^5	3×10^5	2.17×10^5
2	3×10^5	3×10^5	3×10^4	3×10^4	3×10^5	1.20×10^5
3	5×10^7	5×10^6	2×10^6	2×10^6	1×10^6	1.67×10^6

1. Titration at zero time.
2. Titration after 30 min, without shaking.
3. Each trial was performed using three replicates and identical virus concentrations.

but that decline was not severe enough to abandon the mechanical shaking required in subsequent experiments.

Virus adsorption to pond sediment. In initial experiments, known concentrations of virus were added to measured amounts of dry sediment to determine 1) the quantity of virus which would adsorb to a given amount of sediment and 2) if pH was associated with the degree of virus adsorption observed. Table 9 contains findings of this study. Results indicated that at 0.1 gm or higher, the adsorption of virus to sediment is relatively constant at each pH. At 0.01 gm of sediment the results were variable at each pH. No pattern was observed for adsorption as related to pH of the buffer in any trial. When 0.01 gm of sediment was used, a mean of 94% of virus was adsorbed at pH 7.0, but when the sediment was increased to 0.1 gm at the same pH, a slight decline was noted. Only when 1 gm or more was used was adsorption 100%.

Because of these inconsistancies, wet sterile sediment was used in remaining studies. Dry sediment was used initially (13). In this study, dry sediment was also autoclaved since the sediment would be added to the cells in later experiments. The use of dry sediment was abandoned since the possibility existed that autoclaving dry sediment may change the physical and chemical properties of the sediment (Lytle, J. and Lytle, T., Gulf Coast Research Laboratory, Ocean Springs, MS, personal communication)

TABLE 9

Per Cent[1] Adsorption by Dry Sediment

pH	Virus[2] Titer	Sediment			
		0.01 gm:%	0.1 gm:%	0.5 gm:%	1.0 gm:%
5.0	2×10^8	3×10^7:85	2×10^6:99	2×10^6:99	2×10^8:100
7.0	3×10^8	2×10^7:94	3×10^7:90	6×10^5:99	3×10^8:100
9.0	2×10^8	1×10^8:50	8×10^6:95	2×10^6:99	2×10^8:00

1. Mean of three trials.
2. Virus titer = initial amount of virus added to sediment.

which would explain variations in results. When wet sediment was used, a 10-gm sample of sediment was dried and reweighed to determine the dry weight of the sample. Adjustment was made so that all samples represented dry weight of the sediment.

Adsorption studies were also conducted using wet, sterile sediment (Table 10) and results were similar for sediment portions of 0.1 gm or higher. Wet sediment samples of 0.01 gm were more consistent than dry sediment and had a mean of 94% adsorption over a pH range of 5.0 to 9.0. Apparently, even a small amount of sediment has the capability of binding large amounts of virus. Virus, too, may tend to aggregate (57) which may account for high titers of virus adsorbing to a small amount of sediment.

Elution studies. Since CCV readily adsorbs to pond sediment, studies were performed to elute sediment-bound virus. Methods to elute enteroviruses were previously described and it was the purpose of these experiments to devise a similar procedure for the elution of CCV. Channel catfish virus is excreted in urine and feces of infected fish (34, 42, 45), therefore making the surrounding environment (pond water and sediment) a potential hazard for disease transmission. Water has been shown to be a factor in disease transmission (34, 42, 44, 45) but studies which relate sediment-associated virus to the transmission of disease were not available.

TABLE 10

Per Cent[1] Adsorption by Wet Sediment

pH	Virus Titer	Sediment			
		0.01 gm:%	0.1 gm:%	0.2 gm:%	1.0 gm:%
5.0	1×10^7	2×10^5:98	1×10^7:100	1×10^7:100	1×10^7:100
7.0	1×10^7	1×10^6:90	1×10^7:100	1×10^7:100	1×10^7:100
9.0	1×10^7	4×10^5:96	1×10^7:100	1×10^7:100	1×10^7:100

1. Mean of three trials.

To determine per cent recovery of viable virus, several studies were performed using various eluents. Titrations of virus in all eluents were performed to determine if the eluent itself destroyed virus infectivity (Table 11).

Table 12 summarizes results from elution attempts with 4% nutrient broth. Nutrient broth had been cited as a satisfactory eluent for enteroviruses (57); however, virus was not recovered in procedures to elute CCV. Tables 13 and 14 summarize results using 3% and 15% beef extract, respectively, in various buffers and at different pH levels. Only eluents supplemented with 1M NaCl in phosphate buffer showed an extremely low recovery (less than 1%) in all cases. Salt concentrations of 2M or greater were toxic to the BB cell line.

Elutions experiments were performed with purified sand and bentonite clay (Fisher Scientific) to determine if any differences in elution patterns were associated with sediment fractions. Eluents containing beef extract in phosphate buffer were used in this group of experiments. Virus was not recovered from bentonite, and again less than 1% recovery was observed using sand (Table 15). Bitton et al. (4) reported that beef extract sufficiently removed enteroviruses from fine sand. It was hoped that the herpesvirus might be more easily removed from sand than a mixture of different sediment types; however, results indicate that the virus does not desorb readily

TABLE 11

Virus Titrations in Eluents

Eluent	Buffer	pH	Control[1] Titer	Eluent[1] Titer
4% NB[2]	Tris (0.2M)	7.5	6×10^7	1×10^6
4% NB	Tris (0.2M)	9.0	4×10^5	1×10^5
4% NB	Tris (0.2M)	10.5	3×10^6	1×10^6
4% NB	PBS[3]	7.5	1×10^7	3×10^6
4% NB	PBS	9.0	1×10^6	1×10^5
3% BE[4]	Tris (0.2M)	7.5	2×10^6	2×10^6
3% BE	Tris (0.2M)	9.0	2×10^6	3×10^6
3% BE	PBS	7.5	1×10^6	1×10^6
3% BE	PBS	9.0	1×10^6	2×10^7
15% BE	Tris (0.2M)	7.5	1×10^6	4×10^6
15% BE	Tris (0.2M)	9.0	2×10^7	2×10^7
15% BE	PBS	7.5	2×10^7	2×10^6
15% BE	PBS	9.0	2×10^7	2×10^7
4% NB	PO_4-1M NaCl	7.5	5×10^6	6×10^6
4% NB	PO_4-1M NaCl	9.0	5×10^6	1×10^6
3% BE	PO_4-1M NaCl	7.5	5×10^6	4×10^5
3% BE	PO_4-1M NaCl	9.0	5×10^6	1×10^5
15% BE	PO_4-1M NaCl	7.5	5×10^6	1×10^6
15% BE	PO_4-1M NaCl	9.0	5×10^6	4×10^7

Table 11 continued

Eluent	Buffer	pH	Control[1] Titer	Eluent[1] Titer
4% NB	GTSA[5]-2M NaCl	7.5	6×10^7	5×10^7
4% NB	GTSA-2M NaCl	9.0	6×10^7	1×10^6
3% BE	GTSA-2M NaCl	7.5	6×10^7	2×10^7
3% BE	GTSA-2M NaCl	9.0	6×10^7	5×10^6
15% BE	GTSA-2M NaCl	7.5	6×10^7	1×10^6
15% BE	GTSA-2M NaCl	9.0	6×10^7	5×10^7
Heparin (100 mg/ml)	Distilled water	8.0	1×10^7	5×10^6
IC[6] (0.5%)	Distilled water	8.0	5×10^5	6×10^4
10% NCS[7]	Tris (0.2M)	7.5	5×10^7	2×10^7
1% Heparin	Tris (0.2M)	7.8	5×10^5	3×10^6
1% Lecithin 0.5% IC	Tris (0.2M)	7.8	2×10^6	2×10^6
3% BE, 0.005% SDS, 0.5% IC	Tris (0.2M)	8.0	6×10^5	1×10^5
3% BE, 0.005% SDS	Tris (0.2M)	7.5	5×10^4	4×10^3

1. Mean of three trials.
2. NB = nutrient broth.
3. PBS = phosphate buffered saline.
4. BE = beef extract.
5. GTSA = glycine-tris-sodium acetate buffer.
6. IC = isoelectric casein.
7. NCS = newborn calf serum.
8. SDS = sodium dodecyl sulfate.

TABLE 12

Elution of CCV from Wet Sediment

Using 4% Nutrient Broth

Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Recovery (%)
4% NB[1]	Tris (0.2M)	7.5	2×10^6	0
4% NB	Tris (0.2M)	9.0	2×10^6	0
4% NB	Tris (0.2M)	10.5	2×10^6	0
4% NB	PBS[2]	7.5	5×10^6	0
4% NB	PBS	9.0	5×10^6	0
4% NB	Phosphate 1 M NaCl	7.5	4×10^6	$2 \times 10^2 < 1$
4% NB	Phosphate 1 M NaCl	9.0	4×10^6	$3 \times 10^2 < 1$
4% NB	GTSA[3] 2 M NaCl	7.5	3×10^6	0
4% NB	GTSA 2 M NaCl	9.0	3×10^6	0

1. NB = nutrient broth.
2. PBS = phosphate buffered saline.
3. GTSA = glycine-tris-sodium acetate buffer.

TABLE 13

Elution of CCV from Wet Sediment

Using 3% Beef Extract

Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Recovery (%)
3% BE[1]	Tris (0.2M)	7.5	2×10^6	0
3% BE	Tris (0.2M)	9.0	4×10^6	0
3% BE	PBS[2]	7.5	4×10^6	0
3% BE	PBS	9.0	4×10^7	0
3% BE	Phosphate 1 M NaCl	7.5	4×10^6	$2 \times 10^2 < 1$
3% BE	Phosphate 1 M NaCl	9.0	4×10^6	$3 \times 10^2 < 1$
3% BE	GTSA 2 M NaCl	7.5	3×10^6	0
3% BE	GTSA 2 M NaCl	9.0	3×10^6	0

1. BE = beef extract (Inolex).
2. PBS = phosphate buffered saline.
3. GTSA = glycine-tris-sodius acetate buffer.

TABLE 14

Elution of CCV from Wet Sediment

Using 15% Beef Extract

Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Recovery (%)
15% BE[1]	Tris (0.2M)	7.5	5×10^6	0
15% BE	Tris (0.2M)	9.0	5×10^6	0
15% BE	PBS[2]	7.5	8×10^5	0
15% BE	PBS	9.0	2×10^6	0
15% BE	Phosphate 1 M NaCl	7.5	2×10^8	$2 \times 10^3 < 1$
15% BE	Phosphate 1 M NaCl	9.0	2×10^8	$3 \times 10^2 < 1$
15% BE	GTSA 2 M NaCl	7.5	3×10^6	0
15% BE	GTSA 2 M NaCl	9.0	3×10^6	0

1. BE = beef extract (Inolex).
2. PBS = phosphate buffered saline.
3. GTSA - glycine-tris-sodium acetate buffer.

TABLE 15

Elution of CCV from Sand and Bentonite

Particulate Fraction	Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Recovery (%)
Bentonite[1]	4% NB[2]	Phosphate 1M NaCl	7.5	4×10^6	0
Bentonite	4% NB	Phosphate 1M NaCl	9.0	4×10^6	0
Bentonite	3% BE	Phosphate 1M NaCl	7.5	4×10^6	0
Bentonite	3% BE	Phosphate 1M NaCl	9.0	4×10^6	0
Bentonite	15% BE	Phosphate 1M NaCl	7.5	4×10^6	0
Bentonite	15% BE	Phosphate 1M NaCl	9.0	4×10^6	0
Sand	15% BE	Phosphate 1M NaCl	7.5	2×10^6	5×10^2 :< 1
Sand	15% BE	Phosphate 1M NaCl	9.0	2×10^6	1×10^3 :< 1

1. Bentonite clay (Fisher Scientific).
2. NB = nutrient broth.
3. BE = beef extract (Inolex).

from sand.

Eluents modified by the addition of chemical supplements were used in an attempt to desorb virus from sediment (Table 16). Results indicated no improvement in virus recovery. Cell toxicity evaluations were made with these eluents in the manner previously described. It was hoped that the presence of heparin would increase the elution of virus from sediment since an earlier study (17) reported that a heparin solution of 100 mg/ml interfered with the electrostatic adsorption of virus to HeLa cells. Perhaps the same mechanism would allow virus particles to desorb from sediment; however, this was not the case.

Milo (32) indicated that lecithin would aid in the stability of HeLa cells with an increase in poliovirus susceptibility. Isoelectric casein has been used (4) to aid in the desorption of virus from different soil types. Sodium dodecyl suflate (SDS) was added to certain eluents in an attempt to reduce the surface tension between virus and sediment particles. Only 0.005% SDS could be added to an eluent since higher concentrations were detrimental to the virus as well as the cell line (Tables 17 and 18). Elution studies with these eluent combinations are summarized in Table 19. Results of this study showed that even with the addition of supplemental chemicals, the recovery of virus from sediment was not increased. Only

TABLE 16

Organic and Inorganic Eluent Combinations

Eluent	pH	Virus[1] Input ($TCID_{50}$)	Recovery (%)
Heparin (1%), Tris (0.2M), 1M NaCl	7.8	5×10^6	0
1% Lecithin, 0.5% IC[2], Tris (0.2M), 1M NaCl	7.8	5×10^6	0
3% BE[3], 0.005% SDS[4], Tris (0.2M), 0.5% IC, 1M NaCl	8.0	5×10^6	0
3% BE, 0.005% SDS, Tris (0.2M), 1M NaCl	7.5	5×10^6	0
Heparin (100mg/ml)	8.0	5×10^6	0
10% NCS[5], Tris (0.2M), 1M NaCl	7.5	5×10^6	0

1. Mean of three trials.
2. IC = isoelectric casein.
3. SDS = sodium dodecyl sulfate.
4. BE = beef extract.
5. NCS = newborn calf serum.

TABLE 17

Effect of SDS[1] on Cell Line

NaCl Concentration	Cell Survival[2]		
	Trial 1	Trial 2	Trial 3
Control[3]	++++	++++	++++
0.005%	++++	++++	++++
0.015%	+++	++	+++
0.025%	++	++	+
0.030%	0	0	0
0.040%	0	0	0
0.050%	0	0	0

1. SDS = sodium dodecyl sulfate.
2. Survival code:
   - No change in cells (++++)
   - 75% survival (+++)
   - 50% survival (++)
   - 25% survival (+)
   - No survival (0)
3. Control = virus diluted in sterile buffer.

TABLE 18

Effect of SDS[1] on Virus Titer

% SDS	Trial 1	Trial 2	Trial 3	Mean
Control[2]	3×10^6	3×10^6	2×10^6	2.67×10^6
0.005%	1×10^6	1×10^5	2×10^5	4.33×10^5
0.015%	0	0	0	0
0.025%	0	0	0	0
0.030%	0	0	0	0
0.040%	0	0	0	0
0.050%	0	0	0	0

1. SDS = sodium dodecyl sulfate
2. Control = virus titered in sterile buffer

TABLE 19

Elution of CCV from Wet Sediment

Using Various Organic and Inorganic Compounds

Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Recovery (%)
Heparin (1%)	Tris (0.2M) 1M NaCl	7.8	4×10^5	0
3% BE[1], 0.005% SDS[2], 0.5% LC[3]	Tris (0.2M) 1M NaCl	8.0	4×10^5	1×10^2:< 1
3% BE 0.005% SDS	Tris (0.2M)	7.5	4×10^5	0
0.5% IC	Tris (0.2M)	8.0	4×10^5	0
Heparin (100 mg/ml)	Distilled water	8.0	4×10^5	0
10% NCS[4]	Tris (0.2M) 1M NaCl	7.5	4×10^5	0
Lecithin (1%) 0.5% IC	Tris (0.2M) 1M NaCl	7.8	4×10^5	0

1. BE = beef extract (Inolex).
2. SDS = sodium dodecyl sulfate.
3. IC = isoelectric casein.
4. NCS = newborn calf serum.

3% beef extract with 0.005% SDS and 0.5% isoelectric casein in tris buffer (0.2M) with 1M NaCl gave a slight recovery (less than 1%).

Since all elution studies gave poor recoveries, the purpose of the next experiment was to determine whether the elution procedure inactivated the virus. In all trials (Table 20) CPE was observed in the cells 24 hours after portions of the remaining sediment pellet was added to confluent cells. It is not known if some interaction between sediment-bound virus in the cell system caused the virus to detatch from the sediment or if extremely small sediment particles with virus attached were phagocytized by the cell with resulting CPE.

Virus survival in sediment. Since most virus appeared to remain adsorbed to the sediment, the next step was to determine how long bound virus would remain viable. This would determine if sediment-bound virus is a potential hazard to live fish in a natural situation. Virus was adsorbed to sediment in the absence of an eluent as earlier described (elution studies) and allowed to dry. After 96 h there was no virus survival. The procedure was repeated; however the incubation period was 24 h. Again, no virus survival was observed. Samples were then taken at 6-h intervals for 24 h in an attempt to determine if the virus remained active when bound to sediment particles. No CPE was observed in the cell line after the

sixth hour. Samples of 0.1 gm were taken at 1-h intervals within a 6-h period. Results from this experiment are given in Table 21.

It should be noted that virus survived after 24 h in elution experiments (Table 20) but not in later virus survival experiments (Table 21) for the same time period. This may be explained by the protective nature of eluent proteins allowing survival of virus when mixed with eluents but not with buffer.

Infective virus was not detected after 6 h which may indicate one of the following conditions: 1) the virus does not survive for long periods of time once attached to sediment; 2) the longer the virus is in contact with sediment, the electrostatic forces become so great that the virus does not desorb from sediment; 3) the envelope of the virus may be damaged when virus is eluted from sediment thereby inactivating the virus; or 4) eluent proteins may protect the virus but interfere with cellular phagocytosis. Based on these results, sediment may only be a factor in transmission of channel catfish virus disease after initial adsorption to sediment.

TABLE 20

Per Cent Virus Remaining Adsorbed to Sediment after Elution

Eluent	Buffer	pH	Virus Input ($TCID_{50}$)	Sediment Virus Titration	Adsorbed Virus (%)
4% NB[1]	Phosphate 1M NaCl	7.5	2×10^6	5×10^3	2.5
4% NB	Phosphate 1M NaCl	9.0	2×10^6	0	0
3% BE[2]	Phosphate 1M NaCl	7.5	2×10^6	2×10^4	1.0
3% BE	Phosphate 1M NaCl	9.0	2×10^6	3×10^4	1.5
15% BE	Phosphate 1M NaCl	7.5	2×10^6	6×10^4	3.0
15% BE	Phosphate 1M NaCl	9.0	2×10^6	6×10^4	3.0
Heparin (100 mg/ml)	Distilled water	8.0	4×10^5	0	0
3% BE, 0.005% SDS[3] 0.5% IC[4]	Tris (0.2M) 1M NaCl	8.0	4×10^5	5×10^3	1.3
3% BE, 00.005% SDS	Tris (0.2M) 1M NaCl	7.5	4×10^5	0	0
0.5% IC	Tris (0.2M) 1M NaCl	8.0	4×10^5	5×10^3	1.3

1. NB = nutrient broth.
2. BE = beef extract (Inolex).
3. SDS = sodium dodecyl sulfate.
4. IC = isoelectric casein.

TABLE 21

Survival Rate of Sediment Bound Virus[1]

Titer ($TCID_{50}$)	Time (h)	Sample Size (gm)	Recovery (%)
2×10^6	0 (control)	0.1	5×10^5:25
2×10^6	1	0.1	5×10^5:25
2×10^6	2	0.1	2×10^5:10
2×10^6	3	0.1	3×10^4:15
2×10^6	4	0.1	0:0
2×10^6	5	0.1	5×10^4:25
2×10^6	6	0.1	3×10^4:15
2×10^6	12	0.1	0:0
2×10^6	18	0.1	0:0
2×10^6	24	0.1	0:0
2×10^6	96	0.1	0:0

1. Virus and sediment mixed on a mechanical shaker (250 rpm) in GTSA buffer, pH 7.5.

CHAPTER V

SUMMARY

1. Channel catfish virus (CCV) is stable at pH values of 4.5 to 11.0 with the optimum pH range being of pH 5.0 to 9.0.

2. CCV is stable at temperatures of 4°C, 25°C, 37°C, 45°C, and 60°C.

3. CCV will tolerate salt concentrations of 0.1M to 4M; however the brown bullhead (BB) cell line will not tolerate salt concentrations in excess of 2M.

4. Agitating virus in buffer on a mechanical shaker seems to have little effect on CCV recovery.

5. CCV adsorbs readily to sediment regardless of the quantity of the sample or the type of sediment.

6. CCV cannot be eluted from sediment by common elution practices used with enteroviruses. No pattern was observed in relation to pH and elution.

7. When CCV is adsorbed to sediment in the presence of eluent, a small percentage of sediment-bound virus can be detected. When only virus and sediment are mixed, virus can be detected up to 6 h.

APPENDIX A

MEDIA AND REAGENTS

Growth Medium

Leibovitz L-15	440.0 ml
Newborn calf serum	50.0 ml
Antibiotic-antimycotic mixture (100x)	5.0 ml
L-Glutamine (29.2mg/ml) solution (100x)	5.0 ml
Gentamycin	1.0 ml

ATV (10X) Versine Dispersant

NaCl	80.0 gm
KCl	4.0 gm
Dextrose	10.0 gm
Trypsin (Difco 1:250)	5.0 gm
$NaHCO_3$	5.8 gm
EDTA	2.0 gm
Distilled water	QS to 1 liter

Glycine-Tris-Sodium acetate buffer (0.1M) (GTSA)

Glycine	0.75 gm
Tris	1.21 gm
Sodium acetate	1.36 gm
Distilled water	QS to 1 liter

APPENDIX B

EXPERIMENTAL EQUIPMENT

Item	Manufacturer
pH meter (Model LSX)	Sargent-Welch Scientific
Centrifuge (Model RC2-B)	Sorvall
Media filter (90mm, 0.45μ)	Millipore Co.
Filter holder	Millipore Co.
Millex filter unit	Millipore Co.
Sterile ampules	Vangard International
Linbro plates	Flow Laboratories, Inc.
Media holding tank for sterilization	Gibco
Vertical flow laminar hood	Baker Co., Inc.
Forma Biofreezer	Forma Scientific
Incubator (Model 20)	Forma Scientific
Inverted Microscope	Olympus
Incubator shaker	New Brunswick Scientific
Petri plates (60mm)	Corning
Pipet-Aid	Willinger Bros., Inc.
Water Bath	Scientific Products
7 x liquid detergent	Flow Laboratoreis, Inc.

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VITA

Yolanda Juanita Brady, daughter of Mr. and Mrs. Lawrence E. Brady, was born August 7, 1956, in Greenwood, Mississippi.

She attended St. Francis of Assisi Elementary School and secondary school in the Greenwood Public School system. In 1974 she was a Delta District III Science Fair winner and attended the International Science and Engineering Fair at Notre Dame University, South Bend, Indiana.

She received a Bachelor of Science degree in Marine Biology in 1978 from the University of Mississippi, where she was involved in numerous activities including Beta Beta Beta Biological Society (President 1977-1978). She attended graduate school at the University of Mississippi during the year 1978-1979 where she was a teaching assistant and laboratory assistant at the Gulf Coast Research Laboratory, Ocean Springs, Mississippi.

She began work on the Master of Science degree in Microbiology at the University of Southern Mississippi in the Fall 1979 and was also a teaching assistant.

She is professionally associated with the Mississippi Academy of Science, the American Society for Microbiology, and the World Mariculture Society.