CHAPTER II

REVIEW OF LITERATURES

History :

Cell culture is the term used to denote growing cells *in vitro* under conditions where the cells are no longer organized into tissues. Most viruses can be grown in cultured cells as well as embryonated eggs or laboratory animals. The cultivation of viruses in cell cultures in an essential part for further detailed study. Over 70 years have elapsed, many kinds of cell cultures were described including the characteristics of the cells (Schmidt 1979).

There are several types of cultured cells :

1. Primary and secondary cell cultures.

When the cells are taken from animals and placed in a container together with the growth medium. After variable period of time depending on types of cells, these cells attach and spread on the surface of the container. Then the cell start dividing mitotically, give rise to a primary cell culture. This culture consists of a variety of cell types, but mainly are of two types, fibroblast-like and epithelium-like. When the culture becomes too growed the are dispersed with tryp-

sin in order to initiate a secondary cell culture. Visually the primary and secondary cell cultures shows very limited growth in vitro.

2. Diploid cell strains and heteroploid cell lines.

2.1 Diploid cell lines are those in which at least 75 % of the cells have the same karyotype as normal cells of the species from which the cell was originally cultured. These lines do not have the property of indefinite serial passage but tend to die out by the fiftieth passage. In the past, diploid cells in serial passage were frequently referred to as "cell strains". The term cell strain is now applied more specifically to cells derived from either a primary culture or cell line by the selection or cloning of cell having specific properties or markers.

2.2 Heteroploid cell lines are those having fewer than 75 % of cells with diploid chromosome constitution. This term does not imply that the cells are malignant or are able to grow indefinitely in vitro. An established cell line is one which has demonstrated the potential to be subcultured indefinitely in vitro. Generally, a culture must be subcultured at least 70 times before it can be considered to be established (Fedroff, 1967).

Permanent cell lines have normally been established in the growth media found optimal for primary cell cultures. Fish cell lines were the first to be described by Wolf and Quimby (1962) who developed the first poikilothermic vertebrate cell line, RTG-2, from cells of rainbow trout gonads (Salmon gairdneri). This cell lines was propagated in mimimum essential medium (MEM) supplemented only with 10 % fetal calf serum. Whithin a year, Clem et al. (1961) initiated trypsinized blue striped grunt (Haemulon flavolineatum) fin cultures which provided GF-1 cells, the first line of marine fish origin. The next to be established was initiated in 1965; it was an epithelial-like cell from the fathead minnow (Pimephales promelas) and was designated FHM (Gravell and Malsberger 1965). This line was cultured from the caudal trunk of the fish in Eagle's basal medium (BME) supplemented only with fetal bovine serum.

Before the propagating fish cell lines were succeeded, Griitzner (1958) made a major contribution by describing the first trypsinization of fish tissue yielding cultivable cells which grew in monolayers. The method which can be considered "second generation" followed those which had been developed for mammalian use, but temperature during digestion was 20°C or less.

In 1957, Wolf and Dunbar cultivated adult trout and goldfish tissues. These tissue could not subcultured

but kept cilia beating for nearly 2 months and goldfish heart beating for 10 month in medium 199 supplemented with 50 % embryo extract and 20 % albumin.

In 1960, Wolf et al. employed a different procedure to use with coldwater teleosts. Trypsinization was used at 4°- 6 °C with unmodified mammalian-type medium. Cells of six teleosts, an amphibian and a reptile were cultured. Subcultures were effected by machanical dispersion as well as trypsin or disodium versenate. Significantly, some tisuese were stored at 4 °Cfor 24 hrs before use. Preparation of monolayer cell culture from enzymically disaggregated fish tissue has been reported by a number of other workers who anticipated or made immediate application in fish virology(Clem 1961, Jensen 1963, Pfitzer 1965, Fryer, Yusha and Pilcher 1965, Kunst and Fijan 1966).

Physiological Salines or Balanced Salt Solution.

Balanced salt solutions are essential for critical functions of any inorganic ionic species when cells are removed from their in vivo milieu. These include :

1. Sodium (Na^+) and potassium (K^+) to regulate toxicity and permeability.

2. Calcium (Ca^{+2}) and magnesium (Mg^{+2}) to maintain the integrity of cell membranes and internal structures.

3. Phosphate (HPO_4^{-2}, H_2PO_4) and/or bicarbonate (HCO_3^-) to control the hydrogen ion concentration through their buffering effect. Most balanced salt solutions including glucose provide a readily available energy source for cells.

The balanced salt solution was first developed by Ringer (1886). He proposed that each cation alone was toxic for cells in the perfused organ, but the toxicity should be neutralized when the true cations were combined in a proportion close to those existing in seawater. The composition of Ringer solution is given in the first column (Table 1).

Balanced salt solutions for use in tissue and cell culture were formulated over the years by Gey, Earle, Hanks, Dulbecco, Puck and Eagle (Table 1). These solutions are used for washing cells and tissue fragments for suspending enzymes that are used to free cells from tissues and to dislodge them from glass and plastic surfaces. Each solution is also used as a basic unit in formulating cell culture media and formulated a different purpose. Gey (1936) increased the phosphate concentration to make it corresponded more closely to physiological conditions of the animal cells in vivo, and in turn, reduced the bicarbonate level. Earle (1943) increased the concentration of bicarbonate in his salt solution to provide a high buffering capacity for neutral the large

Table 1. Composition of balanced salt solutions (Kuchler 1977).

Component	Formula weight	Ringer's mg/L	Gey's mg/L	Earle's mg/L	Hank's mg/L	Dulbeco mg/L	Eagle'MEM mg/L
NaCl	58.44	6500.0	8000.0	6800.0	8000.0	8000.0	6100.0
KCL	74.56	140.0	375.0	400.0	400.0	400.0	200.0
$CaCl_2.H_2O$	146.99	-	225.0	265.0	186.0	-	-
$CaCl_2$	110.99	120.0	170.0	200.0	140.0	100.0	-
$MgSO_4.7H_2O$	246.50	-	70.3	200.8	200.8	-	-
$MgCl_2.6H_2O$	203.31	-	210.1	-	-	-	200.1
Na_2HPO_4	267.96	-	226.0	-	90.0	1500.0	-
$NaH_2PO_4.H_2O$	138.0	-	-	140.1	-	-	1400.0
K_2HPO_4	136.9	-	-	-	60.4	200.0	-
$NaHCO_3$	84.01	200.0	227.0	2200.0	350.0	-	2000.0
Phenol red	-	-	-	10.0	10.0	-	10.0
Glucose	186.16	-	1000.0	1000.0	10.0	-	1000.0

amounts of lactic acid produced by growing cells *in vitro* (Kuchler 1977). The level of calcium (Ca^{+2}) in the salt solution developed by Hanks and Wallace (1949) was lower than other solution. The concentration of phosphate in this solution was carefully adjusted so that the solution lacked of bicarbonate and could be sterilized as a unit autoclaving at 121°C. Magnesium was also added as the sulphate salt. Dulbecco and Vogt (1954) used phosphate only to buffer their salt solution at pH 7.6. This salt solution was developed to prepare trypsin solution for disaggregating tissue to effect suspension of cells. The higher pH was used to achieve maximum trypsin activity within the physiological pH range. Eagle (1971) developed a spinner salt solution in which all the calcium was removed to minimize the clumping of cells (Kuchler 1977).

For freshwater teleosts, Earle's balanced salt solution (BSS), Dulbecco and Vogt's phosphate buffered saline (PBS), and Hanks'BSS are commonly used. (Wolf and Dunbar 1957, Gravell and Malsberger 1965, Wolf and Quimby 1976a, Bower and Plumb 1980, Fryer MacCain and Leong 1981, Wolf and Ahne 1982, Fijan et al.1983). More than 20 years of experience with fish cell and tissue culture has confirmed that these physiological salines are wholly suitable, without modification, for cell and tissue culture application with materials from

freshwater teleosts (Wolf and Ahne 1982).

The suitability is further reinforced by the general similality of the inorganic blood constituents of man and several representative freshwater teleosts. In lieu of the usual phosphate or bicarbonate buffer system, physiological saline may be buffered with Tris, HEPES (N - 2-hydroxyethylpiperazine - N-2-ethanesulfonic acid TRICINE (Eagle 1971). The levels range from about 10 to 20 mM, but bicarbonate should be included also, at a level of about 9-15 mM (Wolf and Ahne 1982).

Cell Culture Media.

Cells cultured in vitro are completely dependent for their survival and growth on the ingredients of the aqueous medium. For growth and multiplication, the medium must contain certain chemical compounds that maintain the cell physically, and others that can be utilized to provide energy for cell function and building blocks for cell structure. Any media that will support the sustained growth of cells in vitro must contain five categories of substances, which include salts, a carbohydrate, amino acids, vitamins, and serum proteins. If the macromolecular fraction of serum and the vitamins are omitted, the mixture will suffice as a maintenance medium and support essential cell functions for several

days. If the amino acids are also omitted, the mixture containing only salts and a carbohydrate is essentially a balanced salt solution. Cell suspended in such a solution may survive as long as 24 hours before they degenerate. If the carbohydrate is excluded from the salt solution, the cells will only survive for a few hours, because there is no exogeneous compound aviable to provide a continuous supply of energy (Parker 1957, Kuchler 1977,).

A. Nutritional factors.

Most fish cell and tissue culture are grown in semisynthetic media with natural supplements. Soret and Saders (1954) were the first to report use of a semisynthetic solution medium 199 for fish tissue culture. The details of reports on fish culture show that three synthetic media have been used far more than others. In order of decreasing frequency of use (Wolf 1973a, Wolf and Ahne 1982), the medium 199 (M-199) (Wolf and Quimby 1962, Koch 1965), Eagle's basal medium (BME) (Fryer, Yusha and Pilcher 1965), Leibovitz L-15 (Wolf and Dunbar 1957, Gravell and Malsberger 1965) have been employed. By far, the most frequently medium, Eagle's minimal essential medium with Earle's salt (MEM) has been used for routine fish cell culture (Table 2) (Gravell and Malsberger 1965, Wolf and Quimby 1976b, McKenzie

Table 2. General purpose culture media for fish cell tissue (Wolf and Quimby 1966).

Culture type	System component	Supplement	
		Requirement	optimal
Explant or monolayer in a closed system	Eagle's MEM with Earle's or Hank's BSS, M 199.	5-20 % Serum (Fetal or calf serum)	5 % whole egg ultrafiltrate
Explant or monolayer in petridish or other open system	Leibovitz' L - 15	As above	As above

and Stephensons 1979, Fijan et al. 1983, Lannan, Winton and Fryer 1984). In the Leibovitz medium galactose is used as an energy source, but in most other media glucose is used and design to maintain pH in the physiological range under normal atmospheric conditions without adding CO_2.

B. Serum addition.

Serum is required for the growth of most cultured cells. The essential components in serum are found in the macromolecule fraction. The requirement for these substances is not nutritional, since they are neither accumulated nor broken down by cells cultured in vitro. The function of serum proteins in promoting cell growth in vitro includes:

1. Regulation of genetic information.
2. Neutralization of trypsin activity.
3. Detoxification of inhibitory components.
4. Protection of cell surfaces from adverse physical phenomena.
5. Stabilization of labile nutrients.
6. Transportation of substrates across the cell membranes.
7. Stabilization of essential substances (Kuchler 1977).

Fish serum, either homologous or heterologous, give mixed results. Among the reports of the more re-

cent workers, heterologous or homologous fish serum has inhibitory (Clem et al. 1961) or toxic effect (Fryer et al. 1965). Where budgets can afford, fetal bovine serum is the serum of choice. It is notable for its lack of toxicity and for its growth stimulating properties. However, there have been lots that are some what toxic to specific cell line (Wolf 1973a, Wolf and Ahne 1982).

The usual level of medium supplementation with serum is 10 %. Some fish cell lines grow satisfactorily with only 2-5 % serum, but the growth rates are slightly reduced. Still further reductions in serum levels are typically accompanied by reduced rates of growth. Serum at levels above 10 % has been used for some primary cell cultures and only a very few fish cell lines. For example, a 15 % level is suggested for the gold fish cell line (GAR) (Mckenzie and Stephenson 1973, Wolf and Ahne 1982).

C. Antibiotics.

Antibiotics routinely used in mammalian tissue culture are suitable at comparable level for fish cells. For routine purposes, many have used media containing 100 IU of penicillin, 100 ug of streptomycin and 25 IU of nystatin per milliliter. In table 3, a suggested guide for the use of antibiotics with fish cells and tissue is given (Wolf 1973a, Wolf and Quimby 1976a, Kuchler 1977).

Table 3. Antibiotics used in fish cell and tissue culture (Wolf 1969).

Antibiotic	Routine use	Remarks
Amphotericin B	2-10 ug/ml	May be toxic at > 5 ug/ml
Chloramtetracycline HCl	50 ug/ml	50 ug toxic to BF-2 cell line
Kanamycin	25-50 ug/ml	100 ug halves growth rate at RTG-2 cells
Neomycin	50-100 ug/ml	-
Nystatin	25-50 ug/ml	100 IU inhibitory for some cells
Penicillin G, Potassium	50-100 ug/ml	2000 IU/ml has been used
Polymyxin B, Sulphate	no data	2000 IU/ml as brief bath.
Streptomycin Sulphate	50-100 ug/ml	2000 IU/ml has been used.

Kanamycin and amphotericin B are more inhibitory or toxic at lower concentrations than other antibiotics. If mold colony is found in a bottle, it is carefully removed and the necessary amount of amphotericin B is added to the medium. Kanamycin has similarly been used with bacterial contamination in the presence of penicillin and streptomycin (Wolf and Quimby 1971).

Temperature and pH.

Fish cell line and tissue culture differ somewhat from mamalian cell cultures in having a greater temperature range for incubation. On the basis of the physiological requirement and environmental preferences, fish are categorized as cold water or warm water, and the temperature factor applies to in vitro culture conditions. Growth of coldwater fish cell extends from 5 to 25°C with an optimal temperature of 20°C. Cells from warmwater fish grow well at 25°to 30°C but may grow at 15°through 35°C and higher (Wolf and Quimby 1962, Fryer et al. 1965, McKenzie and Stephenson 1973, Wolf 1973a) (Table 4).

The pH of medium is necessary for good growth of fish cells and most cells can grow well at the range of 7.2 to 7.8. Primary cultures and lower densities of cells will usually grow better at 7.3 - 7.4 than at pH 7.8. Many fish cell cultures grow well, but with

Table 4. Proximate range of incubation temperature for fish cell or tissue cultures (Wolf and Ahne 1982).

Representative ecological niche	Representative fish	Temperature range (°C) low	near optimal	hight
Coldwater	Salmonids (Trout and Salmon)	2- 4	20	25-27
Coolwater	Percids and Esocids (Perch and Pike)	4-10	20-23	25-30
Warmwater	Centrachids, Cichilds and Cyprinids (Bluegills and Tilapia)	7-13	25-27	30-37

somewhat reduced activity, at pH 7.0 - 7.2 and as high as 7.0 - 7.8 (Wolf and Quimby 1966, Wolf 1973a).

Choices of Tissues and Their Preparation.

1. Choices of tissue for culture.

Ovarian and embryonic fish cells are the most readily cultivated, and their state of active mitosis lends impetus to continued divisions in culture. Embryonic tissue has the advantages of being bacteriologicaly sterile, of having a low propability of harboring latent virus, and of having relatively large amounts of cell storage products and a small component of differentiated cells (Fryer et al. 1965, Li et al. 1965, Kelly et al. 1973a, Ahne 1979b, Bower and Plumb 1980).

Gonads, preferably juvenile or immature organs, provide a greater population of undifferentiated tissue and more actual germinal tissue (Wolf and Quimby 1962, Noga et al. 1977).

Kidney, speen, swim bladder, heart and liver may also be used, but young animals are preferable (Nicholson and Bnyre 1973, Noga et al.1977, Wharton et al.1977, Watanabe et al. 1978, Ellender et al. 1979). Fryer (1964, 1981) found that rainbow trout hepatomatous tissue readily yeilded viable cells, and he established a permanent line of cells from such tissue.

Caudal peduncle, the trunk posterior to the anus and fin, will usually yeild successful cultures providing that young animals are used and that they are adequately decontaminated (Clem et al. 1961, Gravell and Malsberger 1965, Middlebrooks et al. 1974).

2. Preparation of fish for obtaining tissue.

To prevent or minimize gross contamination from regurgitated food or feces, food should be withheld from donor fish for a day or more before use. Clean and healhty specimens free of external lesions are preferred, otherwise there is risk of encountering systemically disseminated bacteria which threaten the survival for culture. The health history of the source population helps in selection of donor. Cells and tissue should be cultured at a temperature similar to the environmental temperature preferred by the donor species. Extended exposure of tissue from coldwater fishes such as salmon and trout to 30°C can be lethal. In contrast, many and perhaps most fish tissues remain viable even if held for a day or two on ice or at 4°- 6°C (Wolf and Quimby 1976a). Donor fish can be killed with a sharp blade to the head, pithed, or euthanized with 1:5000 tricine methanesulfonate (MS 222), 1:2500 benzocaine, or a lethal concentration of any other fish anesthesia. Muscle contractions of freshly killed fish can

jeopardize aseptic techniques ; however, the problem can be avoided by refrigerating the carcass for an hour or more before organ or tissue removal is attempted.

2.1. Internal organ.

Prior to opening the fish, the area of incision or when feasible, the entire fish should be topically disinfected or sterile. It is advantageous to remove scales from heavily scaled fish. Wolf and Dunbar (1957) used procedures developed by Dr.S.F.Skiszko for bacteriological examination of fish. The fish is bathed for several minutes in a 1:1000 solution of benzalkonium chloride (10 % Roccal or Zephiran). Excess disinfectant should be rinsed off in chlorinated tap water or sterile physiological saline. Before opening the body cavity, one should further clean the surface by sponging it with 70 % alchohol (ethanol or isopropanol) (Gravell and Malsberger 1965, Wolf and Quimby 1976a, Wolf and Ahne 1982).

2.2. External tissue.

The use of antibiotics in culture media has greatly reduced, but not eliminated, the problems associated with all culture in growing internal fish tissue with minimal microbial contamination. When fin, skin, barbel, cornea, caudal trunk or other external

tissues are to be used, external decontamination with strong disinfectants should be avoided because it can damage or destroy many of the cells intended for culture. Instead, the tissue can be decontaminated in antibiotic solution or cultured with stronger than usual levels of antibiotics. A 1 or 2 hours decontamination at 4 °- 15 °C mixture of 500 IU polymyxin B, 500 g neomycin, and 40 IU zince bacitracin per milliliter of sterile water has proved effective in greatly reducing or eliminating the gram-negative bacteria so commonly found in and on fish (Wolf and Quimby 1976a, Wolf and Ahne 1982).

The caudal trunk portions of healthy fishes were surface sterilized by immersion for approximately one minute in freshly prepared calcium hypochlorite solution. The calcium hypochlorite solution was prepared by adding one part calcium hypochlorite powder to 10 parts distilled water. Undissolved material was removed by passing the solution through a coarse filter paper surface. The sterile fish was rinsed immediately with 70 % ethyl alcohol (Gravell and Malsberger 1965).

Noga and Hartmann (1981) established a gill cell line by using tissue that was first decontaminated with two washings of calcium and magnesium-free saline, followed by 2-24 hours stirred suspensions of the gill in a culture medium containing 400 IU penicillin, 400 ug

streptomycin, and 10 g amphotericin B. After decontamination, the gills were minced and the fragments planted (Wolf and Ahne 1982).

3. Preparation of primany or original monolayer Cell Culture.

3.1. Planting minced tissue.

Minced to proper size and either washed in PBS or left unwashed, the tissue pieces typically intended for trypsinzation can be planted directly in medium. One to two mm fragments are distributed at the rate of one fragment per 1-1.5 cm of growth area. Moist fragments may be distributed with a narrow steriled spatula or transferred and spread with a large - bore pipette. After closure is secured, the specemen is stood on edge or bottom to allow tissue adherence and liquid drainage and left to incubate for 1 or 2 hours at a suitable temperature for incubation. After the fragments have adhered, the fluid that has drained may be pipetted off and the fragments gently covered with culture medium. A plastic flask or a glass bottle should be prefered (Wolf and Dunbur 1957, Wolf et al. 1960, Wolf 1973a, Kuchler 1977, Parker 1977, Wolf and Ahne 1982).

3.2. Enzymatic disaggregation.

The most commonly used method of initiating

primary cultures of fish cells is that of dispersing tissue by a stirred enzymatic digestion. The most widely employed enzyme is trypsin. The usual final concentration is 0.25 %, using products with an activity of 1:250 (Wolf and Quimby 1962, Wolf 1973a, Wolf and Quimby 1976a, Ahne 1979, Fijan et al. 1983).

Typical enzymatic digestion consists of preparatory processing for a half of hour or more, during which cellular and tissue debris are released. Because the first materials released have little growth potential, they are discarded and only cells and the small tissue fragments obtained from subsequent treatments are harvested for culture. The ratio of tissue to digestion mixture is not critical. Up to several volumes of minced tissue are used for each 5 to 20 volumes of digestant (McFalls et al. 1967, Wolf and Ahne 1982).

Disaggregation or partial digestion of tissue to strain fish cells for primary monolayer culture follows the general procedures used avian and mamalian tissues (Kalter 1963, Rovozzo and Bruke 1973). An important exception however is temperature. To maintain viable fish cells throughout the procedures, temperatures should not exceed 20°- 21°C.

In 1958, Griitzner introduced modern methodology in fish cell culture. She used a relatively brief digestion period and culture liver and kidney cells from

tench in media which were diluted from mammalian concentration trypsin at 0 .25 %. This trypsin was used in Dulbecco or Vogt's PBS. After 10 minutes of mixing on magnetic stirrer,the supernatant was discarded and fresh trypsin solution was added. Harvests of separated cells were made at 30 minute intervals, cooled with ice, and sedimented by gentle centrifuge during digestion not exceeding 20°C .

In 1960, Wolf found that slight modification of the so-called "cold trypsinization" used by Bodian (1956) for monkey kidney cells consistently gave cultivable cells from these and other fish and also from amphibians and reptiles. The digestion itself is carried out on magnetic stirrer at 4 °C. Harvests of sample cells were made at intervals of several hours or overnight (Jensen 1963, Li and Stewert 1965, Gravell and Malsberger 1965, Kunst and Fijan 1966, Robert 1966, Wolf and Quimby 1976a).

Storage and Preservation of Fish cells.

Freezing and long - term storage of living cells at ultra - low temperatures has been practiced for many years (Scherer and Hoogasion 1954, Steelberg et al.1958). Fish cell cultures may be preserved by freezing and holding at -80°C or lower, by the same procedures used routinely with cells from hemeotherm vertebrate. The

culture of cold-blooded animal cells have the advantage of being amenable to low-temperature incubation, and consequently cells can be stored for considerable periods of time without being frozen (Wolf 1973a, Wolf and Ahne 1982).

1. Freezing.

In general, cells are suspended in a medium having 10 % or more serum and 5-10 % of either glycerol or dimethyl sulfoxide (DMSO = Cryoprotactants) as a protective additive (Dougherty 1962). The cells are allowed to equilibrate at 5 °C for 30-60 minutes. For the best results freezing should be done slowly by cooling 1 °C/ minute to -25 °C, these transfering to -65 °C or lower (Rovozzo 1973, Kuchler 1977, Hsiung 1982).

The best single source of published data on freezing fish cells is the American type Culture Collection. The following tabulation lists all lines that freeze well in various media (Table 5).

Table 5. Fish cell lines that freeze well in the media given (Wolf and Quimby 1969).

Cell line	Freeze medium
FHM (CCL 42)	85 % Eagle's MEM (Hanks'BSS), 10 % calf serum, 5 % DMSO.
RTG-2 (CCL 55)	80 % Eagle's BME, 15 % fetal bovine serum, 5 % DMSO.
GF (CCL 58)	75 % Eagle's BME with nonessential amino acids (Hanks' with 0.196 M NaCl), 10 % calf serum, 10 % human serum, 5 % DMSO.

When cells are recovered from a frozen storage, the ampules or vials should be thawed rapidly by using a container of water at room temperature (20°C).The frozen cells should be agitated rapidly until they are completely thawed. Thawed cells can be transferred to six or eight volumes of fresh culture medium and allowed to equilibrate for 1 or 2 hours,while the cryoprotectant diffused out of the cells. Most viable cells settle and adhere to the growth surface during equilibration. Afterward, the medium is withdrawn and replaced with an appropriate volume for the culture vessel (Plumb and Wolf 1969, Wolf and Ahne 1982).

2. Low temperature incubation.

Some kinds of fish culture have been grown at temperatures of 5°- 10°C (Wolf and Mann 1980, Kelly and Miller 1978). The rainbow trout gonad (RTG-2) cell lines from a coldwater fish can be grown from 4°- 26 °C, and the rate of protein synthesis and glucose utilization have been measured through that temperature range (Wolf and Quimby 1962). RTG-2 has been incubated at 4°C for 2 years without being subcultured. At the end of 2 years, the medium was acidic, but after the culture was moved to 20°C for a day or two and the medium was then replaced with a fresh medium, the culture resumed mitosis and was subcultured within a week. Some warm

water and cold water fish cell lines have been maintained at temperature of 12°- 15°C, with transfer intervals of 3 to 4 months (Wolf and Quimby 1967, Plumb and Wolf 1969).

Fish Cell Lines.

Cell lines selected for inclusion in the American Type Culture Collection are fully characterized using the folowing criteria (Steelberg et al. 1970) :

1. The comparative viabilities of the cell population just prior to freezing, and from representative frozen ampules, are determined by a dye-exclusion test or by growth reponse.

2. The medium requirements of the reference culture are usually those employed in the isolation and/or continued propagation of the cell by the original investigator. The medium is antibiotic-free and, unless otherwise specified, contain fetal bovine serum.

3. Growth potential of cells is determined by inocula of freshly thawed ampules. Number of cells yields in a given period of time are recorded.

4. When a cell line can be grown from a single cell, direct plating efficiency of the cell from a thawed ampule is determined.

5. The morphologic appearance of strain and or living cells are recorded and photographed.

6. The diploid number of the species of origin (2n) is recorded, as well as the stem line number of the cell line and, when applicable, deviations such as chromosomal polymorphism,aneuploidy, translocation or breaks are noted. The chromosome frequency, distribution and morphology in at least 50 representative cell are determined, plus karyotype analysis of at least 10 cells within the model number. An estimate of the frequency of polyploid cells in the population is established by comparing the total number of chromosomes in 100 to 200 mataphases under low magnification. These observations are made on cells from the first or second passage following recovery from frozen preserved reference stock ampules.

7. Cell lines are tested and show to be free of mycoplasma, bacteria, fungi,protozoa and cytopathic virus. The presence or absence of virus-like particles by electron microscopy are recorded when appropriate.

8. The species of origin of the reference lines are verified by testing with specific antisera using one or more of the following tests or equivalent: mixed agglutination, fluorescent antibody reaction, indirect hemagglutination, cytotoxic-antibody dye exclusion and agar gel immunodiffusion. When distinct karyotypes clearly indicate species, these are used in conjunction with a serological reaction.

9. A determination of susceptibility or in susceptibility to polioviruses is made as an indication of primate or non primate lines and other viruses may be used for complete characterization.

10. Other specific characterization tests such as tumorigenicity, biochemical markers, drug susceptibility, isoenzyme analyses, electron microscopy, etc. are applied to reference culture when indicated to verify their identities and characteristics.

11. Cell lines of limited life expectancies in culture are called finite cell lines and must meet the criteria listed for permanent cell lines. In addition they must be shown to have a useful life expectancy after recovery from the frozen state.

12. Reference cells are usually prepared by the cooperating laboratories in batches of 100 to 200 ampules, each containing one to five million cells in 1 ml of medium. The cooperating laboratories are reponsible for the accuracy of all characterizations and for ensuring that the viability of the cell is adequate to permit recovery with the characteristics described.

Selection of Cell Lines on the Basis of Special Characteristics and Uses.

In adding permanent cell lines to the collection, information must be obtained as to the history or the

prototype of the cells. It seems clear, however, that most culture cells fall rather sharply into one of three categories (Krooth et al. 1965, Hayflick 1965):

1. Those that show considerable variation are heteroploid, grow indefinitely as permanent cell lines and may be epithial-like (human) or fibroblast-like.

2. Those that show little variation retain the karyotype of the donor, have a finite life span of about 20 to 50 passages are non malignant and are usually fibroblastic.

3. Those often isolated from peripheral blood, diploid in karyotype, are seemingly permanent, fail to attach to glass or plastic surfaces, and are lymphocyte in morphology. The latter have been isolated from humans in case of acute luekemia in children and adults (Foley et al. 1965, Moore et al. 1966, Clarkson et al. 1967) from normal individuals (Moore et al. 1967) and from Burkitt's lymphoma victims (Epstein et al. 1964).

For fish lines, the survey includes six widely used lines that have been characterized and are designated as Certified Cell Lines (CCL). They are in the repository of the American Type Culture Collection (ATCC) (Wolf and Quimby 1973a and Wolf and Mann 1980).

Fish cell lines which have been established and those which are under development are all of teleost origin. In 1979, there were 61 lines of fish cells

presenting 17 families and 36 donor species or hybrids of teleost (bony) fish. There were no cell lines from elasmobranch or more primitive classes of vertebrate (Table 6) (Fedoroff 1966, Wolf and Mann 1980). Most of the lines are from freshwater or anadromous species; only eight, about 22 % are from strictly marine species. Because the impetus to initiate fish cell line stems from the needs of virological research and diagnosis, about two-thirds of all the cell lines initiated have come from sport and commercial fishes. Age of donor material varies from embryo through juvenile or sub-adult to mature (Fryer et al. 1965, Nicholson and Bryne 1973).

Table 6. A Listing of Teleost Fish Cell Lines (Wolf, 1979).

Family & Scientific Name	Common name[a]	Tissue[b]	Cell Line Abbreveation	Cell Representory Number	Cell Morphology	Temp.[degree-C] Range/Optimum
Anabaenidae						
Trichogaster trichopterus	Golden gourami	N-peduncle	GP	-	E	15-39/30
Carangidae						
Caranx mate	Omoka	N-decapitated larvae	-	-	F	25-27/-
Centrarchidae						
Lepomis macrochirus	Bluegill	N-caudal trunk	BF-2	CCL 91	F	15-33/25
Micropterus salmoides	Largemouth bass	N-caudal trunk	LBF-2	-	F	15-33/25
Cichidae						
Petrophyllum scarlare	Angelfish	N-eyeball	AE	-	-	15-40/30
Clariidae						
Clarias batrachus	Walking catfish	N-gill	G1B	-	E	18-37/25
Clarias batrachus	Walking catfish	N-gonad	GD11	-	Mixed	18-37/25
Clarias batrachus	Walking catfish	N-kidney	K1K	-	F	18-37/25
Clupeidae						
Alosa sapidissima	American shad	N-whole caudal trunk	ASF-1		F	15-20/15
Cyclopteridae						
Cyclopterus lumpus	Lumpus	N-fin	-	-	F	18-20/-
Cyclopterus lumpus	Lumpus	N-testis	-	-	F	18-20/-
Cyprinidae						
Carassius auratus	Glodfish	N-fin	GAR	CCL 71	F	20-25/25
Carassius auratus	Goldfish	N-fin	KGC-1	-	E	25-32/29
Carassius auratus	Goldfish	N-fin	KGL-1	-	E	25-32/29
Cyprinus carpio	Carp	N-fin	KGCP-1	-	E	25-32/29
Cyprinus carpio	Carp	A-epithelioma papilosumcyprini	EPC	-	E	15-30/-
Cyprinus carpio Carassius auratus	Carp/goldfish	N-epithelial cell	KGCF-1	-	E	25-32/29
Pimephales promelus	Fathead minnow	N-caudal trunk	FHM	CCL 42	E	0-36/34
Rhodeus ocellatus	-	N-fin	KGR-1	-	E	25-32/29
Tinca tinca	Tench	N-ovary	TG	-	E	2-32/22-27
Esocidae						
Esox lucius	Northern pike	N-fin epithelium	NPF	-	F	4-25/20-25
Esox lucius	Northern pike	N-gonad	PG	-	F	15-25/20-22
Esox lucius	Northern pike	A-sarcoma	PS 12	-	F	-/18
Esox masquinongy	Muskellunge	A-lymphosarcoma	-	-	-	-
Ictaluridae						
Ictalurus nerubosus	Brown bullhead	N-caudal trunk	BB	CCL 59	E	4-34/25-30
Ictalurus punctatus	Channel catfish	N-ovary	CCO	-	F	20-30/30
Percichthyidae						
Morone saxatilis	Striped bass	N-fry	SBF-1	-	Mixed	15-20/20
Percidae				-		
Stizostedion vitreum	Walleye	N-whole eyed eggs	WF	-	-	4-25/-
Stizostedion vitreum	Walleye	N-caudal trunk	WF2	-	F	9-22/15

Family & Scientific Name	Common name	Tissue	Cell Line Abbreveation	Cell Representory Number	Cell Morphology	Temp.[degree-C] Range/Optimum
Stizostedion vitreum	Walleye	A walleye dermal sarcoma	WC1	-	F	10-30/25
Poeciliidae						
Gambusia affinis	Mosquitofish	N-embryo	KTG-1	-	-	25-32/29
Poecilia helleri	Guppy	N-whole embryo	GE-4	-	F	-28/22
Xiphophorus helleri	Green swordtail	N-whole embryo	SWT	-	F	-26/30
Xiphophorus maculatus	Southern platyfish	N-whole embryo	XH-A1	-	F	/25
Pomadasyidae						
Haemulon sciurus	Bluestriped grunt	N-fin	GF-1	CCL 58	F	15-25/21
Salmonidae						
Oncorhynchus kisutch	Coho salmon	N-embryo	CSE-119	-	F	4-27/21
Oncorhynchus masou	Yamame	N-kidney	YNK	-	F	4-29/-
Oncorhynchus nerka	Sockeye salmon	N-embryo	SSE-5	-	E	4-27/20
Oncorhynchus nerka	Sockeye salmon	N-embryo	SSE-30	-	E	4-27/21
Oncorhynchus nerka	Kokanee	N-fry	KF-1	-	Mixed	10-20/20
Oncorhynchus tshawytscha	Chinook salmon	N-embryo	CHSE-114	-	F	4-27/21
Oncorhynchus tshawytscha	Chinook salmon	N-embyro	CHES-214	-	E	4-27/21
Oncorhynchus tshawytscha	Chinook salmon	N-embryo	CHES-214	-	E	4-27/21
Salmon gairdneri	Rainbow trout	N-spleen	RBS	-	F	9-22/15-22
Salmon gairdneri	Rainbow trout	N-fry	RTF-1	-	F	4-26/20
Salmon gairdneri	Rainbow trout	A-hepatoma	RTH-149	-	E	4-27/21
Salmon gairdneri	Rainbow trout	N-gonad	RF	-	F	15-25/20
Salmon gairdneri	Rainbow trout	N-gonad	RTG-2	CCL 55	F	4-26/20
Salmon gairdneri	Rainbow trout	N-ovary	RTO	-	F	18-24/-
Salmon gairdneri	Steelhead trout	N-embryo	STE-137	-	E	4-23/21
Salmon gairdneri	Rainbow trout	A-nephroblastoma	RTN	-	Mixed	10-25/20
Salmon salar	Atlantic salmon	N-heart, liver, kidney	AS	-	F	4-28/20
Salmon salar	Atlantic salmon	N-heart	ASH	-	F	15-25/20
Salmon salar	Atlantic salmon	N-ovary	ASO	-	F	15-25/20
Sciaenidae						
Bairdiella chrysura	Silver perch	N-swimbladder	SP-1	-	F	18-32/26&32
Bairdiella chrysura	Silver perch	N-spleen	SP-2	-	F	18-32/26-32
Cynoscion arenarius	Sand seatrout	N-fin	WTF	-	F	20-35/27.5
Cynoscion nebulosus	Spotted seatrout	N-fin	STF	-	F	20-35/27.5

A : abnormal
N : normal

E : epitheloid
F : fibroblast

f : persistantly infected with IPN virus

Fish Virus

Since 1955, studies of fish cells and tissue have been conducted. A variety of fish viruses and virus-like agents have now been recognized, and most of them are associated with pathological conditions. Some of the fish viruses have been well characterized and classified into established viral taxonomic grouping while others have been only partly characterized or demonstrated solely by electron microscopy and their classification is tentative.

All the virus isolated from fish are cytopathic and cell infection commonly results in the destruction of the cell culture. The destruction starts with the formation of syncytia for herpesvirus, by the separation of fusiform cells in the case of birnavirus and other "IPN related virus", and by the formation of dense, round cells in the case of rhabdovirus (Kinkelin 1986).

The literature regarding fish virology presently shows that there are at last 32 viruses. 17 have been isolated in cell culture and the other 15 have been visualized for the most part rather convincingly by transmission electron microscopy (Table 7) (Wolf and Mann 1980).

Table 7. A listing of virus isolated from, or known to occur in fish, 1979 (Wolf and Mann 1980).

Name of the agent of the disease it causes and abbreviation	Major grouping	Present status
DNA virus :		
Channel catfish virus (CCV) (ATCC VR 665)	Herpesvirus	Isolated
Herpesvirus salmonis	"	"
Virus of epithelioma papilosum	"	E.M.
Turbot herpesvirus	"	"
Lymphocystis virus (LV) (ATCC VR 342)	Iridovirus	Isolated
Piscine erytrocytic necrosis virus	"	E.M.
RNA virus :		
Bluegill virus (BGV) (ATCC VR 604)	Orthomyxo virus	Isolated
Eel virus (EV-2)	"	"
Eel American (EVA)	Rhabdovirus	"
Eel Europe (EVEX)	"	"
Haemorrhagic septicemia virus or Egtved virus (VSH)	Rhabdovirus	Isolated
Infectious haemorrhagic necrosis virus (IHNV)(ATCC VR 714)	"	"

Name of the agent of the disease it causes and abbreviation	Major grouping	Present status
Spring viremia of carp (SVC)	"	"
Pike fry rhabdovirus (PFR) (Grass carp rhabdovirus)	"	"
Agents of provisional or unknown virus		
Pleuronectid papilloma virus	Ungrouping	E.M.
Walleye sarcoma virus	"	"
Walleye epidermal hyperplasia virus	"	"
White sucker virus	"	"
Brown bullhead papilloma virus	Ungrouping	E.M.
Atlantic salmon papilloma virus	"	"
Golden shiner virus (GSV)	(Reovirus-like)	Isolated
Infectious pancreatic necrosis virus (IPNV)(ATCC VR 299)	" (Birnavirus)	"
Eel virus (EV-1)	Ungrouping	"
Eel virus,European (EVE)	"	"
Grunt fin agent (GFA) (ATCC VR 683)	Ungrouping	Isolated
Intraerythrocytic virus of rainblow trout	"	"
Carp gill necrosis virus	(Iridovirus)	"

Name of the agent of the disease it causes and abbreviation	Major grouping	Present status
Atlantic salmon fibrosarcoma virus	Oncovirus-like	"
Northern pike sarcoma virus	Ungrouping	"
Epitheliocystis agent (bluegill, striped bars and white perch)	"	"

Up to now, about fifty viruses of pathological and economical importance have been either isolated from diseased and apparently healthy fishes or evidenced by electron microscopy of injured fish tissues. They are about 5 virus families and one unclassified group. Most of the fish viruses can be tentatively classified as follow : (Wolf and Mann 1980, Kinkelin 1986).

DNA Virus :

1. Herpesviridae.

1.1 Isolation in cell cultures.

a) Cyprinid herpesvirus (Schubert 1966).

b) Ictalurid herpesvirus or Channel catfish virus (CCV) (Wolf and Darlington 1971, Plumb et al. 1974).

c) Salmonid herpesvirus(Wolf et al.1978).

d) Nerka virus (Sano 1976).

1.2 Detection by electron microscopy (Herpesvirus-like agent).

a) Epithelioma papillosum of cyprinus (Wolf 1973b).

b) Herpesvirus scophthalmi (Buchanan and Madeley 1978).

Electron micrographs indicate that channel catfish virus or fish herpesvirus possess characteristics of herpesvirus morphology (Wolf and Darlington 1971,

Plumb et al.1974, Buchman and Madely 1978). The piscine herpes virion is surrounded by an envelope and contains an icosahedral nucleocapsid composed of two layer, the outer capsid with 162 hollow, elongated capsomers and the inner core. The following dimensions of the herpes-virus were detected (Table 8) :

Table 8. Dimensions of herpesviruses detected in fish (McAllister 1979).

Virus	virion dimensions (nm)		
	Enveloped particle	outer capsid	Inner core
Channel catfish virus	170-200	95-105	40-50
Herpesvirus salmonis	150	80-90	50-60
Nerka virus	230	Nd[a]	Nd[a]
Herpesvirus scophthalmi	200-220	85-100	25-30
Epithelioma papillosum-associated virus	140-150	110	50

([a] Nd = Not determined)

Stability. They are moderately stable when suspended in a cell culture medium supplemented with serum. There was little loss of infactivity when was stored for 9 months at -80°C (Wolf 1973b, Wolf et al. 1978).

Cell culture susceptibility. BB (Brown bullhead) and CCO (Channel catfish ovary), two cell lines of ictalurid origin, will support replication of channel catfish virus (Wolf and Darlington 1971). Herpesvirus salmonid appears to replicate only in cells of salmonid origin. The other cell lines, RTG-2, RTF-1, CHSE-214 and KF-1 will support virus replication (Wolf et al. 1978).

Clinical signs. Diseased fish may vary, with some or all of the following signs being present : distension of the abdomen with the peritoneal cavity filled with a clear-straw colored fluid, exophthalmia, pale or haemorrhagic gills, haemorrhagic areas at the base of fins and throughout the skin, particularly on the ventral surface. Internally, haemorrhagic areas appear thoughout the musculature, liver, kidney and spleen, the stomach and the intestine are filled with mucoid secretion and are void of food.

A generalized viremia is established within 24 hours after experimental infection. The kidney, liver, spleen and intestine become involved in virus replication in 24 - 48 hours after infection with virus isola-

tion from brain tissue possible after 48 hours. Peak of virus titers occur in kidney and intestine 72 hours after infection. In spleen, brain and liver, the peak titer occurs 96 hours after infection. Only a small amount of virus is found in the musculature (Plumb 1972).

2. Iridoviridae.

2.1 Isolated in cell culture.

a) Lymphocystis virus (LV) (Dunbar and Wolf 1966).

2.2 Detection by electron microscopy.

a) Piscine erythrocyts necrosis virus (PENV) (Laird and Bullock 1969).

Electron micrographs of tissue affected with lymphocystis virus, also of infected cell lines, indicated that both lymphocystis virus and the virus of PEN possess the morphological characteristics of iridovirus. The capsid has icosahedral symmetry with lamellar construction on the dimension of both viruses vary between 150-300 nm. Both LV and PENV have been observed in several genera of fish (Walker 1962, Wolf et at. 1966, Hows and Christmas 1971, Kelly and Robertson 1973).

Stability. Infectivity is retained after 5 days at 18°- 20°C in water, after lyophilization for 105 days at the same temperature, and at -20 °C for two years. It is inactivated by glycerol and ether (Wolf 1964, Wolf 1968, Roberts 1978).

Cell culture susceptibility. LV will replicate in BF-2, GFBF-1, blue striped ground kidney, but not in BB, FHM, RTG-2 or blue gill ovary (Wolf 1962, Wolf et al. 1966, Zwilleberger and Wolf 1968, Wolf and Carlson 1965, Midlige and Malsberger 1968).

Pathogenesis of PEN is not well defined. Most researchers report that infected fish display no external signs. However, pale or white gills can result from the severe anemia often associated with the disease (Rohovec and Amand 1981, Smail 1982).

Microscopic observation of stained blood smears from infected fish reveals the presence of cytoplasmic inclusion bodies in erythrocyte (Grober et al. 1980, Smail 1982).

The external lymphocystis lesions are raised growths generally granular, wart - like or nodular. Individual lesions, in the early stages, may be hardly visible. Color of the lesion is usually light and may be gray or cream-colored. Mature lesion may become slightly haemorrhagic, but they seldom become necrotic (Wolf 1968).

RNA Virus :

1. Birnavirus.

1.1 Isolated in cell culture.

a) Infectious pancreatic necrosis virus (IPNV) (Wolf et al. 1960).

b) Branchionephritic virus (Sano et al. 1981).

c) Eel virus 1 (McAllister et al. 1977).

1.2 IPN related virus (Kinkelin 1986).

a) Golden shiner virus (GSV).

b) Atlantic menhaden virus.

c) Yellow tail ascites virus.

The electron micrographs of negative stained and thin sectioned IPNV is non-enveloped, icosahedral and approximately 70 nm in diameter (recorded variously as 55-74 nm) (Roberts 1978). The RNA genome consisting of two segments of large size class double strand RNA (Kelly and Loh 1972, Dobos et al. 1977). The virus can be separated into three groups (Macdonald and Goneer 1981, Okamato et al. 1983).

Group 1 IPN serotype I (VR 299), USA.

Group 2 IPN serotype II (Ab), Europe.

Group 3 IPN serotype III (Sp), Europe.

IPNV serotype I or VR 299 is the reference strain classified in the American type culture collection (ATCC) as an American strain. The two European strains, discoveried in Denmark, are called Sp., and Ab strain. Both VR 299 and Sp can cause serious mortality in fry, whereas strain Ab causes low mortality and does not produce CPE in the FHM fish cell line.

Stability of virus. IPN is quite stable. Infec-

tivity, whether associated with the whole fish, tissue homogenates or a cell culture medium, can be preserved for years by freezing at -20 °C or lower, storage at 4 °C in 50 % glycerol, or lyophilization (Malsberger and Cerini 1963, Wolf 1964, Wolf et al. 1969, Tu et al. 1975, Roberts 1978).

Cell culture susceptibility. The RTG-2 cell line has been extensively used in IPN virus studies, and AS, BB, BF-2, CHSE-214, GF cell provide comparable yields. Some IPN virus strains appeare to have restricted cell culture host range (Wolf Mann 1980, Scherrer and Cohen 1975).

Symptoms include overall darkening, protruding eyes, abdominal distension, and at time haemorrhages in the central area including bases of fins. Internally. Multiple petechiae occur in the pyloric caecal area, and the liver and spleen are pale. The digestive tract is almost universally without food. A clear to milky mucous occurs in the stomach and intestine. Many of the infected fish exhibit an abnormal type of swimming, characterized by a corkscrewing or whirling motion (Wolf 1966, Grobery et al. 1980).

Eel virus Europe is similar to IPNV, and is closely related to the Ab strain of IPNV (Okamoto et al. 1983).

2. Rhabdoviridae.

2.1 Isolated in cell culture.

a) Infectious haematopoietic necrosis virus (IHNV) (Amend et al. 1970).

b) Viral haemorrhagic septicemia (VHS) (Jensen 1963) or Egtved virus.

c) Eel vesiculovirus (EVA/EVX) (Sano et al. 1976).

d) Spring viraemia of carp (SVC) (Fijan et al. 1971).

e) Pike - fry rhabdovirus (PFR) or Grass rhabdovirus (Kinkelin 1973).

f) European perch rhabdovirus (Malsberger and Lautenslager 1980).

All of the fish rhabdovirus isolated possess characteristic animal rhabdovirus morphology. In thin - section preparation IHN and VHS virions measure 185 nm in lenght and 65 nm in diameter while SVC and PFR virions measure 160 nm in lenght and 90 nm in diameter. Only negatively stained preparations have been used to determine the dimensions of eel virus of American (143 x 68 nm), the rhabdovirus of Europe eel (172 x 92 nm), the GRV (120 x 70 nm) (Amend and Chamber 1970, Bootsma et al. 1973, Hill et al. 1975, Clerx et al. 1975).

Stability, they are moderately stable under a

variety of storage conditions. Infectivity can be preserved for years at -20 °C or lower in cell medium supplemented with 2-10 % serum and lyophilization (Clerx et al. 1975, Kinkelin et al. 1975, Roberts 1978, McAllister 1979).

Clinical signs and histopathological of the virus in this group are as follow :

IHNV : Affected fish may show a variety of clinical signs, but none of these alterations in appearance or behavior is IHN specific (Parisot et al. 1965). In chronic cases, abdominal swelling, exophthalmia (pop-eye), anemia (pale gills), haemorrhages at the base of the fins, dark coloration, and fecal casts are typical clinical signs of the disease. Frequently a subdermal haemorrhagic area is present just posterior to the skull about the lateral line (Amend 1970, 1974). Histopathological. Internally, the liver, spleen and kidney are usually pale. Necrosis granular cell of the laminar propria, stratum compactum, and stratum granulosum of the alimentary tract are distinguishable pathological characteristics of IHNV (Yasutake 1965, Amend 1974). The stomach is filled with a milky fluid and the intestine with a watery, yellow fluid that sometimes includes blood. IHNV is endemic to America and other parts of the world.

VHS : The clinical signs developed a triphasic

pattern. The first, acute phase is characterzied by high mortality, with affected fish dark, lethargic and showing haemorrhages at the base of fins, in the gills, in muscle, and visceral peritoneal vessels. Dead fish at this stage often have massive haemorrhages in the abdominal cavity. The second stage is one of chronic disease where the predominant feature is very black coloration, and anemia. The gills are very pale and exophthalmos is a common feature. Organ haemorrhages within the abdomen are the main feature. The third clinical stage, from which virus is not usually isolated, is associated with the cessation of mortality, but affected fish show nervous signs manifested by a distinctive looping swimming behaviour. Such fish may show the darkness and exophthalmia of the chronic disease, but the main feature is swelling and discoloration of the kidney (Ghittino 1965, Fijan 1971, Wolf 1972, Roberts 1978).

Eel vesiculovirus are two rhabdovirus which have also been isolated from cell in Japan. The first, from young American eels (Anguilla rostrata), was designated EVA, the second, from Europe eels (A. anguilla), was designated EVEX. The disease produced by these virus in rainbow trout fry were clinically indistinguishable from VHS clinical manifestations were limited to external haemorrhaging (McAllister et al. 1977, Hill et al.1980).

SVC : Clinical signs are gathering of fish with water outflows, darkening, petechial haemorrhages especially of the skin and gills, loss of balance, exophtalmia and abdominal dropsy. Internally there is commonly a fibrinous peritonitis, pectechial haemorrhages over viscera and catarrhal or necrotic enteritis (Ahne 1979b, Roberts 1978).

PFR : Affected fish show loss of equilibrium, often swimming near the surface, and show marked cranial distension, exophthalmia and haemorrhages at gills and skin. There are also petechial haemorrhages throughout the muscle. Histopathological changes consistently occur in the kidneys but are variable in the liver, heart, pancreas, gastrointestinal tract and muscle (Kinkelin, Bootzma and Galimord 1973, Roberts 1978, McAllister 1979).

Cell susceptibility. Replication of IHN and VHS virus has been reported in a variety of piscine cell lines, most notably CHSE-214, FHM, RTG - 2 and STE-137 (Nims et al. 1970, Wolf and Quimby 1973). SVC virus will replicated in BB, BF - 2, EPC and FHM. PFR virus replicated in BB, BF-2, FHM and RTG. Replication of the two eel rhabdovirus have been monitored only in a piscine cell line eg. RTG - 2 (Fijan et al. 1971, Hill et al. 1975, Sano 1976, 1977). The fish rhabdovirus matures at the cell surface budding off from the cell membrane

cytoplasma. Inclusion bodies can be observed (Roberts 1978, McDaniel 1979).

3. Retroviridae.

Several proliferative lesions occurring in fish are considered to be caused by retrovirus infection. Viral etiology has been proposed based on electron micrographs which reveal retrovirus - like particles in association with affected tissue (McAllister 1979).

a) Atlantic salmon fibrosarcoma virus (Carlisle 1977, Duncan 1978).

b) Esox epidermal sarcoma or Esox sarcoma (Papas et al. 1976).

c) Esox epidermal hyperphasia (Sonstegard 1976).

d) Wallege dermal sarcoma (Yamamuto 1976).

e) Wallege epidermal hyperplasia virus (Walker 1962).

f) White sucker epidermal pappilloma (Sonstegard 1977).

4. Unclassified and putative fish virus associated with neoplasia.

a) Stomatopapilloma of eel – associated virus (Deys 1976, McAllister et al. 1977).

b) Brown bullhead papilloma - associated virus (Edwards et al.1977).

c) Atlantic salmon papilloma – associated

virus (Ljungberg 1963, Carlisle 1977).

d) Pleuronectic epidermal papilloma-associated virus (Wellings et al. 1965, Brooks et al. 1969).

5. Unclassified and putative fish virus.

5.1 Isolated in cell culture.

a) Bluegill virus (Hoffman et al. 1969). This virus was isolated from bluegills (Lepomismacro chirus) in West Verginia (Hoffman et al. 1969). It was 70 - 80 nm in diameter, spheriodal virus particle and resembled a myxovirus. This virus can be stored for month at -70 °C in cell culture medium supplemented with 10 % serum. The bluegill virus replicated only in cell lines of piscine origin. The BF-2 cell line is commonly used, and the virus reportedly replicates in RTG-2 as cell (Hoffman et al.1969, Nicholson and Bnyre 1973, Wolf and Quimby 1973, Wolf and Mann 1980).

b) Grunt fin agent (GFA)(Clem et al.1965). Clem et al. (1965) isolated an infected cytopathogenic agent. Electron micrographs of partially purified GFA preparations occasionally contain ellipsoidal particles which are centrally placed. The particles are 120 - 140 nm in diameter on the long axis and 100 nm in diameter on the short axis. GFA can be preserved for an extended period by freezing at -65 °C and replicates well in cell lines derived from the blue striped grunt, the lane snap-

per (Lutjanus synagris) and the goldfish (Garassius auratus).

c) Ulcerative dermal necrosis-associated virus (UDN).

In 1972, Rolat et al. discovered that Atlantic salmon, seatrout were infected with a desease called "Ulcerative dermal necrosis"(UDN). Electron micrographs revealed cytoplasmic concentration of virus - like particles. The particles appeared to be unenveloped icosahedral 30 - 33 nm in diameter and were detected only in diseased fish (Lountma and Janatuinen 1970).

Table 9. Technical information on the most suitable fish cell lines.

Virus group	Virus	Fish cell line	Temperature °C cell	virus
	CCV	BB	27/4-24	25-30
Herpesvirus	Herpesvirus-salmonids	RTG-2	21/4-26	10-15
		CHES-214	21/4-27	
DNA				
Iridovirus	LV	BF-2	25/15-33	22
	IPNV Ab	RTG-2,BF-2	-	15-20
		RTG-2,BF-2		
	IPN Sp	CHES 214		15
Birna-		FHM	30/0-35	
virus	IPN VR299	The same as IPNV sp		
	Branchione phritis virus	The same as IPNV Ab		
	Eel virus	CHES 214		15-20
RNA				
	IHNV	RTG-2, EPC	-	10-15
Rhabdo-		BF-2, BB, FHM		
virus	VHS	"		"

Virus group	Virus	Fish cell line	Temperature °C cell	virus
	SVC	FHM,BF-2,	25	15-20
		EPC	"	
Rhabdo-	PFY	"	"	"
virus	Eel rhabdo-	EPC,FHM	"	20
	virus			

-Retrovirus

-Unclassified group.