CHAPTER V

DISCUSSION

Freshwater fish cell cultures

The caudal trunk was the material which was used for preparing fish cell cultures. In the past, all attempts to cultivate cells from normal spleen, liver, swimming bladder, kidney and ovary tissues from adult stripe snakehead fish (*O. striatus*) in Eagle's MEM (with Earle's BSS) with 10 %fetal bovine serum, 10% tryptose phosphate broth, 10 % Tris HCl and antibiotics were unsucessful, excepted for ovaries and swimming bladder which formed confluent cell sheets. However, they could not be subcultured. Then, the caudal trunks of fry of stripe snakehead fish (size 2-3 cm) were used for culture which had formed a confluent monolayer. It could be grown for more than twenty passages (Watfanodorn 1985). But ovarial and embryonal tissues consistently provided good culture material (Wolf 1973a, Wolf and Quimby 1976a). In experiments, it was difficult to select fish which had immature ovaries for culture. For this reason, the caudal trunks of freshwater fish were used for culture because there were regeneration and division in this part (Denton 1973).

Cell attachment from properly prepared trypsinization usually began within hours after the cultures were seeded. Depending on seeding density and quality of the preparation, confluent culture could be expected within 2 to 5 days. Compared with primary cell cultures prepared from minced fish tissue fragments, cultures from trypsinized preparations were more homogeneous and developed more rapidly. Although the preparation time was greater, the trypsinization method lended itself more readily to large scale work. Primary monolayer cultures prepared from trypsinized tissue were suitable for virological applications and for initiation fish cell lines (Wolf and Quimby 1976b). So that, the trypsinized method was used for the preparation of freshwater fish cell cultures.

The tissues were trypsinized at room temperature and collected the loose cells every 15 to 30 minutes which was the method used by Gravell and Malsberger (1965). It was different from the method of Wolf and Quimby (1976a) which trypsinized at 5°C, 16 hrs. To prevent gross contamination from regurgitated food, feces or body surface, the caudal trunks were treated with 50 % clorox and 500 ug/ml of kanamycin. It was different from method of Wolf and Quimby (1976a) in which the internal organs were immersed in cool to cold hypochlorite solution containing 10 % house-hold bleach

per liter of water for 5 minutes, but the external organs, were immersed in solution containing 500 IU polymyxin B, 500 ug neomycin and 40 IU of bacitracin for 1 hr.

Morphology and Subculture of Cells.

At the time when this thesis was prepared the cell cultures of O. lucius and A. testudineus had undergone 120 and 70 subculture passages respectively to initiating a fish cell line. It was considered an adequate demonstration of establishment of a cell line, although the criteria for establishment of the cell lines of most species remained uncertain (Clark 1972, Schmidt 1979).

Cell cultures of C. carpio could be subcultured for about fifty passages. Thereafter, the cells began to degenerate. A cell line could not be established, if the conditions eg. pH, temperature, supplement or medium were changed. Induction of the cells with chemical agents or irradiation might be possible to establish a cell line.

Under these conditions, cell culture of Lebeo rohita did not survive more than fifteen passages. They began to degenerate as the same as C. carpio in fifty passages and in the same way as A. testudineus which had been prepared from larger fishes (10-11 cm). During passages when the medium or temperature were changed, they

began to clump after forming a monolayer. Thus, these cell cultures did not use for furture study.

The morphology of cell culture from A. testudineus, C. carpio, L. rohita and O. lucius was fibroblast-like just like many kinds of fish cell lines (Wolf and Mann 1980).

Optimal Growth Temperature and Growth Curve of the Cell.

There was a clear and direct relationship between temperature and the growth curve of the cells. Fish categories as coldwater or warmwater species and the temperature factor applied in vitro to culture condition. Growth of salmon and other coldwater fish cells extended from 5-25 °C while the optimal growth was near 20 °C. Fathead minnow and other warmwater fish cells generally did well at 25-30°C, but many grew at 15° through 35 °C or higher (Wolf 1973a), which had nearly the same range as the temperature tested in this study. At 4 °C, O. lucius, A. testudineus, could not grow but C. carpio, could be maintained for a short time. At 16°and 22 °C, C. carpio could be survived more than one month but A. testudineus, O.lucius could be survived for only a month. The optimal temperature for them was 28°C. At 37°C, O. lucius could be grown well for 7 days. After that it degenerated like the C. carpio.

Thus, 28°C seemed to be the optimal temperature

for the cell cultures from A. testudineus, C. carpio and O. lucius which stimulated a logarithmic growth phase of the cells within 3-7 days. Under these conditions, it was suitable to use cell cultures for studying viruses.

Sterility of the Cell Culture.

Microbial contamination was not detected in any of the examinations using thioglycollate broth and saboraud's agar plate.

Mycoplasma contamination was a major problem in cell cultivation. Mycoplasma, prokaryote was grown at or below the cytoplasmic membrane and was resistable to many kinds of antibiotics. Normally primary cultures were freed from mycoplasma whereas cell lines were contaminated. This condition indicated that the mycoplasma might be transferred from persons handling the cells or that some ingredients in the medium. Kanamycin and aureomycin could suppress but seldom eliminated a mycoplasma infection. An efficient aseptic technique and avoidance of mouth pipetting were important means to reduce the occurrence of mycoplasma infection in the culture. It had been reported that it was possible to eliminate a mycoplasma infection by passaging cells in a medium containing specific antiserum against the contaminating mycoplasma (Goran and Wadall 1983).

Viral Multiplication in the Cell Cultures.

Study of the ability of these cell cultures to detect the three viruses showed a lack of sensivity in the cell cultures of C. carpio. This cell culture did not develop a cytopathic effect (CPE) after being inoculatedat $10^{2 \cdot 5}TCID_{50}$/50 ul and undergoing five passages.

Conclusion

1. Primay cell cultures could be prepared from the caudal trunks of some species of freshwater fish :

Ophicephalus lucius

Anabas testudineus

Cyprinus carpio

Lebeo rohita.

2. Two species of freshwater fish could be estalished as cell lines :

Anabas testudineus

Ophicephalus lucius

3. Infectious pancreatic necrosis virus, snakehead fish viruses and carp viruses could be multiplied in cell cultures from A. testudineus, O. lucius but could not multiply in C. carpio.

4. The methods of building up primary and continuous cell lines were established.