Asian Fisheries Science 3(1990):299-307.
Asian Fisheries Society, Manila, Philippines.
https://doi.org/10.33997/j.afs.1990.3.3.003

Studies on a Cell Culture from the Hepatopancreas of the Oriental Shrimp, *Penaeus orientalis* Kishinouye

HU KE

Yellow Sea Fisheries Research Institute
19 Laiyang Road
Qingdao, China

WANG LIPING, DUAN YUMEI and ZHANG SHUJI

Shandong Institute of Marine Material Medica
Qingdao, China

Abstract

Hepatopancreatocytes from hepatopancreas tissue of the oriental shrimp, *Penaeus orientalis* Kishinouye were cultured in artificial medium *in vitro*. The new migrating cells could be observed from blind fragments of hepatopancreas tubules, and new cells also formed a confluent monolayer sheet. One primary culture and four subcultures were set up; some were subcultured for 17 and 28 generations, more than five months *in vitro*. The subcultured cells and fresh hepatopancreas tissue were studied histologically. The morphology of embryonic cells from hepatopancreas tissue was similar to that of cells obtained from subcultures which were considered to originate from embryonic cells of hepatopancreas tubular epithelium.

Introduction

Penaeid shrimps (Crustacea, Decapoda) are one of the important marine Crustacea which can be cultured on a commercial scale. The development and rapid expansion of prawn farming has occurred in China, Taiwan, Japan and throughout much of Southeast Asia, the main species of penaeid shrimp being cultured are the oriental shrimp (*Penaeus orientalis* Kishinouye), the giant tiger shrimp (*P. monodon* Fabricius) and the Kuruma shrimp (*P. japonicus* Bate).

At present, several viruses of shrimp, such as baculovirus penaei (BP) (Couch 1974), *P. monodon* baculovirus (MBV) (Lightner and Redman 1981; Lightner et al. 1983a), baculoviral midgut gland necrosis virus (BMN) (Sano et al. 1981), infectious hypodermal and hematopoietic necrosis virus (IHHNV) (Lightner et al. 1983b), hepatopancreatic parvo-like virus (HPV) (Lightner and Redman 1985) and reoviridae (RV) (Tsing and Bonami 1987) are known to adversely affect the culture of a number of penaeid shrimps. Diseases caused by some of these viruses have been reported to cause great losses to shrimp production.

The development of cell cultures for shrimp would provide a useful tool for studies on cellular metabolism and virus isolation *in vitro,* and can provide models for studying the biology of shrimp under standardized conditions. For these purposes, numerous tissue cells from different species of Crustacea have been studied, but to date, no subcultures have become available (see Peponnet and Quiot 1971; Patterson and Steward 1974). Chen et al. (1986) were the first to successfully culture cells from Crustacea, gonad cells from *Penaeus monodon* being subcultured for three passages.

In this paper, we describe the results of our attempt to culture hepatopancreas cells of *Penaeus orientalis.*

Materials and Methods

The penaeid shrimp *(P. orientalis)* used in this study was obtained a commercial shrimp farm in suburban. Qingdao during July 1987 and July 1988. The shrimp, of 5-10 cm body-length farmed 2-3 months, were chosen and taken to the laboratory for tissue cell culture.

To obtain hepatopancreas tissue for cell culture, shrimp were washed with clean seawater and the body surface sterilized several times with 2% tincture of iodine and 75% ethanol. The sterilized shrimp were then attached to the bottom of a dissecting tray and hepatopancreas tissue was carefully removed so as to avoid injury to the digestive system using sterilized forceps and scissors. The tissue was then minced into small pieces of about 1 cubic mm and washed several times with culture medium. The tissue fragments were pipetted up and down several times to facilitate dissociation into free cells and a few connective tissue strands. The cell suspension was made up with culture medium and 0.5 ml of the thoroughly mixed

cells was added to 0.5 ml of 0.2% trypan blue and allowed to stand for two minutes to determine cell viability. The cells were suspended in culture medium that was composed of balanced salt solution (Patterson and Steward 1974) and 199 TC medium (Nissui Seiyaku Co., Ltd. Japan), supplemented with 15% fetal bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin; seeded to culture flasks and incubated at 27-28°C.

For histological examination, subcultured cells were transferred to Leighton's culture tubes containing 5 x 21 mm glass cover slips. After culturing for several days, monolayer cultures which grew on the cover slips were fixed with 75% ethanol, and samples were stained as monolayers with hematoxylin and eosin (H & E). The fresh hepatopancreas tissue was cut into small pieces and the tissue fragments incubated in 0.25% trypsin (Difco Lab, USA) at 30°C. At 15-minute intervals, tissue fragments were dispersed to obtain free cells and a few connective tissue strands. The cell suspension was then washed with culture medium and centrifuged at 600 rpm for 10 minutes. The cells were spread in a thin layer on clean grease-free slides, fixed with 75% ethanol and stained with H & E.

Results

During July 1987-July 1988, four subcultures and one culture were set up. Some cultures were terminated by microbial contamination at an early stage of cultivation. Other cultures were maintained for short periods but deteriorated before or after subculturing. Consequently, only two subcultures remained alive for more than five months and had been subcultured for 28 and 17 passages. The history of the five cultures is shown in Table 1.

The cells and tissue fragments of the hepatopancreas were usually suspended in the medium at first. The small number of cells which happened to attach themselves to the glass surface of culture vessels after the culture was set up in 24 hours could be easily freed from the glass by gentle flushing of medium upon them. By three to four days postculture, cell migration from explant fragments of tissue started, and some aggregations of cells were growing out to form centers of multiplication. A number of migrating cells could be observed from blind fragments of hepatopancreas tubules (Fig. 1a). When the number of growing cells increased, they formed a confluent cell monolayer (Fig. 1b). The cells were mostly single, but double or

Table 1. The history of primary culture and subcultures from prawn hepatopancreas.

Culture designation	Date culture initiated	Number of passages	Status of cultures
POH-1	17/7/1987	2	Lost, 31/7/1987
POH-2-1	9/9/1987	28	Lost, 24/2/1988
POH-2-2	9/9/1987	17	Lost, 28/7/1988
POH-3	11/11/1987	1	Lost, 20/11/1987
POH-4	7/7/1988	5	Lost, 5/8/1988

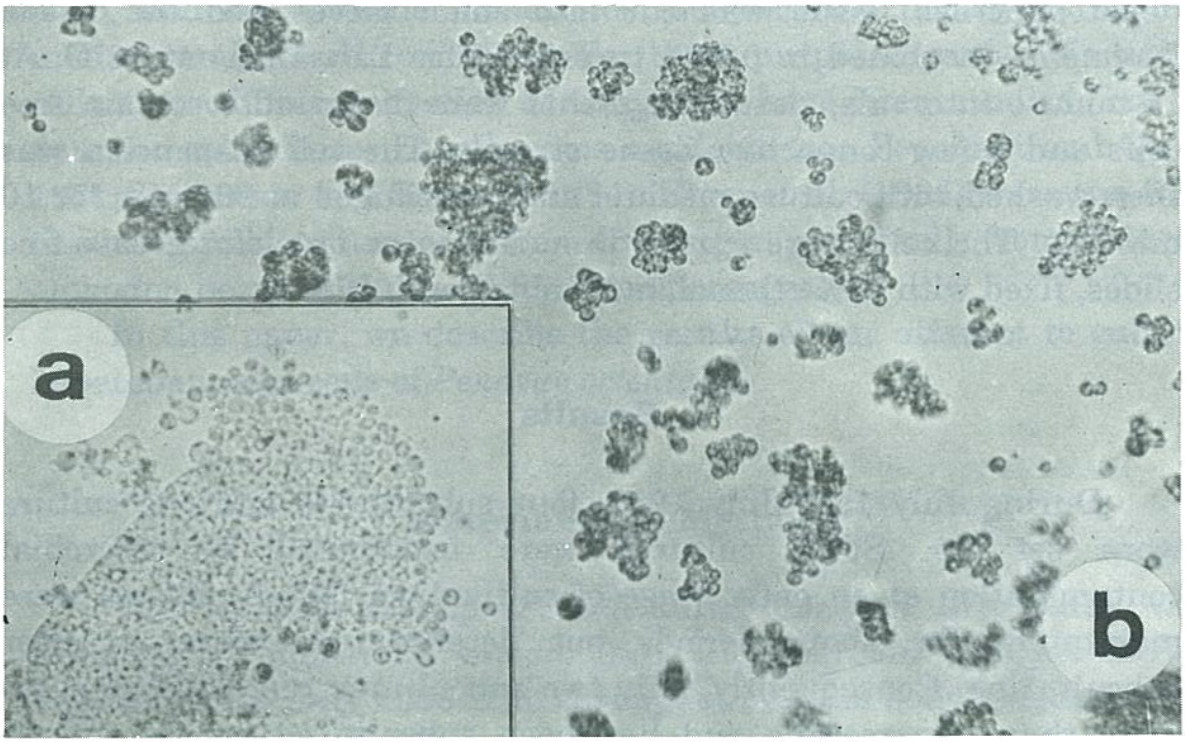

Fig. 1. (a) Cells growing from fragments of hepatopancreas tubules. 120X magnification. (b) The confluent monolayer of cells. 120X magnification.

multiple layers of cells, as well as cell aggregates were frequently observed near multiplication centers. After 9-12 days, subcultures were made by flushing the cell monolayers with culture medium or by simply shaking the flask and transferring the resultant cell suspension into new flasks with appropriate amounts of fresh medium. Subculturing was repeated at 6 or 7-day intervals.

In primary culture and subculture the cells were mostly spherical and about 8-15 μm in diameter, each with a large nucleus and small cytoplasm (Figs. 2-4).

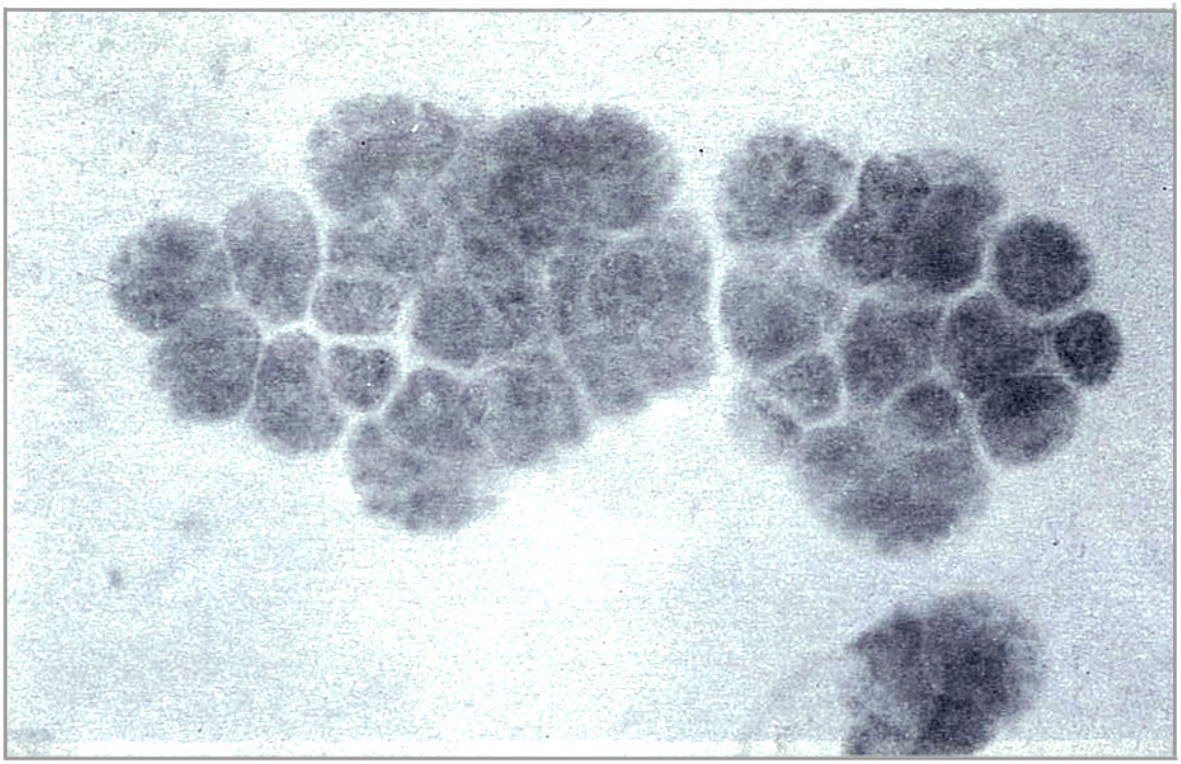

Fig. 2. Subculture cells stained with hematoxylin and eosin. 120X magnification.

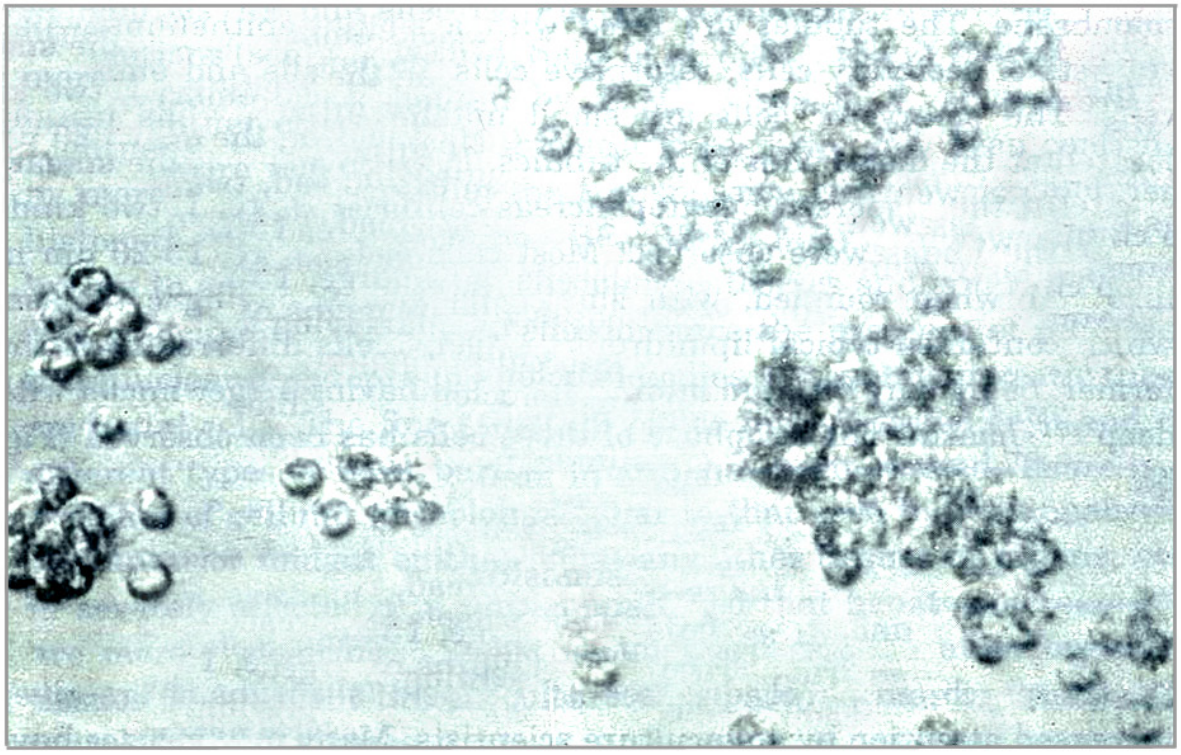

Fig. 3. Phase-contrast photomicrograph of POH-2-1 cells (17 passages). 250X magnification.

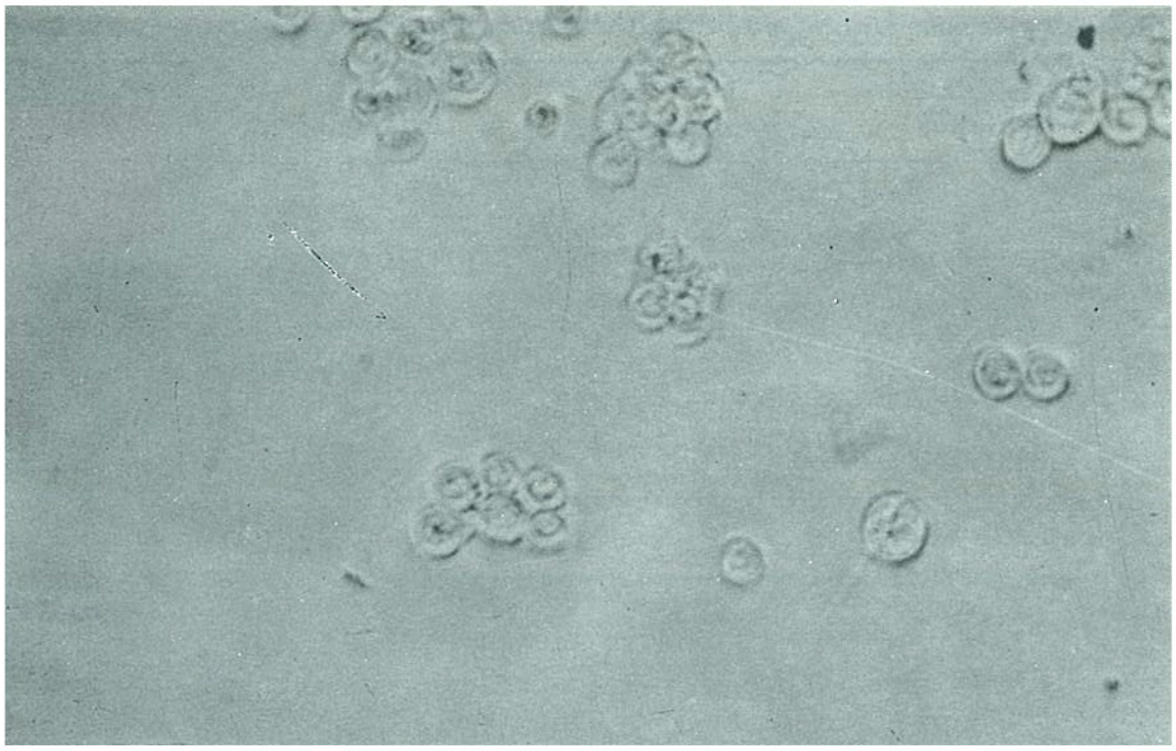

Fig. 4. Phase-contrast photomicrograph of POH-2-2 cells (12 passages). 250X magnification.

Histologically, the hepatopancreas of shrimp consists of a number of blind tubules which are enclosed by the tenacious membrane. The tubules are lined with a simple epithelium which consists of secretory cells, resorptive cells, fiber cells and embryonic cells. The embryonic cells are small undifferentiated cells usually located at the distal ends of the tubules. Upon examining the smears on which the dispersed hepatopancreas cells were spread, two kinds of spherical cells were observed. Most cells were large, 15-20 μm in diameter when rounded, with small nuclei, and having cytoplasm which contained typical lipid droplets. A few cells differred from the former, being only 8-15 μm in diameter, and having larger nuclei with deep chromatin. The telophase of these cells has been observed (Fig. 5).

Discussion

Research on viral diseases of penaeid shrimp has received increased attention by aquaculture scientists. Many new viruses have been recognized in major commercial shrimp species. BP and BMN were reported to be potentially serious diseases in hatchery-reared

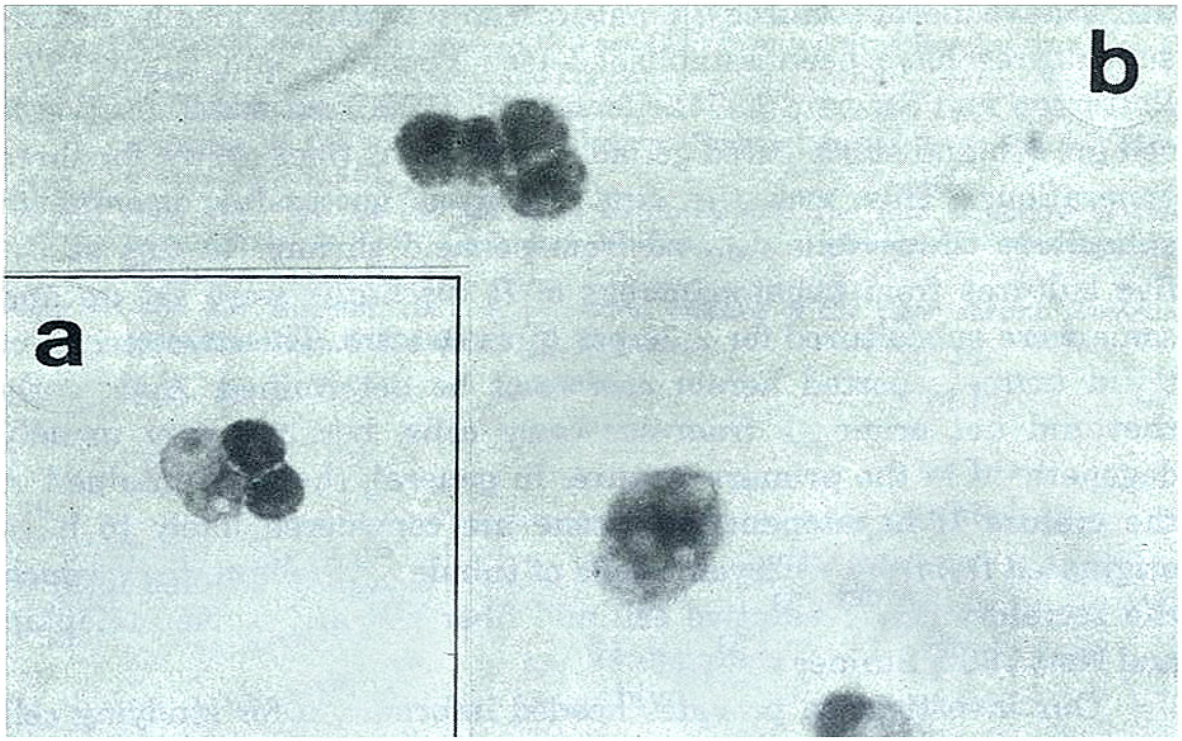

Fig. 5. Fresh hepatopancreatic cells dispersed by trypsin and stained with hematoxylin and eosin. (a) 400X magnification; (b) 1,000X magnification.

larval and early postlarval stages of their host species and cumulative mortalities have accounted for approximately 70-90% of original populations (see Couch 1974; Sano et al. 1981). Heavy mortalities and high infection were caused by IHHN virus in *P. stylirostris* (see Lightner et al. 1983b). All shrimp viruses have only been confirmed by more recent work using the transmission electronmicroscope and histopathological observation. BP, BMN, MBV, HPV and RV are known to adversely affect hepatopancreatocytes and anterior midgut. The principal feature of these lesions are the presence of prominent intranuclear or cytoplasm inclusion bodies in hepatopancreatic tubule epithelial cells. The fine structure of the virus-infected cells and the different types of virus particles found has been studied. There is no evidence of cellular pathology similar to that seen in hepatopancreas and anterior midgut epithelium in any other tissues or organs, even in severely affected prawn. It is indicated that hepatopancreatocytes are more susceptible to most shrimp viruses than other organs or tissues of shrimp. The cell culture of hepatopancreas is thus helpful to isolate these viruses.

The different tissues and organs of the lobster, crab and crayfish, including hemocyte, lymphoid gland, ovary, hepatopancreas and

heart have been cultured in natural and synthetic media, but no subcultures have been established (see Peponnet and Quiot 1971; Patterson and Steward 1974). Chen et al. (1986) obtained observable cell growth and subcultured gonad cells of giant tiger prawn for three generations. This was the first reported successful attempt to subculture tissue cells derived from penaeid shrimp. In this study, five cultures from hepatopancreas of *P. orientalis* were set up and some were subcultured for 2-38 passages *in vitro*. The exact origin of the cultures reported herein could not be determined. Apparently they did not originate from secretory cells, because they usually degenerated in the primary culture. In general, the cells obtained in the culture of hepatopancreas tissue are considered likely to have originated from the embryonic cells of tubule epithelium, from which the secretory cell, resorptive cell and fiber cell are derived (Hopkin and Nott 1980; Stainer et al. 1968).

Our investigation provides needed information for studying cell cultures derived from hepatopancreas tissue of penaeid shrimp. Investigations will be extended to identify more characteristics of the cell culture, such as metabolism, nutritional requirements and sensitivity to viruses, after which culture cells can be supplied for various experiments.

References

Chen, S.N., S.C. Chi, G.H. Kou and I.C. Liao. 1986. Cell culture from tissues of grass prawn, *Penaeus monodon*. Fish Pathol. 21: 161-166.

Couch, J.A. 1974. An enzootic nuclear polyhedrosis virus of pink shrimp: ultrastructure, prevalence, and enhancement. J. Invert. Pathol. 24: 311-331.

Hopkin, S.P. and J.A. Nott. 1980. Studies on the digestive cycle of the shore crab, *Carcinus maenas* (L.) with special reference to the B cells in the hepatopancreas. J. Mar. Biol. Assoc. U.K. 60: 891-907.

Lightner, D.V. and R.M. Redman. 1981. A baculovirus-caused disease of the penaeid shrimp, *Penaeus monodon*. J. Invert. Pathol. 38: 299-302.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983a. Observations on the geographic distribution, pathogenesis and morphology of the baculovirus from *Penaeus monodon* Fabricus. Aquaculture, 32: 209-233.

Lightner, D.V., R.M. Redman and T.A. Bell. 1983b. Infectious hypodermal and hematopoietic necrosis (IHHN), a newly recognized virus disease of penaeid shrimp. J. Invert. Pathol. 42: 62-70.

Lightner, D.V. and R.M. Redman. 1985. A parvo-like virus disease of penaeid shrimp. J. Invert. Pathol. 45: 47-53.

Patterson, W.D. and J.E. Steward. 1974. *In vitro* phagocytosis by hemocytes of American lobster *(Homarus americanus)*. J. Fish. Res. Board Can. 31: 1051-1056.

Peponnet, F. and J.M. Quiot. 1971. Cell cultures of Crustacea, Arachnida and Merostomacea, p. 341-359. *In* C. Vage (ed.) Invertebrate tissue culture, Vol. I. Academic Press, New York and London.

Sano, T., T. Nishimura, K. Oguma, K. Momoyama and N. Takeno. 1981. Baculovirus infection of cultured Kuruma shrimp, *Penaeus japonicus* in Japan. Fish Pathol. 15: 185-191.

Stainer, J.E., M.A. Woodhouse and R.L. Griffin. 1968. The fine structure of the hepatopancreas of *Carcinus maenas* (L.) (Decapoda: Brachyura). Crustaceana, 14: 56-66.

Tsing, A. and J.R. Bonami. 1987. A new viral disease of the tiger shrimp *Penaeus japonicus* Bate. J. Fish Dis. 10: 139-141.