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養殖草蝦細菌性肝胰臟炎之組織病理之研究

Histopathological Study of the Bacterial Induced Hepatopancreatiitis of Cultured Shrimp (Penaeus monodon Fibricius)

劉　國　鈞

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Abstract

Hepatopancreatic tissue of cultured shrimp (Penaeus monodon) were studied with both light and electron microscopes. The structure of the hepatopancreatic tissue were greatly altered by the presence of microoganism aggregates in the hepatopancreatic tubules. The distruction of the hepatopancreatic tissue can be observed both on the light and electron microscopic levels. Based on the observed morphological characteristics, the microorganism was tentatively identified as bacillus bacterium.

緒　　言

臺灣中南部及東北部爲草蝦 (Penaeus monodon) 之集中養殖區，由於業者急於短期獲利之心理，採取高密度方式養殖，而導致疾病之頻繁發生和大量死亡，造成嚴重損失，已引起各方之注意，先後已有多次調查報告之提出和防治方法之建議 (Lia *et al.*, 1985, Cheng *et al.*, 1986)。甲殼動物的疾病已有多種文獻可供參考 (Fisher 1978, Johnson 1977, 1978; Lightner 1983)。疾病發生誘因複雜，例如養殖池的溫度，酸度，氧氣含量和鹽度等物理性的改變。化學性方面如遺留之餌料和代謝生成物所衍生之毒性物質。生物性之藻，菌，原生動物及病毒等共同集合成一極爲複雜又相互影響之疾病誘因。故任何疾病的發生，都不易做單純的探討，本報告中所觀察的細菌性肝胰臟炎，當也無法獨立於蝦生長環境中複雜的疾病誘因之外。僅將所觀察結果做爲進一步探討之參考。

材 料 與 方 法

平均二十五隻一公斤重之養殖草蝦，發生不明原因死亡及活動性、攝食性減低等現象，蝦體無明顯傷害。解剖後將肝胰臟固定，切成小片，部分做光學顯微鏡觀察之處理，部分則做電子顯微鏡觀察之處理。做光學顯微鏡觀察處理之材料包埋於石蠟中，切片後用伊紅和蘇木紫染色。做電子顯微鏡觀察的材料，則用 Epon 和 Araldite 混合液包埋，超薄切片用醋酸鈾和檸檬酸鉛染色。利用 JEOL 電子顯微鏡，在 60 KV 加速電壓下觀察。

結　　果

上市草蝦肝胰臟呈土黃色至淺灰色，分爲多個小葉，間有結締的被膜相隔，小葉由許多小管縱向排

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列而成。消化管由中央通過 (Fig. 1) 小管內壁由單層柱狀上皮組織所構成 (Fig. 1, 2, 3)。小管間之空隙卽爲血淋巴 (hemolymph) 循環所經過之處。

病蝦之肝胰臟略呈灰黃色，表面略具不規則之變色斑塊，體積上並不見顯著之差異。在光學顯微鏡下，部分肝胰小管出現病變，可觀察到病變包括：肝管腫大變形，病變嚴重的肝胰管內壁的細胞變形，空泡、消失及肝胰管中充滿無定形顆粒狀物質(Fig. 4, 5, 6)。在高倍電子顯微鏡放大倍率的影像中，這些顆粒在形態上顯示出微生物的特徵，外有細胞壁，內有細胞膜。由隨意取樣的方式，量度二十個顆粒的直徑平均爲 1.3 微米，最大直徑爲 2.1 微米最小直徑爲 0.8 微米。顆粒的切面多呈橢圓形 (Figs. 7, 8)，故判定爲桿狀細菌的一種 (Alam 1987)。在嚴重桿狀菌集結之肝胰小管，管徑增大。並觀察到小管破裂及游離細胞圍繞在呈現病變之小管四周 (Fig. 7)。這類細胞略呈圓形，具有巨大的細胞核位於中央，不具上皮細胞之特徵。原來肝胰管的結構已遭破壞。電子顯微鏡放大下肝胰管的周圍具肌上皮組織 (myoepithelium) (Fig. 8)。肌上皮細胞中之肌纖維呈紡錘形，小管間血球細胞呈圓形，細胞質中含有圓形顆粒 (Fig. 9)。在嚴重病變肝胰小管間，肌上皮組織遭破壞。在嚴重的情況下，肝胰小管間崩潰，小管間隙消失 (Fig. 10)。在病變過程中可以見到肌上皮細胞及血球細胞破裂，肌纖維萎縮及血球細胞之細胞核濃縮，所含顆粒出現在細胞外的現象 (Fig. 10)。

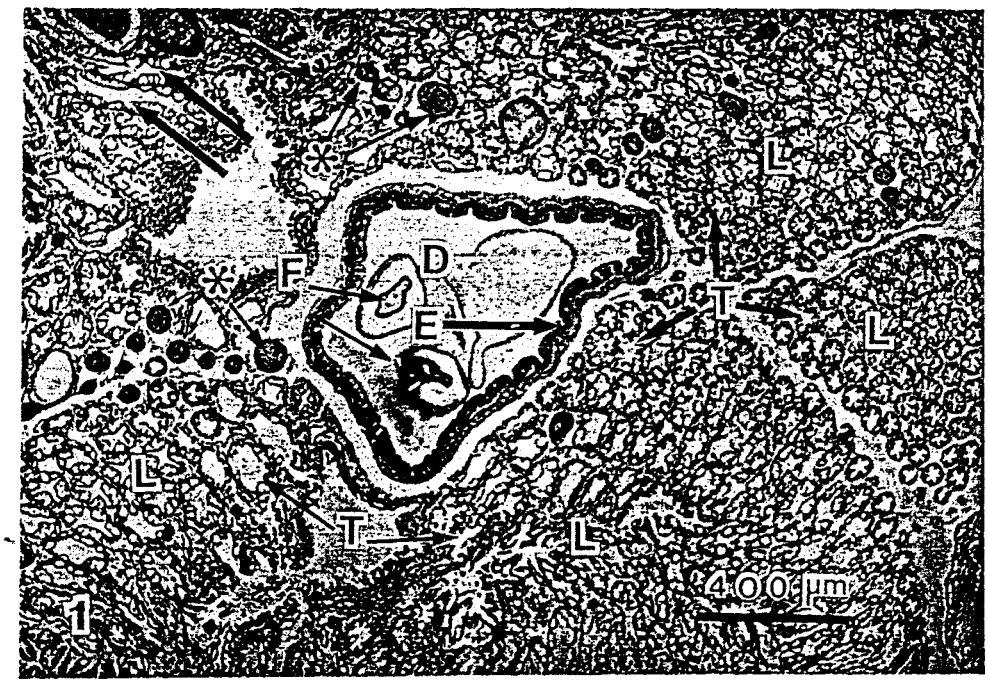

Fig. 1. Light microscopic picture of the hepatopancrease of cultured shrimp. It consists of many lobules (L), There is a connective tissue covering each lobule. The digestive track (D) passes through the central area of the hepatopancrease. There are food particles (F) left in the digestive track. The microvilli of the simple columnar epithelium (E) is distingushable. The hepatopancreatic lobules consist of many tubules (T). In this figure the tubules were cross sectioned and only a few tubules were longitudinally sectioned (Arrows). The arrangement of the tubules is parallel to the digesttive track in this section. A few of the tubules (Star) show pathological changes.

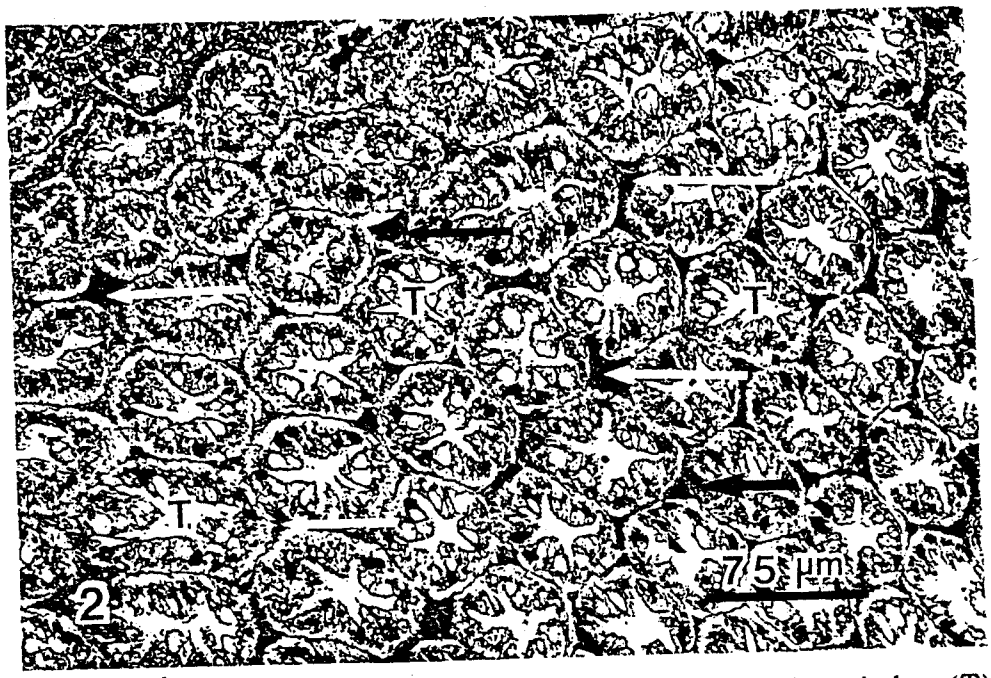

Fig. 2. The cross section of the normal hepatopancreatic tubules (T). The averaged diameter of the tubules is about 80 micron. The intertubular space (Arrows) is the areas where hemolymph circulates.

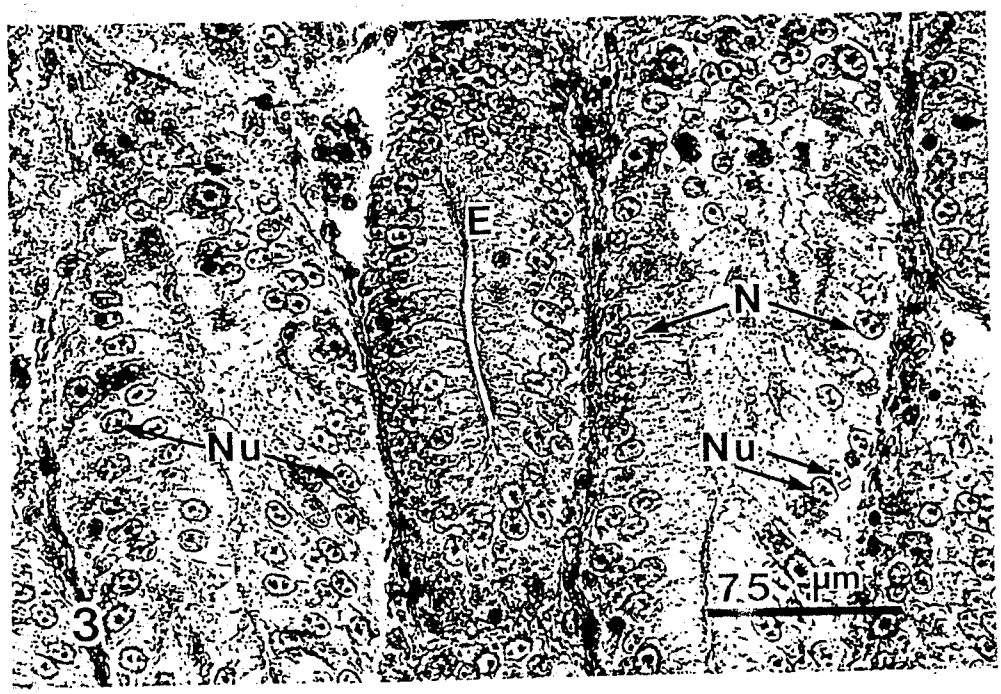

Fig. 3. The longitudinally section of normal hepatopancreatic tubules. The columnar epithelial cells (E) contain large nucleus (N), located at the lower part of the cells. Many nuclei contain more than on mucleoli (Nu).

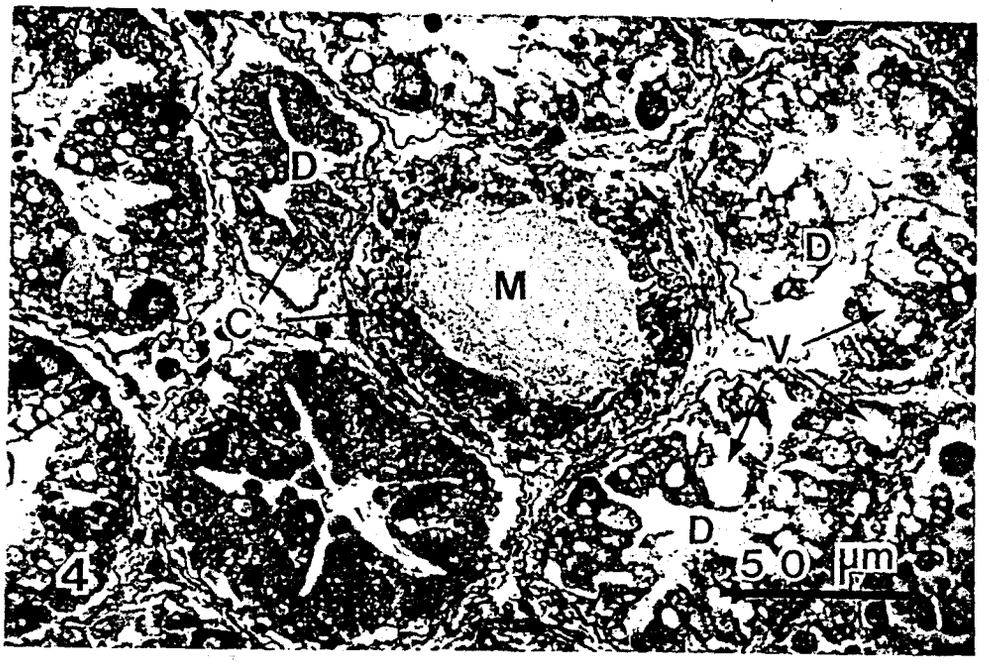

Fig. 4. Cross section of diseased hepatopancreatic tubules show disruppted tubules (D) with epithelial cells (C) loss their normal columnar shape, with large vacuoles (v), and tubule with large mass of amorphrous material (M).

Fig. 5. Longitudinally section of tubules show serious pathological condition. The tubules were greatly enlarged by the accumulation of amorphrous material (M) in the lumen of the tubules. The tubular epithelium (E) loss its cellular morphology.

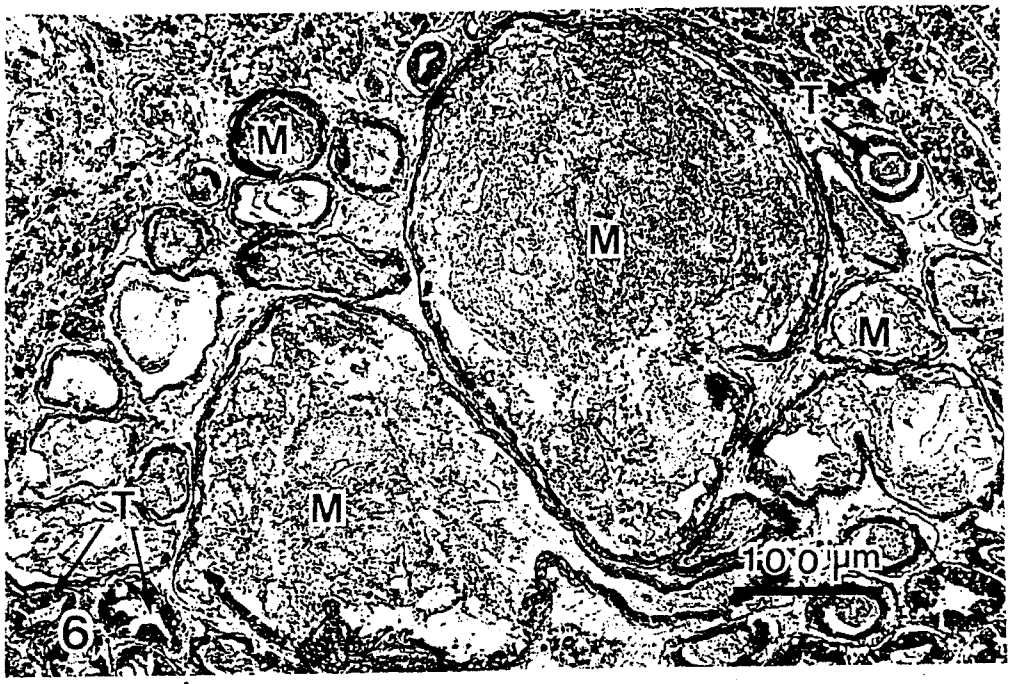

Fig. 6. Cross section of tubules show serious pathological condition. The tubular lumen are greatly enlarged by the accumulation of amorphrous material (M). Some of the tubules (Arrows) are still distinguishable.

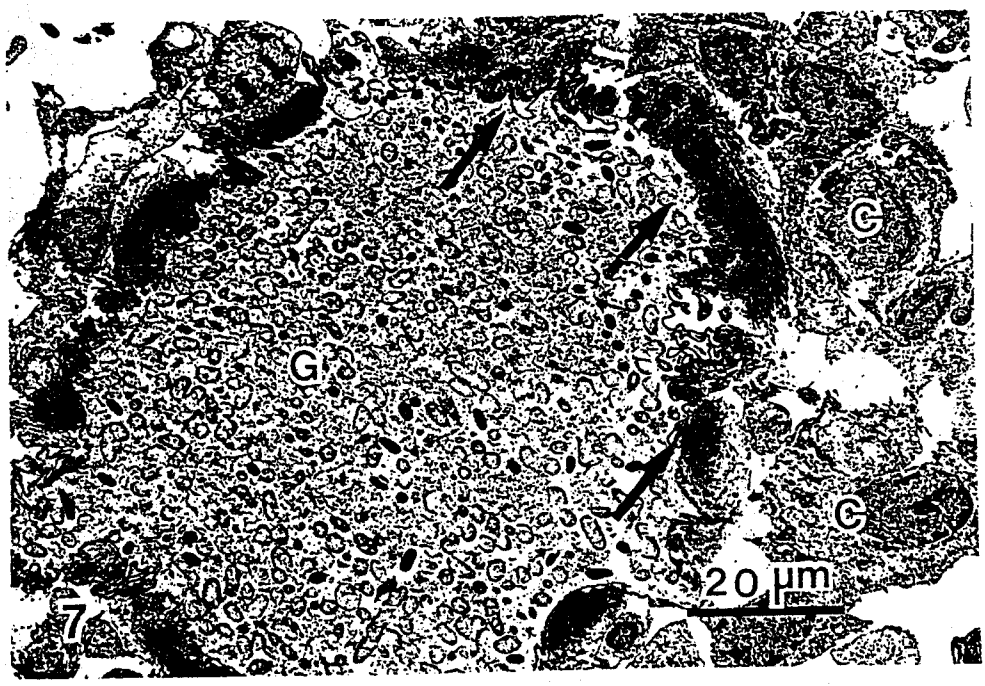

Fig. 7. Under electron microscope the amorphrous material in the diseased tubules show granular nature (G). The tubular epithelial cells (Arrows) loss their cellular morphology. The cells (C) arround the tubule are not epithelial in nature. They may be hemocytes from the intercellular space.

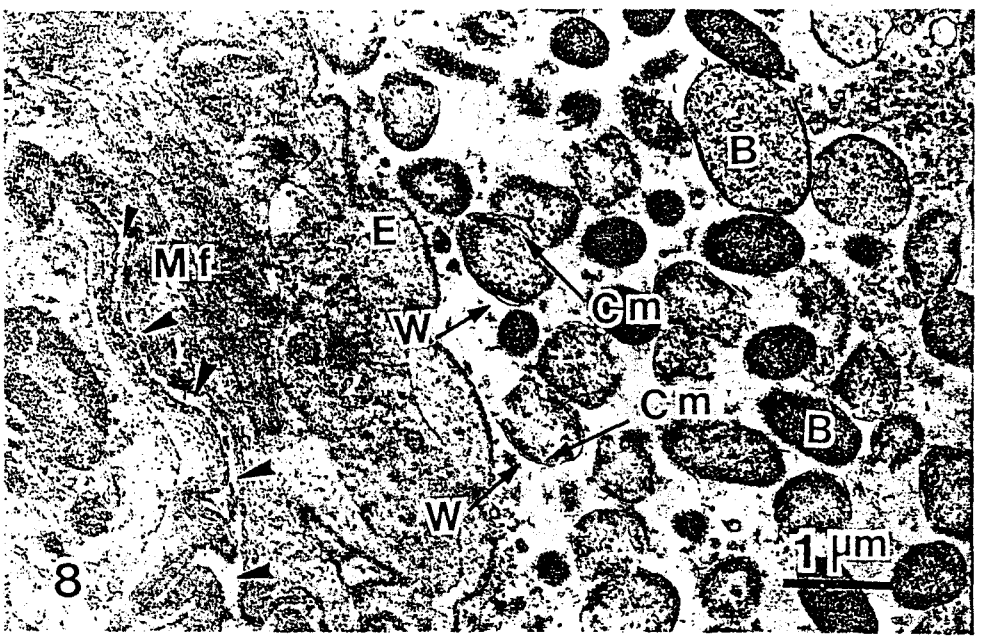

Fig. 8. Electron-micrograph of higher magnification. The granules (B) of the tubule are microoganism in nature. They show both cell wall (W) and cell membrane (Cm). The epithelium (E) and myoepithelium were distroyed. The myofilaments (Mf) were the indication where the myoepithelium used (to be located.) The intertubular space (Arrows) was reduced to a narrow space.

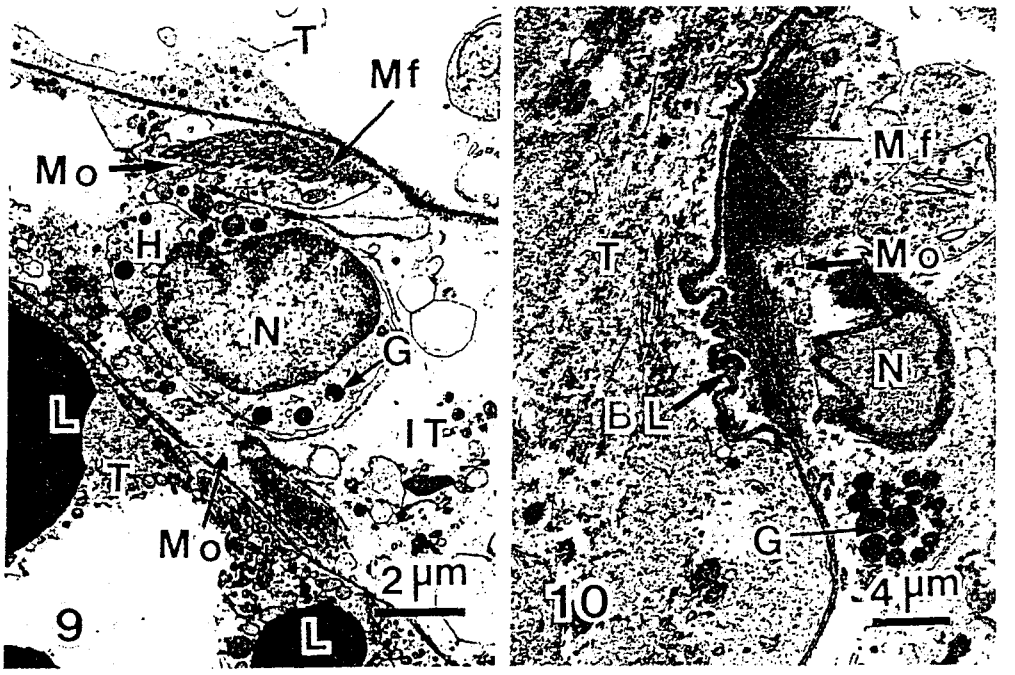

Fig. 9. Electronmicrograph shows the intertubular space under normal condition. The myoepitheial cells (Mo) and hemocyte (H) located between tow tubular cells. The myoefilaments (Mf) were spindle in shape. The hemocyte is round with a large nucleus and many dense granules (G), (L) indicates the lipid droplets in the tubular cells.

Fig. 10. Electronmicrograph of part of a diseased tubular cell (T) and part of the intertubular space. The condensed myofilaments (Mf) indicates the distroyed myoepithelial cell, and the condensed nucleus (N) and granules (G) indicate a distroyed hemocyte. The basement menbrane (BL) is folded, which indicated the disrupption of a tubular organization.

討　　論

在近年來國內多篇關於養殖草蝦疾病之研究報導中，已有關於各種生物和環境因子病原和疾病種類的報導。其中亦見關於肝胰臟病毒性肝胰臟炎的報告 (Cheng *et al.* 1988)。唯細菌性肝胰臟炎的組織病理性研究尚屬首次。由於觀察之情況顯示，病原可能爲桿狀菌之一種。由於對肝胰臟小管的破壞和集結的範圍及造成傷害程度，可以確定細菌曾在其中繁生過一段長時間才能造成，至於確切的檢定尚需進一步之研究。肝胰管的周圍肌上皮組織，在其他甲殼動物已有報導(Dell and Moriarty 1983)，具有保護及促進分泌的作用，如遭破壞，自然影響到血液的循環和小管的功能，肝胰臟爲營養器官，根據已有之報導，蝦類肝胰管內具有形態和功用不同的細胞(Miyawaki *et al* 1961; Miyawaki and Tanoue 1962; Dall and Moriarty 1983)，小管細胞之破壞和阻塞，影響到肝胰細胞的功能，同時大量桿狀細菌的繁生亦可能生成有毒性的代謝產物，造成草蝦死亡。但更進一步的細菌分離培養與室內感染才能使這一病害的眞正原因得到確切的證明。

摘　　要

光學與電字顯微鏡對不明原因死亡之病蝦肝胰組織之觀察結果之描述，正常與病變組織之比較與病原之初步鑑定。

謝　　辭

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參 考 資 料

Aleam, I. E. Fundamemtals of Microbiology. The Benjanion Cremmings Co. INC. 1987.

Fisher, W. S., E. H. Nilson, J. Steengeregen and D. V. Lightner. (1978) Microbal Disease of Cultured Labsters. A Review. Aquaculture. 14: 115-140.

Johnson, S. K. (1978) Handbook of Shrimp Disease, Sea Grant Publ. TAMU-G 75-603. Texas A and M University.

Johnson, S. K. (1978) Some disease Problem in Crayfish and Fresh Water for Shrimp Culture. FDDL-S11 pp. 1-4.

Lia, C. G. H. Kao, S. N. Chen and J. Y. Lai. (1985) Priliminary investigation on the Diseases of Cultured Prawn in Ping Tung Area. COA Fisheries Series of Fish Disease Res. (VII). 86-94.

Lightner, D. V. (1978) Gill Disease: A Disease of Wild and Cultured shrimp. International Council for the Bxperimentation of the Sea. Coppenhagen. Denmark.

Miyawaki, M., M. Matsuzaki and N. Sasaki. (1961) Histoochemical Studies on the Hepatopancreas of the Crayfish, Procambarus clarkii. Kumamoto. J. Sci. 6: 1-11.

Miyawaki, M. and S. Tanoue. (1962) Electron MIcroscopy of the Hepatopancreas in the Crayfish, Pracombarus clarkii. Kumanoto J. Sci. 7: 1-11. 1962.

Tseng-Jou Cheng and Cheng-I. (1986) Liu. Pathological Study on Major Disease of Cultured Grass Shrimps (Peaneus monodon Fabricius) COA Fisheries Series NO 8. Fish Disease Res. (VIII): 75-68.